# The effects of dietary ricinoleic acid from castor oil on the zootechnical traits and haemato-biochemical profile of lactating Kankrej cows

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#### **ABSTRACT**

The aim of the present study was to evaluate the effects of dietary ricinoleic acid from castor oil on the milk yield, composition, fatty acid profile and haemato-biochemical profile in lactating Kankrej cows. Twenty lactating Kankrej cows were assigned to the following groups: control (CON), basal diet without any additive and treatment (RAS), basal diet with 2 g/animal/day of ricinoleic acid from castor oil. Dietary ricinoleic acid had no (P>0.05) effect on dry matter intake and feed efficiency. Milk yield (milk, 4% fat corrected milk and energy corrected milk) were not affected (P>0.05) by the feeding of ricinoleic acid. The milk composition showed no significant differences between the groups. A significantly (P<0.05) higher percentage of C4:0, C6:0 and C8:0 was found in the milk from the RAS group than the CON group. A lower (P<0.05) milk C16:0 percentage was observed in the RAS group as compared to the CON. The percentages of C18:2 and C18:3 were found to be increased in the RAS group. There was a decrease (P = 0.055) in milk saturated fatty acids but an increase in milk unsaturated fatty acids percentage in the RAS as compared to the CON group. The milk polyunsaturated fatty acids were significantly (P<0.05) increased in the RAS group. There was no difference (P>0.05) in haemato-biochemical profile between the CON and RAS groups. It was concluded that supplementation of 2 g/animal/day ricinoleic acid from castor oil had no effect on milk yield, milk composition and haemato-biochemical profile. However, it increased the milk fat percentages of unsaturated fatty acids and polyunsaturated fatty acids, and decreased milk saturated fatty acids, without any adverse effect on the health status of the cows.

Key words: Kankrej cow; milk yield; fatty acid profile; blood metabolites; ricinoleic acid

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## Introduction

Ruminal biohydrogenation of the dietary unsaturated fatty acids (UFA) ingested by ruminants results in the production of saturated fatty acids (SFA) at the expense of the UFA. Milk and dairy products are a major source of SFA in the human diet, but also serve as a versatile source of nutrients (KLIEM and SHINGFIELD, 2016). Public health policies recommend a population wide decrease in the consumption of SFA to lower the incidence of cardiovascular and metabolic diseases. Altering milk fat composition offers the opportunity to replace the SFA in milk fat with monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). Animal scientists are experimenting with various alternative strategies to manipulate the ruminal biohydrogenation process, in order to obtain meats and milk with a lower SFA content, which would be of great value for consumer health. To avoid the use of synthetic molecules or antibiotics in livestock farming, animal scientists are focusing on the use of plant bioactive lipid compounds as potential modulators of ruminal fermentation and enhancers of animal productivity (HAUSMANN et al., 2018; PAWAR et al., 2019). Plant bioactive lipid molecules, such as ricinoleic acid from castor oil may have several beneficial responses in animal production. Ricinoleic acid (12-hydroxycis-9-18:1) is the main fatty acid component (about 90%) of castor oil, which is obtained by pressing castor seeds (TORRENTES-ESPINOZA et al., 2017). Ricinoleic acid acts as a functional oil by providing health benefits, viz. antimicrobial (FERREIRA et al. 2002), anti-inflammatory (VIEIRA et al. 2000), anti-oxidative (TREVISAN et al. 2006), and gastro-protective (HAMAD and MUBOFU, 2015) properties, besides its nutritive properties. The addition of ricinoleic acid/castor oil improved rumen fermentation, increasing the molar proportion of propionate and decreasing ammonia nitrogen concentration, and a relative abundance of methanogenic archaea has been reported in in vitro and in vivo research (SERADJ et al., 2017; KANG et al., 2018; KONDA et al., 2019). Propionate is the most important substrate for hepatic gluconeogenesis (accounting for 60-74% of the total substrate) and

is highly associated with milk production in dairy cows (ASCHENBACH et al., 2010; HAMMON et al., 2010). Recent studies have shown that castor oil/functional oil supplementation increased the milk yield of dairy cows (GANDRA et al., 2014; FERREIRA DE JESUS et al., 2016; MATLOUP et al., 2017; JOSHI, 2019). In vitro studies showed that ricinoleic acid modulates the biohydrogenation of UFA, and increases the content in vaccenic and rumenic acids, leading to the increased concentrations of trans-18:1 and CLA isomers (MORALES et al., 2012; ALVES et al., 2017). To test these hypotheses, we assessed the effects of the dietary addition of ricinoleic acid from castor oil on milk yield, composition, fatty acid profile and blood parameters in lactating Kankrej cows.

