

Effects of Soybean and Rice Bran Oil Supplementation on Nutrient Utilization, Lactation Performance, Milk Fatty Acid Profile and Ruminant Fermentation in Surti Goats

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1 **Effects of soybean and rice bran oil supplementation on nutrient utilization, lactation**
2 **performance, milk fatty acid profile and ruminal fermentation in Surti goats**

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10 **Short Title:** Soybean and rice bran oil supplementation in Surti goats

11 **Abstract**

12 A study was conducted to observe the effect of soybean and rice bran oil supplementation on
13 nutrient utilization, lactation performance and ruminal fermentation in Surti goats. Twenty-
14 four multiparous lactating Surti does were distributed into four homogenous groups for entire
15 lactation of 150 days. Control group (CON) was offered a basal diet consisting of compound
16 concentrate mixture, green jowar and pigeon pea straws without any oil supplementation,
17 while other treatment groups were additionally supplemented with soybean oil @ 3% of DMI
18 (SBO), rice bran oil @ 3% of DMI (RBO) and equi-proportional blend of soybean oil and rice
19 bran oil @ 3.0 % of DMI (SRBO). DM, CP, NDF and ADF intake (g/d) and digestibility (%)
20 remained statistically ($p>0.05$) similar amongst dietary treatments groups. EE intake (g/d)
21 and digestibility (%) was significantly ($p<0.01$) improved in all three oils supplemented
22 groups as compared to control but values between oils supplemented groups remained at par
23 ($p>0.05$). Nitrogen balance of experimental groups remained unaffected ($p>0.05$) and all the
24 animals were under positive nitrogen balance. Milk yield (kg/d), milk fat, SNF, protein,
25 lactose, FCM and ECM yields (g/d) were significantly ($p<0.05$) increased in oils
26 supplemented groups as compared to control. Feed efficiency in terms of MY/DMI and
27 FCM/DMI significantly ($p<0.05$) improved in SBO, RBO and SRBO as compared to CON.
28 Soybean and rice bran oil supplementation either alone or in combination significantly
29 reduced ($p<0.05$) SCFA, MCFA, SFA with increased LCFA and PUFA in milk. However,
30 values between oils supplemented groups remained at par ($p>0.05$). Oil supplementation
31 increased ($p<0.001$) oleic acid (C18:1 n-9) and linoleic acid (C18:2 n-6) in SBO, RBO and
32 SRBO as compared to CON while, linolenic acid (C18:3 n-9) remained non significant
33 amongst treatments. Lipid quality indices (LQI) like atherogenicity index, thrombogenicity
34 index and hypocholesterolaemic/hypercholesterolaemic (h/H) index were significantly
35 improved in all the oils supplemented groups as compared to control. Rumen pH, total

36 nitrogen and its fractions (ammonia N, TCA precipitable N and soluble N) remained similar
37 ($p>0.05$) amongst treatment except TVFA. Thus, supplementation of soybean oil and rice
38 bran oil either alone or in combination in lactating goat can be effectively used to improve
39 both nutritional quantity and quality of milk.

40 **Key words:** Milk fatty acid, Soybean oil, Rice bran oil and Surti goat

41 **Introduction**

42 With highest goat population in world, dairy goat farming is gaining importance in Indian sub
43 continent, which provides food and nutritional security to the millions of small and marginal
44 farmers (Haenlein 2004). Goat milk and milk products consumption is considered beneficial
45 for humans due to its technological advantage with regards to smaller size of fat globules,
46 lower amount of casein and higher mineral compounds over cow milk (Attaie and Richter
47 2000; Gomes et al. 2013). Goat milk production and composition, especially (Fatty acid
48 profile and flavour) is a genetic trait, but it can be effectively altered by the dietary
49 supplementation with lipids (Nudda et al. 2006). During last decade, there is an increased
50 interest in researchers to reduce saturated fatty acids and enrich product with beneficial
51 polyunsaturated fatty acids (PUFA) or conjugated linoleic acid (CLA), which could offer
52 potential health benefits to consumers (Bouattour et al. 2008). Fatty acid content in goat milk
53 is affected by the fat content, type of predominant fat and physical form of the dietary fat
54 supplement (Nudda et al. 2014). Vegetable fat supplements are usually more effective in the
55 form of free oil than in the form of seeds at increasing the milk FA content (Nudda et al.
56 2014). Plant oils from different oilseeds sources have different FA compositions and
57 accordingly exert different effects on milk FA profile (Gómez-Cortés et al. 2008). Feeding
58 PUFA rich vegetable oils like soybean oil (Almieda et al. 2019; Li et al. 2009) and rice bran
59 oil (Maia et al. 2006) has been shown to affect milk yield and composition. However, to the
60 best of our knowledge, there is meager information about response of vegetable oil

61 supplementation to dairy goats in Indian tropical conditions. Therefore, present experiment
62 was planned with hypothesis that, supplementing soybean oil or rice bran oil would have
63 beneficial effects on production performance and milk fatty acid composition.

64 **Materials and Methods**

65 **Animals, feeding and experimental design**

66 The feeding trial was carried out at Livestock Research Station (LRS), Navsari Agricultural
67 University, Navsari, Gujarat, India. Twenty-four multiparous lactating Surti goats were
68 randomly distributed into four homogenous groups on the basis of live body weight
69 (28.10 ± 1.04 kg), parity (4.47 ± 0.47) and previous standard lactation yield (133.39 ± 5.00
70 kg/150d) up to entire lactation of 150 days. Control group (CON) was offered basal diet
71 without any oil supplementation, while other treatment groups were additionally
72 supplemented with soybean oil @ 3% of DMI (SBO), rice bran oil @ 3% of DMI (RBO) and
73 equi-proportional blend of soybean oil and rice bran oil @ 3.0 % of DMI (SRBO). Basal diet
74 consisted of compound concentrate mixture, green jowar and pigeon pea straws.
75 Experimental animals were individually fed during entire experiment according to ICAR
76 feeding standard (2013). Compound concentrate mixture mixed with oil was fed once daily at
77 08.30 h, green sorghum at 10.00 h and 16.00 h while pigeon pea straws were fed *ad libitum*
78 during night hours. *Ad libitum* drinking water was available at all the time during experiment.
79 At mid of experiment a metabolism trial of seven days was conducted to assess the nutrient
80 utilization efficiency (digestibility). During metabolism trial, all the animals were shifted to
81 the metabolic cages, having facilities for separate urine and faeces collection. Daily feed
82 intake and output of faeces and urine was recorded and representative samples were collected
83 for further processing and analysis. Samples were analysed for proximate composition
84 (AOAC 2005), fibre fractions (Van soest et al. 1991) and fatty acid analysis (O'fallon et al.
85 2007).