## Materials and methods

The use of the animals and the experimental procedure were approved by the Institutional Animal Ethics Committee (IAEC) constituted as per the Article 13 of the rules of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), laid down by the Government of India. The IAEC approval number is VETCOLL/IAEC/2018/13/PROTOCOL-2.

Animals, feeding and experimental design. Twenty primiparous Kankrej cows (average 21 days in milk; 8.84 kg/d of milk yield and 340 kg of body weight) were selected from the herd maintained at the Livestock Research Station, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat, India. It is located in semi-arid region at a latitude of 24.35 ° North and longitude of 72.59 ° East, and an elevation of 189 meters above mean sea level. The cows were randomly assigned to the following treatments: control (CON), basal diet (consisting of concentrate mixture, green lucerne fodder and jowar hay) without feed additive; 2), Ricinoleic acid (RAS), basal diet with 2 g/d/cow of ricinoleic acid added. Ricinoleic acid was mixed thoroughly into concentrate and provided once daily for period of 56 days. The basal diet was formulated to meet

Composition	Concentrate mixture	Jowar hay	Green Lucerne
Dry matter	95.55	89.73	19.92
Crude protein	18.59	2.29	18.76
Crude fibre	6.58	37.58	22.64
Ether extract	2.43	1.36	2.93
Ash	8.25	10.51	9.38
NFC	35.53	28.22	27.80
NDF	35.2	57.62	41.13
ADF	19.05	45.17	21.15

Table 1. Chemical composition (% DM basis) of concentrate, Lucerne and jowar hay in the diet of lactating Kankrej cows

NFC - Non-fibre carbohydrate, calculated by equation: NFC (% of DM) = 100 - (CP + NDF + EE + ash); NDF - Neutral detergent fibre; ADF - Acid detergent fibre.

ICAR (2013) nutrient requirements. The chemical composition of feeds and fodders fed to the experimental animals is given in Table 1.

Sampling and chemical analysis. The feeds offered and subsequent left-overs were recorded for each animal in order to calculate feed intake. Samples of feeds and fodders were collected, composited and dried at 60 °C in a forced air oven for 48 h, and ground to pass through a 1-mm screen using a Wiley mill (Star Scientific Instruments, Delhi, India). The feeds samples were analysed for dry matter (DM, method 934.01), ash (method 942.05), crude protein (method 976.05) and ether extract (method 973.18) according to AOAC (2007). Neutral detergent fibre and acid detergent fibre were determined as per VAN SOEST et al. (1991). Nonfibre carbohydrate was calculated according to NRC (2001). The cows were milked twice a day, and the individual milk yield for each cow was recorded daily using an electronic weighing balance. The 4% fat corrected milk (FCM) was calculated as stated by GAINS (1928): milk yield (kg) x 0.4 + fat yield (kg) x 15. Energy corrected milk (ECM) was determined according to DAVIDSON et al. (2008):  $0.327 \times \text{milk yield (kg/day)} + 12.86 \times \text{fat yield (kg/day)}$ day) +  $7.65 \times$  protein yield (kg/day). Milk samples were collected at fortnightly intervals for analysis of milk composition (fat, solids-not fat (SNF),

protein and lactose) using an EKOMILK Ultra Pro Milk Analyser (Everest Instruments Pvt. Ltd.).

Determination of milk fatty acid profile. Milk fatty acids were analysed by isolating milk fat by centrifugation and methylation using sodium methylate, according to O'FALLON et al. (2007). Fatty acid methyl esters were analysed using a gas chromatograph (Thermo Scientific Ceres 800) with flame ionization detector and capillary column (60 m  $\times$  0.25 mm  $\times$  0.20 mm). The initial oven temperature was 120 °C, held for 5 min, subsequently increased to 240 °C at a rate of 2 °C min<sup>-1</sup>, and then held for 60 min. Nitrogen at a flow rate of 1 ml/min was used as the carrier gas. Both the injector and the detector were set at 260 °C. As an internal standard fatty acid Heptadecanoic acid C17:0 (Catalogue number H3500, Sigma-Aldrich, Bangalore, India) was used, and a mix of FAME standards (Supelco 37 Component FAME Mix, Sigma Aldrich, Bangalore, India) was used to generate a calibration curve. Fatty acids were identified by comparing their retention times with the fatty acid methyl standards, and were expressed as a percentage of the total fatty acids.