86 **Sampling and analysis of milk composition**

87 Goats were hand milked twice a day and fortnightly milk samples were collected for
88 chemical composition i.e. fat, solid not fat (SNF), protein and lactose using infrared
89 spectrophotometry (MilkoScan™ FT1 120). Samples for milk fatty acid were collected in
90 separate airtight plastic bottle container for each animal and stored at -20 °C (ultra low
91 temperature freezer) until laboratory analysis.

92 **FA analysis**

93 Fatty acid were analysed as per direct transesterification method of O'Fallon et al. (2007).
94 FAME was prepared by base-catalysed methanolysis of glycerides (KOH in methanol)
95 according to international standards (ISO-IDF 2002) and stored in vial at -20°C for further
96 analysis. FAME was analysed on gas chromatography mass spectrometer (GCMS-QP 2010
97 Plus) equipped with an auto sampler injector. The FAME was separated in 60 m capillary
98 column (60 m x 0.25mm x 20 um) film thickness with mass spectrometer. Helium was used
99 as carries gas at a flow rate of 1 mL/min. The injector and detector temperatures were 250°C
100 and 240°C, respectively. The temperature programme was follow as: Initial temperature held
101 at 100°C for 5 min after sample injection, then programmed to increase at 2 °C/min to 240 °C
102 and held there for 5 min. Sample (1uL) were injected by split injection (split ratio 50:1). The
103 mass spectrometer was operated at source temperature 230°C; interface temperature 240°C
104 with electrospray ionization (EI) method and scan range of 50-1000 m/z. Identification of
105 FAME was performed from the retention time by using standards of 37 individual FAME
106 (Supelco, Bellefonte, PA) was used to determine response factors. The peak areas in the
107 chromatograph were calculated and normalized using response factors.

108 **Sampling and analysis of rumen liquor**

109 Rumen liquor samples were collected from each experimental animal by stomach tube.
110 Collection was done two hours after morning feeding of concentrates at 15, 75 and 150 days

111 of experiment. Rumen liquor was filtered through four layer muslin cloth and pH was
112 immediately measured with digital pH meter. Strained rumen liquor (SRL) of 50 ml was
113 collected in the airtight plastic bottle container and kept at -20°C (ultra low temperature
114 freezer) until laboratory analysis. Ruminal ammonia-N was determined according to AOAC
115 (2005). Total volatile fatty acids (TVFA) production was determined by steam distillation
116 process using Markham micro-distillation apparatus (Barnet and Ried 1956). Total nitrogen,
117 TCA precipitable nitrogen was estimated according kjeldahl method (AOAC 2005) while
118 soluble nitrogen was determined by difference between total nitrogen and TCA precipitable-
119 nitrogen.

120 **Statistical Analysis**

121 Data for body weight, milk yield, milk composition and ruminal parameters of animals were
122 analyzed by using the PROC MIXED procedure of SAS with repeated measures (version 9.3;
123 SAS Institute Inc., Cary, NC) using Tukey's HSD (honestly significant difference) multiple
124 comparison test. Following statistical model was used.

$$125 \quad Y_{ijk} = \mu + A_k + D_i + T_j + (D_i \times T_j) + e_{ijk}$$

126 Y_{ijk} = Dependent variable; μ = Overall mean; A_k = Random effect of animals; D_i =
127 Fixed effect of diet, T_j = Fixed effect of time; e_{ijk} = Residual error

128 The statistical model contained fixed effects of treatment and experimental period
129 and their interactions, random effects of animal and residual error. Parameters related to
130 digestibility of feed, milk fatty acid and nitrogen balance were analysed by one-way anova
131 using tukey's HSD. Differences were declared significant at $p < 0.05$, with values of $p < 0.10$
132 being interpreted as a trend towards significance.

133 **Results and Discussion**

134 **Proximate and fatty acid composition of experimental feeds**

135 Proximate and fatty acid composition of experimental feed offered is presented in table 1.
 136 Roughage: concentrate ratio was tried to maintain at 70:30 during whole experimental period.
 137 Experimental diet was isonitrogenous in nature but due to addition of oil it was not isocaloric
 138 (Adeyemi et al. 2015; Bouattor et al. 2008).

139 **Table 1: Proximate and fatty acid composition of experimental composition of**
 140 **experimental diet (% DM basis)**

| Attributes | CON | SBO | RBO | SRBO |
|------------------------------------|-------|-------|-------|-------|
| Dry matter | 67.37 | 64.69 | 64.69 | 64.69 |
| Organic matter | 85.10 | 82.20 | 82.20 | 82.20 |
| Crude Protein | 12.09 | 11.95 | 11.95 | 11.95 |
| Ether Extract | 2.55 | 5.44 | 5.44 | 5.44 |
| Neutral detergent fiber | 50.35 | 49.82 | 49.82 | 49.82 |
| Acid Detergent fiber | 36.98 | 36.63 | 36.63 | 36.63 |
| ME (MJ/kg DM) | 10.36 | 11.08 | 11.07 | 11.07 |
| FA composition (g/100 g FA) | | | | |
| C10:0 | 0.65 | 0.49 | 0.49 | 0.49 |
| C12:0 | 0.79 | 0.60 | 0.60 | 0.60 |
| C14:0 | 1.27 | 0.98 | 1.05 | 1.01 |
| C16:0 | 21.24 | 18.55 | 20.03 | 18.83 |
| C16:1 | 0.15 | 0.13 | 0.16 | 0.14 |
| C18:0 | 11.00 | 9.53 | 8.86 | 9.38 |
| C18:1 | 14.77 | 16.63 | 20.02 | 18.69 |
| C18:2 | 25.26 | 30.96 | 26.72 | 29.17 |
| C18:3 | 9.27 | 8.61 | 8.33 | 8.32 |

| | | | | |
|-------|------|------|------|------|
| C20:0 | 0.79 | 0.69 | 0.83 | 0.60 |
| C22:0 | 2.24 | 1.79 | 1.77 | 1.79 |
| C24:0 | 1.42 | 1.10 | 1.19 | 1.14 |

141 **Nutrient intake and digestibility**

142 Dry matter intake (DMI; g/d) was similar ($p>0.05$) amongst treatment groups with diet but
143 significantly ($p<0.01$) affected with time. Non-significant DMI was observed when in
144 lactating ewes when supplemented with soybean oil and sunflower oil (Titi and Rahman
145 2013) and with soybean oil (Bouattour et al. 2008) in goats. Statistically similar DMI with
146 addition on unprotected oil in to ration of lactating goats indicates adequate inclusion level of
147 plant oils and both oil either alone or in combination does not hampers the fiber digestion
148 (Adeyemi et al. 2015). In contrary, Bernard et al. (2009) found depressed DMI with
149 sunflower (130 g/d) and linseed oils (130 g/d) supplementation on maize silage based diet in
150 lactating goats.

151 Average initial and final body weight (kg) of treatment groups was similar ($p>0.05$)
152 amongst treatments. Numerically, increase in body weight with time would suggest that
153 animals were under a positive energy balance and that energy and protein provided by all
154 diets were adequate to meet the requirements for milk production (Titi and Rahman 2013).
155 Although diet effect on body weight was not reflected, which might be due to extra available
156 energy might have been diverted to milk production and composition (Table 4).

157 Nutrient intake and apparent digestibility of experimental groups is mentioned in table
158 2. Intakes and digestibility of DM, CP, NDF and ADF for all the treatment groups remained
159 statistically ($p>0.05$) similar. Almieda et al. (2019) found non significant apparent
160 digestibility with supplementation of soybean, sunflower linseed or fish oil at 5.1 % of DMI.
161 Non significant digestibility of fiber fractions indicates that ruminal fiber digestion was not
162 hampered by addition of oil and inclusion level of oil was adequate for optimal ruminal

163 environment (Adeyemi et al. 2015). Present findings were further, supported by ruminal pH
 164 of experimental animal which was within range (Table 7). In contrast, Lunsin et al. (2012)
 165 found decreased fiber fractions digestibility with increasing level of rice bran oil in cattle.

166 EE intake and digestibility was significantly ($p < 0.01$) higher in all three oils
 167 supplemented groups as compared to control. However, values between oils supplemented
 168 groups remained at par ($p > 0.05$). Increased EE digestibility can be attributed to the fact that
 169 non-fatty acids lipids in the diet are relatively less digestible and diluting this fraction with
 170 easily digestible fatty acids from oil, increases the digestibility of EE fraction (Adeyemi et al.
 171 2015; Li et al. 2009). In contrast, when goats were supplemented with soybean, linseed and
 172 fish oil (Almieda et al. 2019) and with flaxseed oil (Kholif et al. 2018) found non-significant
 173 ether extract digestibility. Due to increased EE intake with oils supplementation, TDN intake
 174 was also significantly ($p < 0.05$) increased in SBO, RBO and SRBO as compared to CON.
 175 However, TDN intake was similar amongst oils supplemented groups.

176 **Table 2: Effect of vegetable oil on nutrient intake and apparent digestibility of**
 177 **experimental animals**

| Attributes | CON | SBO | RBO | SRBO | SEM | P value |
|------------------------------|--------------------|--------------------|--------------------|--------------------|-------|---------|
| Nutrient Intake (g/d) | | | | | | |
| Dry matter | 975.31 | 1016.27 | 1008.91 | 994.54 | 28.42 | 0.751 |
| Concentrate | 350.21 | 354.71 | 350.07 | 367.57 | 5.83 | 0.144 |
| Dry fodder | 511.97 | 576.59 | 516.71 | 542.08 | 16.72 | 0.622 |
| Green Fodder | 256.53 | 252.99 | 263.01 | 222.99 | 11.19 | 0.625 |
| Body weight (kg) | 28.21 | 28.41 | 28.11 | 29.43 | 0.63 | 0.437 |
| Crude protein | 118.12 | 124.92 | 116.82 | 122.96 | 2.27 | 0.568 |
| Ether extract | 23.41 ^b | 58.27 ^a | 56.58 ^a | 54.01 ^a | 3.08 | <0.001 |
| Neutral detergent fiber | 652.63 | 699.72 | 670.21 | 659.47 | 15.97 | 0.762 |

| | | | | | | |
|----------------------|---------------------|---------------------|---------------------|----------------------|-------|-------|
| Acid detergent fiber | 490.44 | 526.72 | 504.32 | 496.10 | 12.11 | 0.755 |
| DCP | 84.85 | 88.83 | 84.19 | 85.05 | 1.82 | 0.823 |
| TDN | 678.46 ^b | 778.97 ^a | 771.68 ^a | 721.44 ^{ab} | 21.73 | 0.049 |

Apparent Digestibility (%)

| | | | | | | |
|-------------------------|--------------------|--------------------|--------------------|--------------------|------|--------|
| Dry matter | 64.92 | 64.08 | 66.10 | 61.03 | 1.53 | 0.708 |
| Crude protein | 71.91 | 71.14 | 72.23 | 69.45 | 1.19 | 0.862 |
| Ether extract | 74.31 ^b | 93.18 ^a | 93.60 ^a | 92.38 ^a | 1.81 | <0.001 |
| Neutral detergent fiber | 67.24 | 65.79 | 68.22 | 63.70 | 1.40 | 0.715 |
| Acid detergent fiber | 65.83 | 65.61 | 66.90 | 62.06 | 1.45 | 0.693 |

178 abc -Means with different superscript in a row differ significantly (p<0.05)

179 **Nitrogen balance**

180 Protein metabolism and turnover rate can be effectively studied by estimation of nitrogen
181 balance. Nitrogen intake from feed for experimental groups remained at par (p<0.05).
182 Nitrogen excretion from faeces, urine and milk and nitrogen retained as percent of intake was
183 also remained statistically (p>0.05) similar (Table 3). Further, nitrogen balance also indicated
184 that all the animals were on positive nitrogen balance throughout experimental period.
185 Adeyemi et al. (2016) also found non-significant nitrogen balance in lactating goat
186 supplemented with blend of canola and palm oil.