Blood sampling and analysis. On the 56<sup>th</sup> day of experimental feeding, blood samples were collected from the jugular vein of each animal into vials (BD Vacutainer® Spray-coated K2EDTA Tubes, BD Franklin Lakes, NJ, USA) with and without EDTA

(BD Vacutainer® Plus Plastic Serum Tubes, BD Franklin Lakes, NJ, USA). The fresh blood samples were analysed for haemoglobin, haematocrit, erythrocytes, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), absolute numbers of leucocytes, neutrophils, lymphocytes and monocytes using Exigo EOS Vet Haematology Analyser (Boule Medical AB, Sweden).

The serum samples were analysed for concentrations of glucose, total proteins, albumin, urea, creatinine, triglycerides, cholesterol, calcium and inorganic phosphorus along with activity

of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT), using a Randox Monaco Analyser (Randox Laboratories Ltd., UK).

Statistical analysis. All the experimental data obtained were statistically analysed using SPSS v.16.0 (SPSS Inc., Chicago IL) as per the standard statistical methods (SNEDECOR and COCHRAN, 1994). Significant differences between means of treatments were assessed by Duncan's test, and the differences between treatments were declared significant at P<0.05.

#### Results

Dietary ricinoleic acid had no (P>0.05) effect on DM intake in lactating Kankrej cows (Table 2). The DM intake was  $8.81 \pm 0.27$  and  $9.15 \pm 0.23$  kg/d in the CON and RAS groups, respectively. Milk yields in terms of kg/d, 4% FCM and ECM were not affected (P>0.05) by feeding with ricinoleic acid as compared to the CON. No differences (P>0.05) were observed in yields of milk fat, SNF, total solids, protein and lactose between the CON and RAS groups. The milk compositions (fat, SNF, total solids, protein and lactose) were comparable without any difference (P>0.05) between the groups. Feed efficiency [milk (kg)/DMI (kg), 4% FCM (kg)/DMI (kg) and ECM (kg)/DMI (kg)] did not differ (P>0.05) between the CON and RAS groups.

Following the dietary addition of ricinoleic acid modified milk fatty acids profile (Table 3) the milk from the RAS group had significantly (P<0.05) higher percentages of short chain fatty acids such as C4:0, C6:0 and C8:0 than the CON group. A significantly (P<0.05) lower percentage of C16:0 long chain fatty acid was observed in the RAS as compared to the CON group. The percentages of C18:2 and C18:3 were found to be significantly (P<0.05) higher in the RAS group than in the CON

group. Feeding of ricinoleic acid resulted in a decrease (P = 0.055) in the percentages of milk SFA (70.82  $\pm$  1.40 vs. 74.27  $\pm$  0.92) and an increase (P = 0.055) in milk UFA (5.73  $\pm$  0.92 vs. 29.18  $\pm$  1.40) in the RAS as compared to the CON group. The PUFA were 2.58  $\pm$  0.10 and 4.65  $\pm$  0.48 g/100g in the CON and RAS groups, respectively, and were significantly (P<0.05) higher in the RAS group than in the CON.

There was no effect (P>0.05) on the mean concentrations of red blood cells (haemoglobin, haematocrits, erythrocytes, MCV, MCH, MCHC) and white blood cells (leucocytes, neutrophils, lymphocytes, monocytes) in the CON and RAS groups (Table 4). No difference (P>0.05) was found in serum concentrations of total proteins, albumin, globulin, creatinine, triglycerides, cholesterol, ALT, AST, ALP, GGT, calcium and inorganic phosphorus between the two groups (Table 5).