187 **Table 3: Effect of vegetable oils on nitrogen balance (g/d) of experimental animals**

| Attributes | CON | SBO | RBO | SRBO | SEM | P value |
|-------------|-------|-------|-------|-------|------|---------|
| N in feed | 18.90 | 19.99 | 18.69 | 19.67 | 0.36 | 0.569 |
| N in faeces | 5.32 | 5.78 | 5.22 | 6.06 | 0.29 | 0.734 |
| N in urine | 6.42 | 6.14 | 5.45 | 6.79 | 0.31 | 0.070 |
| N in milk | 3.18 | 4.00 | 4.17 | 2.73 | 0.35 | 0.435 |
| N balance | 3.98 | 4.07 | 3.85 | 4.09 | 0.40 | 0.863 |

| | | | | | | |
|---------------|-------|-------|-------|-------|------|-------|
| N retained | 21.07 | 20.36 | 20.60 | 20.77 | 1.54 | 0.980 |
| % of N Intake | | | | | | |

188 **Milk yield and composition**

189 Milk yield (kg/d) was significantly ($p < 0.05$) increased in SBO (+25.97%), RBO (+32.46%)
 190 and SRBO (+10.38%) as compared to CON. While, amongst the oil supplemented groups,
 191 RBO found highest increase in milk production which was significantly apart from SBO and
 192 SRBO. Increased available metabolisable energy with oil supplementation might be the
 193 reason behind increased milk yield. G3mes-Cort3s et al. (2008) concluded that increased milk
 194 yield was due to greater energy content of the oil supplemented diets and not by a greater
 195 DMI. Various scientists found increased milk yield with vegetable oils supplementation in
 196 goat (Bernard et al. 2009; Hassan et al. 2020; Ibrahim et al. 2020; Mele et al. 2008) and sheep
 197 (Ferreira et al. 2018; Nudda et al. 2015). In contrast to present result, Almieda et al. (2019);
 198 Bernard et al. (2015) and Bouattour et al. (2008) and found no effect of PUFA rich oil
 199 supplementation on milk yield in goats. Differing responses of milk yield to dietary fat may
 200 relate in part to the genetic potential of the goats for milk yield, the supply of energy from the
 201 basal diet and the effects of the dietary fat on DMI and nutrient partitioning (Manso et al.
 202 2011).

203 Milk fat concentration and yield was increased ($p < 0.01$) significantly with diet in
 204 SBO, RBO and SRBO as compared to CON but values remained unaffected with time. Many
 205 researchers found combined effect of increased milk fat concentration along with milk yield
 206 (Bernard et al. 2009; Bernard et al. 2015; Morsy et al. 2015; Li et al. 2016) while some
 207 reported only increased milk fat (Bouattour et al. 2008; Li et al. 2009) with PUFA rich oils
 208 supplementation in lactating goats. In contrast, Reyes et al. (2018) found similar milk fat and
 209 milk production with canola and soybean oil supplementation (20ml/d/goat). Neither diet nor
 210 sampling time had significant ($p > 0.05$) effect on milk SNF, protein and lactose concentration.

211 However, milk SNF, protein and lactose yields were increased, which was highest in RBO
 212 followed by SBO, SRBO and CON. Increased milk yield and milk fat concentration was
 213 directly reflected in increased yield of milk fat, SNF, protein and lactose in oils supplemented
 214 groups as compared to control. Almieda et al. (2019) and Manso et al. (2011) found
 215 statistically similar milk protein and lactose concentration. However, ewes were
 216 supplemented with linseed (Nudda et al. 2015) found improved milk protein and lactose
 217 concentration.

218 Milk composition in terms of percentage is not sufficient enough to evaluate the milk
 219 quality. So various methods formulated that estimates relative energy value required to
 220 produce milk when milk yield is adjusted with amount of fat (fat corrected milk) and amount
 221 of fat, protein and lactose (energy corrected milk). The FCM, SCM and ECM yields (kg/d)
 222 were significantly increased ($p < 0.01$) with oils supplementation but remained unaffected
 223 ($p > 0.05$) by time. Titi and Rahman (2013) observed increased ECM for ewes supplemented
 224 when with soybean and sunflower oil @ 3 % of DMI. However, Bouattour et al. (2008) found
 225 non-significant ECM yield in soybean oil supplemented group as compared to control in goat.
 226 Milk energy output (MJ/d) and milk energy content (MJ/kg) was significantly ($p < 0.01$)
 227 increased with oil supplementation. Feed efficiency in relation to milk yield and FCM yield
 228 was significantly ($p < 0.01$) improved in SBO, RBO, and SRBO with diet and time in all three
 229 oil supplemented groups as compared to control. Increased milk production without affecting
 230 DM intake of animal reflects efficient utilization of feed. Morsy et al. (2015) also found
 231 increased feed efficiency in relation to DM in sunflower and soybean oils supplemented
 232 groups as compared to control in goats.

233 **Table 4: Production performance of experimental Surti does supplemented with**
 234 **vegetable oil**

| Parameters | CON | SBO | RBO | SRBO | SEM | P value | | |
|------------|-----|-----|-----|------|-----|---------|------|-----|
| | | | | | | Diet | Time | DxT |

Yield

| | | | | | | | | |
|-------------------------|--------------------|---------------------|---------------------|---------------------|------|--------|-------|-------|
| Milk (kg/d) | 0.77 ^b | 0.95 ^{ab} | 1.02 ^a | 0.85 ^{ab} | 0.06 | <0.007 | 0.216 | 0.993 |
| Fat (g/d) | 28.16 ^c | 47.19 ^{ab} | 52.11 ^a | 39.83 ^b | 2.88 | <0.001 | 0.322 | 0.959 |
| SNF (g/d) | 62.18 ^b | 78.11 ^{ab} | 80.47 ^a | 66.46 ^{ab} | 4.58 | <0.012 | 0.110 | 0.987 |
| Protein (g/d) | 23.54 ^b | 29.80 ^{ab} | 33.63 ^a | 25.88 ^{ab} | 1.96 | <0.002 | 0.638 | 0.947 |
| Lactose (g/d) | 31.69 ^b | 40.09 ^{ab} | 41.79 ^a | 34.65 ^{ab} | 2.54 | <0.019 | 0.265 | 0.945 |
| Total solid (g/d) | 92.20 ^b | 125.33 ^a | 128.81 ^a | 94.00 ^b | 8.82 | <0.002 | 0.171 | 0.974 |
| FCM (kg/d) ¹ | 0.73 ^c | 1.10 ^{ab} | 1.19 ^a | 0.94 ^{bc} | 0.08 | <0.001 | 0.291 | 0.978 |
| SCM (kg/d) ² | 0.70 ^c | 1.02 ^{ab} | 1.09 ^a | 0.86 ^{bc} | 0.06 | <0.001 | 0.204 | 0.972 |
| ECM (kg/d) ³ | 0.64 ^c | 1.19 ^{ab} | 1.42 ^a | 0.79 ^{bc} | 0.12 | <0.001 | 0.265 | 0.885 |

Concentration (%)

| | | | | | | | | |
|--|-------------------|--------------------|-------------------|--------------------|------|--------|-------|-------|
| Fat | 3.70 ^b | 4.97 ^a | 5.02 ^a | 4.69 ^a | 4.60 | <0.001 | 0.239 | 0.652 |
| SNF | 8.10 | 8.08 | 7.84 | 7.85 | 7.95 | 0.062 | 0.265 | 0.836 |
| Protein | 3.11 | 3.02 | 3.19 | 3.03 | 3.09 | 0.053 | 0.084 | 0.984 |
| Lactose | 4.09 | 4.38 | 3.98 | 4.03 | 4.12 | 0.507 | 0.797 | 0.748 |
| Milk energy output (MJ/d) ⁴ | 2.15 ^c | 3.17 ^{ab} | 3.47 ^a | 2.70 ^{bc} | 0.19 | <0.001 | 0.401 | 0.957 |
| Milk energy content(MJ/kg) ⁵ | 2.82 ^c | 3.30 ^{ab} | 3.37 ^a | 3.17 ^b | 0.05 | <0.001 | 0.379 | 0.577 |

Milk efficiency

| | | | | | | | | |
|---------|-------------------|-------------------|-------------------|--------------------|------|--------|--------|-------|
| MY/DMI | 0.81 ^b | 1.08 ^a | 1.09 ^a | 0.87 ^{ab} | 0.07 | <0.014 | <0.001 | 0.996 |
| FCM/DMI | 0.77 ^b | 1.20 ^a | 1.27 ^a | 0.96 ^{ab} | 0.08 | <0.001 | <0.003 | 0.994 |

235 D- Diet; T- Time; DxT- Diet and time interaction; SEM- Standard error of mean; abc-Means with
 236 different superscript in a row differ significantly (p<0.05)

237 ¹Fat corrected milk (kg/d) = 0.4*milk (kg) + 15*fat (kg) (Tyrell and Reid 1965).

238 ²Solid corrected milk (kg/d) = 12.3× fat (kg) + 6.56× SNF (kg) – 0.0752× milk yield (kg) (Tyrell and
 239 Reid 1965).

240 ³Energy correct milk (kg/d) = Milk (kg/d)*[38.3*fat (g/kg) + 24.2*protein (g/kg) + 16.54*lactose
241 (g/kg) + 20.7]/3140 (Sjaunja et al. 1991).
242 ⁴Milk energy content (MJ/kg) = 4.184*2.204*[(41.63*fat (g/100g) + 24.13*protein (g/100g) +
243 21.60*lactose (g/100g)-11.72)/1000] (Tyrell and Reid 1965).
244 ⁵Milk energy output (MJ/d) = Milk energy (MJ/kg)* milk yield (kg/d) (Morsy et al. 2018)

245 **Milk fatty acid profile**

246 Both the oils either alone or in combination significantly decreased (p<0.001) short and
247 medium chain FA and increased (p<0.001) long-chain FA in the milk. Decreased
248 concentrations of short and medium chain fatty acids (mostly *de novo* synthesized) in PUFA
249 supplemented groups are consistent with previous studies in lactating goats (Almieda et al.
250 2019; Bernard et al. 2015; Pascual et al. 2019), sheep (Ferreira et al. 2018; Nudda et al. 2015)
251 and cows (Lunsin et al. 2012). This response is might be due to supplementation of PUFA
252 rich oils increases the concentration of CLA isomer (*trans*-10, *cis*-12), which is a powerful
253 inhibitor of the *de novo* fatty acids synthesis in mammary gland due to the reduction of the
254 key lipogenesis enzymes (*acetyl-CoA carboxylase* and *fatty acid synthetase*) synthesis
255 (Palmquist et al. 2005). Short and medium chain FAs are synthesized from acetate and β-
256 hydroxybutyrate in mammary gland. The *de novo* synthesis does formation of fatty acids of
257 up to 16 carbons, reducing short and medium chain fatty acids content in the milk. In
258 addition, the capric, caproic and caprylic fatty acids are responsible for the characteristic odor
259 in the caprine milk and meat products (Ibrahim and Soryal 2014), which may create some
260 consumers resistance for meat, milk and their derivatives (Chilliard et al. 2003). Therefore,
261 the reduction of SCFA and MCFA may be important to increase acceptability of these
262 products by consumers. Furthermore, MCFA reduction is desirable because the lauric,
263 myristic and palmitic fatty acids are associated with cardiovascular diseases development
264 (Ferreira et al. 2018). Moreover, the addition of both PUFA rich oils alone or in combination
265 decreased (p<0.05) saturated FA concentrations and increased (p<0.001) unsaturated FA
266 concentrations and MUFA and PUFA contents in the milk. Stearic (C18:0), oleic (C18:1 n-9)

267 and linoleic acids (C18:2 n-6) were increased, whereas linolenic acid (C18:3 n-3) was similar
268 in soybean oil and/or rice bran oil supplementation with comparison to control.

269 The increases in C18:0 and C18:1 fatty acid may be a consequence of higher intakes of these
270 FA in the diet along with biohydrogenation process of unsaturated C18 FA in the rumen.
271 Oleic acid can also be increased by the action of mammary Δ^9 desaturase on C18:0. Grummer
272 (1991) indicated that it is easier to increase the C18:0 content of milk fat with MUFA and
273 PUFA rich supplements than to provide stearic acid (C18:0) *per se*. Increased C18:0, content
274 in milk of the oils supplemented groups could be related to higher milk fat observed as
275 compared to CON. C18:0 is a major regulating factor of mammary lipid secretion in goats
276 and positively correlated with milk fat content (Chilliard et al. 2006).