Table 2. The effect of supplementation of ricinoleic acid on dry matter intake, milk yield and milk composition in lactating Kankrej cows (n = 20)

Parameters	CON	RAS	P value				
Dry matter intake (DMI)							
DMI (kg/d)	$8.81 \pm 0.27$	$9.15 \pm 0.23$	0.341				
Yield (kg/d)	Yield (kg/d)						
Milk	$9.92 \pm 0.47$	$9.42 \pm 0.54$	0.489				
4% FCM	$10.30 \pm 0.61$	$9.67 \pm 0.55$	0.448				
ECM	$11.15 \pm 0.59$	$10.58 \pm 0.59$	0.505				
Fat	$0.42 \pm 0.03$	$0.39 \pm 0.02$	0.453				
Solids not fat	$0.81 \pm 0.04$	$0.77 \pm 0.05$	0.459				
Total solids	$1.23 \pm 0.06$	$1.16 \pm 0.07$	0.430				
Protein	$0.32 \pm 0.01$	$0.32 \pm 0.02$	0.821				
Lactose	$0.44 \pm 0.02$	$0.42 \pm 0.02$	0.706				
Milk composition (%) <sup>¥</sup>							
Fat	$4.23 \pm 0.16$	$4.18 \pm 0.07$	0.799				
Solids not fat	$8.22 \pm 0.10$	$8.17 \pm 0.13$	0.759				
Total solids	$12.45 \pm 0.14$	$12.35 \pm 0.17$	0.668				
Protein	$3.30 \pm 0.06$	$3.40 \pm 0.03$	0.163				
Lactose	$4.40 \pm 0.07$	$4.50 \pm 0.02$	0.192				
Feed efficiency							
Milk (kg)/DMI (kg)	$1.13 \pm 0.06$	$1.04 \pm 0.07$	0.313				
4% FCM (kg)/DMI (kg)	$1.17 \pm 0.07$	$1.07 \pm 0.07$	0.297				
ECM (kg)/DMI (kg)	$1.27 \pm 0.07$	$1.17 \pm 0.08$	0.327				

FCM - fat corrected milk; ECM: energy corrected milk. CON - Basal diet without additive; RAS - Basal diet + 2 g/animal/day of ricinoleic acid; \*Milk samples were collected at fortnightly intervals for analysis of milk composition

Table 3. The effect of supplementation of ricinoleic acid on the milk fatty acids profile (g/100 g) in lactating Kankrej cows (n = 20)

Fatty acids	CON	RAS	P value
C4:0	$2.24^{a} \pm 0.18$	$2.79^{b} \pm 0.07$	0.012
C6:0	$1.46^{a} \pm 0.10$	$1.99^{b} \pm 0.13$	0.004
C8:0	$1.08^{a} \pm 0.05$	$1.73^{b} \pm 0.15$	0.001
C10:0	$2.23 \pm 0.16$	$2.39 \pm 0.14$	0.464
C12:0	$2.58 \pm 0.18$	$2.96 \pm 0.20$	0.181
C14:0	$13.55 \pm 0.51$	$13.10 \pm 0.43$	0.506
C14:1	$0.87 \pm 0.05$	$0.90\pm0.08$	0.810
C15:0	$0.51 \pm 0.05$	$0.63 \pm 0.06$	0.138
C15:1	$0.16 \pm 0.02$	$0.13 \pm 0.01$	0.150
C16:0	$32.19^{b} \pm 0.95$	$26.06^{a} \pm 1.05$	0.001
C16:1	$2.47 \pm 0.10$	$2.52 \pm 0.11$	0.732

Table 3. The effect of supplementation of ricinoleic acid on the milk fatty acids profile (g/100 g) in lactating Kankrej cows (n = 20) (continued)

Fatty acids	CON	RAS	P value
C17:0	$0.64 \pm 0.04$	$0.71 \pm 0.04$	0.178
C17:1	$0.20 \pm 0.01$	$0.18 \pm 0.01$	0.220
C18:0	$16.03 \pm 0.79$	$16.84 \pm 0.56$	0.412
C18:1	$19.44 \pm 0.89$	$20.81 \pm 1.43$	0.427
C18:2	$1.67^{a} \pm 0.10$	$2.90^{b} \pm 0.36$	0.004
C18:3	$0.91^{a}\pm0.05$	$1.75^{b} \pm 0.20$	0.001
C20:0	$0.19 \pm 0.02$	$0.22 \pm 0.02$	0.335
C22:0	$1.58 \pm 0.12$	$1.39 \pm 0.11$	0.255
SFA	$74.27 \pm 0.92$	$70.82 \pm 1.40$	0.055
UFA	$25.73 \pm 0.92$	$29.18 \pm 1.40$	0.055
MUFA	$23.15 \pm 0.88$	$24.53 \pm 1.46$	0.430
PUFA	$2.58^a \pm 0.10$	$4.65^{b} \pm 0.48$	0.001
U/S	$0.35 \pm 0.02$	$0.42 \pm 0.03$	0.057