277 The activity of Δ^9 desaturase is essential in the process of UFA synthesis. The
278 product: precursor ratio has been used as a possible indicator of Δ^9 desaturase activity (Lock
279 and Gransworthy 2002). The desaturase indexes for Δ^9 desaturase like C16:1, C18:1 and Δ^6
280 desaturase were decreased ($p < 0.05$) for SBO, RBO and SRBO as compared to CON. This
281 may be attributed to the increase in the C18:0 concentrations in the milk, probably arising
282 from ruminal biohydrogenation of linoleic acid (Ferreira et al. 2018). According to
283 Simpuolus et al. (1999), recommended range of the n-6:n-3 for human health is between 5:1
284 and 10:1. Present finding supported that observed ratio was well within normal limit when
285 supplemented with soybean oils (Bouattour et al. 2008; Manso et al. 2011) and rice bran oil
286 (Maia et al. 2006).

287 Lipid quality indices (LQI) like atherogenic (AI), thrombogenic (TI), peroxidisability
288 (PI), hypercholesterolaemic and hypocholesterolaemic (h/H) indices are presented in table 5.
289 Atherogenicity index indicates relationship between the sum of main SFA and that of main
290 classes of UFA, the former being considered as proatherogenic and later being considered as
291 antiatherogenic (Ulbricht and Southgate 1991). Atherogenicity index was reduced ($p < 0.01$) in

292 SBO, RBO and SRBO as compared to CON. Reduction in short and medium chain fatty
293 acids might be responsible for decreased atherogenicity index of milk (Ibrahim et al. 2020,
294 Bouattour et al. 2008). Thrombogenicity index (TI), indicates a tendency for clots to form in
295 the blood vessels, was significantly lower in oils supplemented groups as compared to CON.
296 Relatively high hypocholesterolaemic/hypercholesterolaemic (h/H) ratio is desirable, because
297 the higher the values of h/H ratio, the lower the proportion of hypercholesterolaemic fatty
298 acids C14:0 and C16:0 and their effect on low density lipoprotein (LDL) increases (Osmari et
299 al. 2011). In present findings, h/H ratio was significantly ($p<0.001$) increased in SBO, RBO
300 and SRBO as compared to CON. Lower AI and TI with higher h/H indices represent healthy
301 animal product with consumer perspective (Janina et al. 2020). Peroxidizability index
302 represents the relationship between FA composition and its susceptibility to oxidation and
303 used to access the stability of PUFA included in food products. However, higher the PI value,
304 greater the protective potential for coronary artery disease (Janina et al. 2020). In present
305 findings, PI was significantly higher in oils supplemented groups as compared to control,
306 which was highest in SBO followed by RBO, SRBO and CON. Elongases, responsible for
307 elongation and extension of long chain fatty acid, was significantly ($p<0.01$) increased in all
308 three oils supplemented groups as compared to control.

309 Pearson correlation between milk fat concentration and milk fatty acid composition is
310 presented in table 6. Significant positive correlation found between milk fat and PUFA
311 ($r=0.42$) especially with linoleic acid ($r=0.41$). This might be due to both the oils are n-6
312 PUFA rich which was reflected in milk fatty acid profile. Further, atherogenicity index ($r=-$
313 0.86) and thrombogenicity index ($r=-0.93$) were also found significant negative correlation
314 with linoleic acid. n-6: n-3 ratio was also negatively correlated with atherogenicity index(
315 0.51). Manso et al. (2011) found positive correlation ($r=0.67$) between linoleic acid and PUFA
316 content of milk.

317 **Table 5: Milk fatty acid composition (% FAME) of experimental animals supplemented**
 318 **vegetable oil**

| FA | CON | SBO | RBO | SRBO | SEM | P VALUE |
|---------------------------|--------------------|---------------------|--------------------|---------------------|------------|----------------|
| C4:0 butyric | 0.06 ^c | 0.05 ^c | 0.16 ^b | 0.26 ^a | 0.02 | 0.001 |
| C6:0 caproic | 0.58 | 0.47 | 0.60 | 0.71 | 0.03 | 0.093 |
| C8:0 caprylic | 1.52 | 1.03 | 0.93 | 0.95 | 0.09 | 0.071 |
| C10:0 capric | 5.00 ^a | 3.34 ^{ab} | 2.60 ^b | 2.47 ^b | 0.32 | 0.009 |
| C12:0 lauric | 3.03 ^a | 1.71 ^b | 1.39 ^b | 1.35 ^b | 0.20 | 0.002 |
| C14:0 myristic | 7.65 ^a | 4.95 ^b | 4.26 ^b | 4.10 ^b | 0.42 | 0.003 |
| C15:0 pentadecanoic | 1.32 ^a | 0.83 ^b | 0.77 ^b | 0.91 ^b | 0.05 | 0.001 |
| C16:0 palmitic | 22.99 ^a | 18.48 ^b | 20.20 ^b | 18.74 ^b | 0.47 | 0.001 |
| C16:1 palmitoleic | 0.71 | 0.70 | 0.76 | 0.76 | 0.10 | 0.996 |
| C17:0 heptadecanoic | 1.34 ^a | 0.80 ^b | 0.70 ^b | 0.77 ^b | 0.06 | 0.001 |
| C18:0 stearic | 12.86 ^b | 17.46 ^a | 18.27 ^a | 18.00 ^a | 0.69 | 0.007 |
| C18:1 n-9 oleic | 35.09 ^b | 38.91 ^{ab} | 40.76 ^a | 40.49 ^{ab} | 0.81 | 0.036 |
| C18:2 n-6 linoleic | 1.44 ^b | 3.02 ^a | 2.83 ^a | 2.86 ^a | 0.16 | 0.001 |
| C18:3 n-3 linolenic | 0.24 | 0.30 | 0.28 | 0.26 | 0.01 | 0.139 |
| C20:0 arachidic | 0.58 | 0.54 | 0.67 | 0.69 | 0.02 | 0.058 |
| C20:4 n-6 arachidonic | 0.13 | 0.17 | 0.13 | 0.16 | 0.01 | 0.307 |
| C20:5 n-3 eicosenoic | 0.02 | 0.02 | 0.01 | 0.01 | 0.01 | 0.064 |
| C22:0 behenic | 0.23 | 0.20 | 0.19 | 0.23 | 0.01 | 0.539 |
| C22:6 n-3 docosahexaenoic | 0.06 | 0.05 | 0.05 | 0.05 | 0.01 | 0.844 |
| C24:0 lignoceric | 0.08 | 0.06 | 0.08 | 0.08 | 0.01 | 0.145 |
| Others | 4.52 | 6.07 | 3.54 | 5.45 | 0.47 | 0.252 |
| SFA | 57.24 ^a | 49.90 ^b | 50.83 ^b | 49.25 ^b | 0.99 | 0.007 |

| | | | | | | |
|-------------------------------------|--------------------|---------------------|--------------------|--------------------|------|-------|
| UFA | 37.69 ^b | 43.17 ^{ab} | 44.82 ^a | 44.58 ^a | 0.93 | 0.012 |
| MUFA | 35.80 ^b | 39.61 ^{ab} | 41.52 ^a | 41.25 ^a | 0.86 | 0.050 |
| PUFA | 1.89 ^b | 3.56 ^a | 3.31 ^a | 3.34 ^a | 0.16 | 0.001 |
| n-6 | 1.57 ^b | 3.19 ^a | 2.96 ^a | 3.01 ^a | 0.13 | 0.001 |
| n-3 | 0.32 | 0.37 | 0.34 | 0.32 | 0.01 | 0.427 |
| n-6/n-3 | 5.06 ^b | 8.78 ^a | 8.87 ^a | 9.78 ^a | 0.58 | 0.009 |
| SCFA (4-10) | 7.15 ^a | 4.88 ^b | 4.29 ^b | 4.38 ^b | 0.42 | 0.038 |
| MCFA (12-16) | 35.71 ^a | 26.67 ^b | 27.38 ^b | 25.86 ^b | 1.04 | 0.001 |
| LCFA (>16) | 52.62 ^b | 62.39 ^a | 64.79 ^a | 64.31 ^a | 1.35 | 0.001 |
| C16:1 desaturase index ¹ | 0.04 ^a | 0.03 ^b | 0.03 ^b | 0.03 ^b | 0.01 | 0.049 |
| C18:1 desaturase index ² | 0.73 ^a | 0.69 ^b | 0.69 ^b | 0.69 ^b | 0.01 | 0.029 |
| D5 desaturase index ³ | 0.86 ^b | 0.90 ^a | 0.92 ^a | 0.92 ^a | 0.01 | 0.010 |
| D6 desaturase index ⁴ | 0.09 ^a | 0.05 ^b | 0.04 ^b | 0.05 ^b | 0.01 | 0.001 |
| Atherogenicity index ⁵ | 1.56 ^a | 0.94 ^b | 0.87 ^b | 0.82 ^b | 0.09 | 0.001 |
| Thrombogenicity index ⁶ | 2.04 ^a | 1.70 ^b | 1.70 ^b | 1.64 ^b | 0.06 | 0.050 |
| h/H index ⁷ | 1.23 ^b | 1.85 ^a | 1.82 ^a | 1.92 ^a | 0.08 | 0.001 |
| Elongase ⁸ | 0.65 ^b | 0.75 ^a | 0.74 ^a | 0.75 ^a | 0.01 | 0.001 |
| Peroxidisability index ⁹ | 4.49 ^b | 6.66 ^a | 6.24 ^a | 5.90 ^a | 0.21 | 0.001 |

319 abc -Means with different superscript in a row differ significantly (p<0.05)

320 ¹16:1 desaturase index=C16:1/(C16:1+C16:0)

321 ²18:1 desaturase index=C18:1/(C18:1+C18:0) (Kelsey et al. 2003)

322 ³Delta 5 desaturase index =C20:4 n-6/C20:3 n-6+ C20:4 n-6

323 ⁴Delta 6 desaturase index=C20:3 n-6/C18:2 n-6+ C20:3 n-6 (Nudda et al. 2008)

324 ⁵Atherogenicity index=(12:0+4*14:0+16:0)/(MUFA+PUFA) (Ulbricht and Southgate 1991).

325 ⁶Thrombogenicity index (TI)=[(C14:0+C16:0+C18:0)/(0.5*MUFA+0.5*PUFAn6 +
326 3*PUFAn3+ (PUFAn3/PUFAn6))].

327 ⁷h/H(hypocholesterolaemic/hypercholesterolaemic)=(C18:1n9+C18:2n6+C20:4n6+C18:3n3+
328 C20:5n3+C22:6n3)/(C14:0+C16:0) (Santos-Silva et al. 2002)

329 ⁸Elongase= C18:0+C18:1 c9/ (C16:0 +C16:1+ (C18:0+C18:1c9))(Oliviera et al.2014)

330 ⁹Peroxidisability index (PI)=(0.025*monoenes) + (1*dienes) + (2*trienes) + (4*tetraenes) +
331 (6*pentaenes) + (8*hexaenes) (Erickson et al. 1992)

332 **Table 6: Pearson correlation between milk fat concentration and milk fatty acid composition**

| | OA | LA | LnA | SFA | MUFA | PUFA | n6:n3 | SCFA | MCFA | LCFA | AI | TI |
|--------------|-----------|-----------|------------|-------------|-------------|-------------|--------------|-------------|--------------|-------------|-------------|-------------|
| Fat | -0.04 | 0.41 * | 0.29 | -0.11 | -0.07 | 0.42 * | 0.27 | -0.01 | -0.27 | 0.44 * | -0.13 | -0.01 |
| OA | | 0.42 * | 0.20 | -0.84 ** | 0.99 ** | 0.41 * | 0.31 | -0.80 ** | -0.66 ** | 0.83 ** | -0.86 ** | -0.93 ** |
| LA | | | 0.22 | -0.61 ** | 0.37 | 0.99 ** | 0.84 ** | -0.52 ** | -0.80 ** | 0.70 ** | -0.65 ** | -0.42 * |
| LnA | | | | -0.24 | 0.19 | 0.28 | -0.28 | -0.24 | -0.31 | 0.30 | -0.30 | -0.23 |
| SFA | | | | | -0.84 ** | -0.61 ** | -0.48 * | 0.84 ** | 0.84 ** | -0.80 ** | 0.94 ** | 0.92 ** |
| MUFA | | | | | | 0.37 | 0.27 | -0.78 ** | -0.62 ** | 0.78 ** | -0.85 ** | -0.94 ** |
| PUFA | | | | | | | 0.82 ** | -0.52 ** | -0.081 ** | 0.70** | -0.66 ** | -0.42 * |
| n6:n3 | | | | | | | | -0.42 * | -0.64 ** | 0.55 ** | -0.51 * | -0.29 |
| SCFA | | | | | | | | | 0.84 ** | -0.90 ** | 0.93 ** | 0.74 ** |
| MCFA | | | | | | | | | | -0.91 ** | 0.93 ** | 0.67 ** |
| LCFA | | | | | | | | | | | -0.94 ** | -0.73 ** |
| AI | | | | | | | | | | | | 0.87 ** |
| TI | | | | | | | | | | | | |