a, b values in a row with different superscripts differed significantly (P<0.05); CON - Basal diet without additive; RAS - Basal diet + 2 g/animal/day of ricinoleic acid; SFA - saturated fatty acids; UFA - unsaturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids; U/S = sum of unsaturated fatty acids/sum of saturated fatty acids.

Table 4. The effect of supplementation of ricinoleic acid on the haematological parameters of lactating Kankrej cows (n = 20)

Parameters	CON	RAS	P value
Haemoglobin (g/dL)	$10.53 \pm 0.23$	$10.36 \pm 0.23$	0.613
Haematocrit (L/L)	$32.34 \pm 1.08$	$31.15 \pm 0.70$	0.365
Erythrocytes (10 <sup>6</sup> /μL)	$6.97 \pm 0.17$	$6.74 \pm 0.17$	0.349
MCV (fL)	$45.88 \pm 0.32$	$44.89 \pm 0.52$	0.121
MCH (pg)	$15.76 \pm 0.32$	$15.24 \pm 0.29$	0.246
MCHC (g/dL)	$34.08 \pm 0.37$	$33.71 \pm 0.26$	0.425
Leukocytes (10 <sup>3</sup> /μL)	$8.19 \pm 0.60$	$7.50 \pm 0.37$	0.338
Neutrophils (10 <sup>3</sup> /μL)	$3.31 \pm 0.23$	$3.50 \pm 0.26$	0.593
Lymphocytes (10 <sup>3</sup> /μL)	$3.53 \pm 0.26$	$3.44 \pm 0.27$	0.812
Monocytes (10 <sup>3</sup> /μL)	$0.70 \pm 0.05$	$0.63 \pm 0.04$	0.308

 $MCV - mean\ corpuscular\ volume,\ MCH - mean\ corpuscular\ haemoglobin,\ MCHC - mean\ corpuscular\ haemoglobin\ concentration;\ CON - Basal\ diet\ without\ additive;\ RAS\ - Basal\ diet\ + 2\ g/animal/day\ of\ ricinoleic\ acid$ 

Table 5. The effect of supplementation of ricinoleic acid on blood biochemical profile
of lactating Kankrej cows $(n = 20)$

Parameters	CON	RAS	P value
Glucose (mg/dL)	$62.10 \pm 1.50$	59.00 ± 1.81	0.204
Total protein (g/dL)	$7.66 \pm 0.11$	$7.71 \pm 0.15$	0.792
Albumin (g/dL)	$2.90 \pm 0.06$	$2.89 \pm 0.07$	0.912
Globulin (g/dL)	$4.76 \pm 0.12$	$4.82 \pm 0.15$	0.760
Urea (mg/dL)	$39.28 \pm 1.69$	$41.48 \pm 1.93$	0.403
Creatinine (mg/dL)	$1.40 \pm 0.11$	$1.27 \pm 0.07$	0.295
Triglycerides (mg/dL)	$34.96 \pm 2.69$	$32.46 \pm 1.40$	0.421
Cholesterol (mg /dL)	$218.9 \pm 16.14$	$190.5 \pm 16.39$	0.232
ALT (U/L)	$41.81 \pm 3.84$	$49.34 \pm 3.39$	0.159
AST (U/L)	$68.98 \pm 4.32$	$67.46 \pm 5.83$	0.836
ALP (U/L)	$227.7 \pm 22.33$	$189.8 \pm 14.06$	0.168
GGT (U/L)	$10.63 \pm 2.52$	$10.11 \pm 1.09$	0.852
Calcium (mg/dL)	$7.70 \pm 0.22$	$7.91 \pm 0.12$	0.424
Phosphorus (mg/dL)	$6.83 \pm 0.22$	$7.32 \pm 0.33$	0.234

ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase. CON: Basal diet without additive; RAS: Basal diet + 2 g/animal/day of ricinoleic acid

#### Discussion

In the current study, cows in the RAS group were fed 2 g ricinoleic acid from castor oil daily which as a functional oil provides health benefits such as antimicrobial (FERREIRA et al. 2002), antiinflammatory (VIEIRA et al. 2000), anti-oxidative (TREVISAN et al. 2006), and gastro-protective (HAMAD and MUBOFU, 2015) properties, and can be used as a phytogenic feed additive as an alternative to antibiotics. There was no adverse effect of ricinoleic acid supplementation on feed intake. Similarly, FERREIRA DE JESUS et al. (2016) found that supplementation of 500 mg/kg DM functional oil (a blend of cashew nut shell oil [CSNL] and castor oil [CO]) had a non-significant (P>0.05) effect on dry matter intake (kg/d and % live weight) in lactating Holstein cows. Additionally, other studies that evaluated CSNL (functional oil) in the diet of dairy cows also described no differences in DM intake (COUTINHO et al., 2014; BRANCO et al., 2015). No impact of functional oils on feed efficiency in dairy cows was reported by FERREIRA DE JESUS et al. (2016) and GHIZZI et al. (2018).

In the present study, there was no effect on milk yield (milk, FCM and ECM) due to dietary ricinoleic acid. Similarly, previous studies (COUTINHO et al. 2014; BRANCO et al. 2015; GHIZZI et al. 2018) reported that feeding functional oil (blend of CSNL and CO) had no impact on milk or FCM yield in dairy cows. However, GANDRA et al. (2014) observed significantly (P<0.05) higher milk (24.16 vs. 22.47 kg/d) and FCM yields (26.08 vs. 23.44 kg/d) in Simmental dairy cows fed with ricinoleic acid (2 g/d) from castor oil. FERREIRA DE JESUS et al. (2016) reported that feeding 500 mg/kg DM functional oil to Holstein cows significantly increased (P<0.05) milk yield (25.1 vs. 24.6 kg/d). The discrepancies between studies on the effect of functional oil on the milk yield of dairy cows might be due to feeding with individual or combinations of oils, types of diet, added levels of oil and the different durations of the experiments. No changes in milk composition were observed in the current study. These findings were in agreement with other studies (COUTINHO et al., 2014; GANDRA et al., 2014; BRANCO et al. 2015; FERREIRA DE JESUS et al., 2016). Nonetheless, PARENTE et al. (2018) found that feeding 30 g/kg DM of castor oil to ewes significantly (P<0.05) increased milk fat and total solid percentages as compared to the control, whereas milk protein and lactose percentages were unchanged.

In the current study, dietary addition of ricinoleic acid altered the milk fatty acid (FA) profile. The milk FA profile depends on the type of animal, the ration composition, type and dose of oil fed, along with the interrelationship between dietary lipid, rumen fermentation, and metabolic changes occurring in the liver, blood, and finally in the mammary gland (KLIEM and SHINGFIELD, 2016; VARGAS-BELLO-PÉREZ et al. 2019; FERLAY and CHILLIARD, 2020). Information on the effects of feeding ricinoleic acid/castor oil/functional oil on the milk FA profiles of dairy cows is scarce. BRANCO et al. (2015) observed that feeding CNSL (functional oil) resulted in a reduction in the concentrations of C4:0 (P = 0.02), C14:0 (P = 0.11) and C18:0 (P = 0.06) compared with the control. COUTINHO et al. (2014), reported milk FA data for lactating cows receiving CSNL functional oil showed a linear decrease in C6:0 and a linear increase in C16:1n-7 concentrations. Recent studies have highlighted that supplementation of lipids and plant bioactive compounds leads to changes in milk fatty acids profile due to changes in the microbial population involved in the biohydrogenation process (TORAL et al., 2018; FATAHNIA et al., 2018). The lower C16:0 concentration of milk fat in RAS cows could be related to the higher C18:2 and C18:3 concentrations in the milk fat. In the mammary glands and other tissues vaccenic acid can be converted to C18:2 by the action of  $\Delta 9$ -desaturase (SHINGFEILD et al., 2013). This suggests that, formation of PUFA acids resulted from the ricinoleic acid which protected them from rumen biohydrogenation, or rapidly passed from the rumen to the duodenum (DOREAU et al., 2009). Long chain fatty acids (C14:0, C16:0 and C18:0) are abundantly present in ruminant milk fat and they are preferentially stored as body fat in humans. They are considered as hyper-cholesterolemic, would have an impact on cardiovascular health parameters (GÓMEZ-CORTÉS et al. 2018).

Elevated concentrations of UFA and PUFA in milk fat have numerous health benefits for humans (SHAHIDI and AMBIGAIPALAN, 2018). Therefore, nutritional interventions that increase the percentages of UFA and PUFA content in the milk fat of dairy cows are of interest. Feeding dairy cows ricinoleic acid in their diet might be one such way.

The results obtained in this study indicate that ricinoleic acid supplementation did not have any adverse effect on the health and welfare of the animals. In accordance with the present findings, GANDRA et al. (2014) reported a non-significant effect on red blood cell and white blood cell counts after supplementation with ricinoleic acid (2 g/d) from castor oil, in Simmental dairy cows. Feeding of functional oil did not impact (P>0.05) haematological indices in feedlot cattle (GANDRA et al., 2012; CRUZ et al., 2014; VALERO et al., 2016). Similar to the present findings, no differences were observed in the blood metabolites of dairy cows fed functional oils in other studies (BRANCO et al., 2015; GHIZZI et al., 2018). On the contrary, a decrease in the concentrations of serum urea in cows fed functional oil was reported by GANDRA et al. (2014) and FERREIRA DE JESUS et al. (2016).

## Conclusions

On the basis of the results of the present study, it was concluded that supplementation of 2 g/animal/day ricinoleic acid from castor oil had no effect on milk yield, milk composition and haemato-biochemical profile. However, it increased the milk fat percentages of unsaturated fatty acids and polyunsaturated fatty acids, and decreased milk saturated fatty acids, without any adverse effect on the health status of the cows.

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## SAŽETAK

Cilj istraživanja bio je procijeniti učinak ricinoleične kiseline iz ricinusova ulja na prinos mlijeka, kemijski sastav mlijeka, profil masnih kiselina u mlijeku te na hemato-biokemijski profil krava pasmine Kankrej u laktaciji. Ukupno 20 krava u laktaciji podijeljeno je u: kontrolnu skupinu (CON), skupinu koja je dobivala osnovnu hranu, bez dodataka i pokusnu skupinu (RAS), koja je dobivala osnovnu hranu i 2 g po životinji dnevno ricinoleične kiseline iz ricinusova ulja. Ricinoleična kiselina nije imala učinka (P>0,05) na unos suhe tvari i iskorištavanje hrane. Dodatak ricinoleične kiseline nije utjecao (P>0,05) na prinos mlijeka (mlijeko, obogaćeno mlijeko s 4 % masnoće i energijski obogaćeno mlijeko). Sastav mlijeka nije pokazao znakovite razlike među skupinama. Uočen je znakovito veći postotak C4:0, C6:0 i C8:0 u mlijeku u skupini RAS (P<0,05) nego u skupini CON. Niži postotak C16:0 u mlijeku (P<0,05) uočen je u skupini RAS u usporedbi sa skupinom CON. Povećan postotak C18:2 i C18:3 pronađen je u skupini RAS. Zapaženo je smanjenje udjela zasićenih masnih kiselina u mlijeku (P = 0,055), dok je postotak nezasićenih masnih kiselina u mlijeku u skupini RAS. Nije bilo znakovite razlike (P>0,05) u hemato-biokemijskom profilu skupina CON i RAS. Zaključeno je da dodatak 2 g ricinoleične kiseline po životinji dnevno ne utječe na prinos i sastav mlijeka te hemato-biokemijski profil, međutim povećava postotak mliječne masti, nezasićenih masnih kiselina i višestruko nezasićenih masnih kiselina, a smanjuje postotak zasićenih masnih kiselina bez posljedica za zdravlje krava.

**Ključne riječi:** krava pasmine Kankrej; prinos mlijeka; masnokiselinski status; krvni metaboliti; ricinoleična kiselina