333 OA- Oleic acid; LA- Linoleic acid; LnA- Linolenic acid; SFA- Saturated fatty acid; MUFA-Mono unsaturated fatty acid; PUFA- Polyunsaturated fatty acid;

334 SCFA- Short chain fatty acid; MCFA- Long chain fatty acid; LCFA- Long chain fatty acid;

335 AI- Atherogenicity index, TI-Thrombogenicity index, * p<0.05; ** p<0.01; *** p<0.001

336 **Ruminal parameters**

337 Ruminal parameters like pH, ammonia nitrogen, total nitrogen and its fraction were at par
338 amongst treatments and within normal range. Ruminal pH was not affected by diet or sampling
339 time and was ideal for normal digestibility of fiber in rumen (Orskov 1994). Reduced fiber
340 digestibility and microbial synthesis have been observed when rumen pH falls below 6.2 (De
341 veth & Kolver 2001). Non significant ($p>0.05$) ADF and NDF digestibility also suggest that oils
342 supplementation did not affect fiber digestion.

343 Ruminal TVFA (mmol/L) was significantly ($p<0.05$) increased with dietary inclusion of
344 soybean oils and/or rice bran oil as compared to control. However, time had no effect on TVFA
345 concentration. TVFA concentration in the rumen depends on many factors including nutrient
346 digestibility, rumen pH, rate of digesta passage from rumen, rate of absorption as well as the
347 microbial population in the rumen and their activities (Morsy et al. 2015). The higher
348 concentration of TVFA in SBO, RBO and SRBO indicates more efficient anaerobic
349 fermentation.

350 Ruminal ammonia-N did not differ ($p>0.05$) significantly with both diet and time. In the
351 present study, the concentrations of rumen ammonia-N (Table 7) was above the minimum
352 concentrations required (≥ 5 mg/dL) for rumen microbial growth and for optimum fiber
353 degradation in the rumen (Morsy et al. 2015).

354 Ammonia-N level depends on mainly upon dietary CP solubility. Non significant
355 ($p>0.05$) ammonia-N concentrations in the treatment groups can be considered as an indicator of
356 optimum ruminal fermentation rate and microbial protein synthesis. Earlier studies have reported
357 an increase (Messana et al. 2013), a decrease (Morsy et al. 2015) or no variation (Adeyemi et al.
358 2015) in ammonia-N concentration following lipid supplementation in ruminants. However, an

359 extensive review by Doreau and Ferlay (1995) suggested little or no effect of dietary lipid on
 360 ammonia-N concentration. Total nitrogen (mg/dl), TCA precipitable nitrogen (mg/dl) and
 361 soluble nitrogen (mg/dl) did not differ either with diet or sampling time.

362 **Table 7: Effect of vegetable oil supplementation on ruminal fermentation of experimental**
 363 **animal**

| Parameters | CON | SBO | RBO | SRBO | SEM | P value | | |
|-------------------------------------|-------------------|-------------------|--------------------|--------------------|------|---------|-------|-------|
| | | | | | | D | T | D x T |
| pH | 6.44 | 6.49 | 6.41 | 6.45 | 0.04 | 0.645 | 0.688 | 0.207 |
| TVFA (mmol/l) | 6.26 ^b | 7.06 ^a | 6.85 ^{ab} | 6.99 ^{ab} | 0.29 | <0.012 | 0.422 | 0.719 |
| Ammonia Nitrogen (mg/dl) | 12.22 | 12.25 | 12.72 | 12.49 | 0.12 | 0.217 | 0.201 | 0.830 |
| Total Nitrogen (mg/dl) | 76.08 | 76.66 | 75.55 | 76.72 | 2.97 | 0.991 | 0.928 | 0.976 |
| TCA Nitrogen (mg/dl) | 29.43 | 31.30 | 30.55 | 30.72 | 1.38 | 0.810 | 0.512 | 0.988 |
| Soluble Nitrogen (mg/dl) | 46.65 | 45.36 | 45.00 | 46.00 | 3.02 | 0.981 | 0.663 | 0.990 |

364 D- Diet; T- Time; DxT- Diet and time interaction; SEM- Standard error of mean

365 abc -Means with different superscript in a row differ significantly (p<0.05)

366 **Conclusions**

367 Addition of soybean oil and/or rice bran oil showed significant improvement in nutritional
 368 quality of milk fat with respect to FA composition and lipid quality indices (LQI) of milk. Both
 369 the vegetable oils either alone or in combination, increased production performance of Surti does
 370 in terms of milk yield, fat content and FCM of milk. However, results were more promising in

371 individual oil supplemented groups as compared to mixture of oil. Supplementation of both the
372 oils alone or in combination had no adverse effect on nutrient utilization and ruminal
373 fermentation of Surti does.

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377 **Competing interest:** The authors declare that they have no competing interest.

378 **Ethical approval:** All applicable international, national and/or institutional guidelines for the
379 care and use of animals were followed. The experiment (no. 052-VCN-ANN-2017) was
380 approved by Institutional Animal Ethic Committee (IAEC/CPCSEA Reg No. 1631/GO/Re-
381 SL/Bi-S/12/CPCSEA)

382 **Consent to participate:** Not applicable

383 **Availability of data and material:** Not applicable

384 **Code availability:** Not applicable

385 **Author's contribution:** APR - Designed research work, statistical analysis of project and
386 manuscript writing. VRP - Sample collection and analysis. LMS and JKM - Execution of
387 research work. All authors read and approved the manuscript.

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