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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(12): 1421-1424

www.thepharmajournal.com
Received: 01-10-2022
Accepted: 05-11-2022

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# Effect of sunflower oil supplementation on hemato-biochemical profile of lactating Kankrej cows

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**Abstract**
A study was conducted to evaluate the effect of sunflower oil supplementation on hemato-biochemical profile of lactating Kankrej cows. Thirty lactating Kankrej cows (20 days post-partum) were assigned randomly into three groups (10 cows per group). The three groups were as $T_1$: Basal diet, $T_2$: Basal diet + 125 ml/animal/day of sunflower oil supplementation and $T_3$: Basal diet + 250 ml/animal/day of sunflower oil supplementation. The duration of experiment was of 90 days. The blood samples were collected at the start (0 day) and end ($90^{th}$ day) of experimental feeding. The mean hemoglobin (Hb) and hematocrit values were comparable ($p>0.05$) among the treatment groups. There was no effect ($p>0.05$) on red blood cells count (erythrocytes, MCV, MCH and MCHC) caused by the dietary addition of sunflower oil. White blood cell count (leucocytes, neutrophils, lymphocytes, monocytes and eosinophils) was not influenced ($p>0.05$) by the supplementation of sunflower oil. The initial serum concentrations of estimated blood biochemical parameters were without any significant ($p>0.05$) difference across the treatment groups. At end of experimental feeding (90d), serum concentrations of glucose, total protein, albumin, globulin, urea, creatinine, cholesterol, ALT and AST were not affected ($p>0.05$) by the dietary supplementation of sunflower oil. However, triglycerides concentration was increased ($p<0.001$) in $T_3$ as compared to the $T_1$ and $T_2$ groups. Based on the results, it may be concluded that sunflower oil supplementation at 125 or 250 ml/d in the diet of lactating Kankrej cows did not have any adverse effect on haemato-biochemical parameters except elevated triglycerides levels in lactating Kankrej cows.

**Keywords:** Blood metabolites, haematological profile, Kankrej, sunflower oil

## Introduction
Out of the total 192.49 million cattle population, 142.11 million was contributed by Indigenous cattle (Livestock Census, 2019) [13]. Among the Indigenous cattle, Kankrej is one of the important breed of cattle in India which is mainly found in the region of north Gujarat and neighbouring districts of Rajasthan. During the early lactation (14 to 100 days after calving), cows achieve peak milk production, undergo weight loss, reduced feed intake and alterations in hemato-biochemical parameters. Assessment of haemato-biochemical profile is very important for monitoring the metabolism and health status of ruminants animals (Carlos *et al*., 2015). [4] The values of haemato-biochemical parameters of cows are influenced by several factors such as breed, age, physiological status, sex, nutrition or season (Antunović *et al*., 2017) [2]. Blood biochemical parameters indicate possible metabolic disorders and disorders caused by inadequate nutrition (Rios *et al*., 2006) [16]. Supplementing the diet with fats/oils to help improve the energy and metabolic status in lactating animals and, therefore, enhance productive and reproductive performance (Castro *et al*., 2019) [5]. Sunflower oil is considered as premium oil, with a correct equilibrium between linoleic acid and tocopherols which helps in washing out cholesterol deposition in the coronary arteries of the heart (Shelake *et al*., 2017) [19]. We hypothesized that dietary supplementation of sunflower oil in lactating dairy cows would improve haemato-biochemical parameters in lactating Kankrej cows. Therefore, a study was conducted to determine the effect of sunflower oil supplementation on haemato-biochemical profile of lactating Kankrej cows.

## Materials and Methods
### Animals and experimental design
The use of animals and the experimental procedure were approved by institutional Animal Ethics Committee (approval No. VETCOLL/IAEC/2020/16/PROTOCOL-6). Thirty lactating Kankrej cows (20 days post-partum) were assigned randomly into three groups (10 cows per group) stratified on the basis of body weight and milk production.

All the selected animals were apparently healthy and free from diseases. The three experimental groups were as $T_1$: Basal diet, $T_2$: Basal diet + 125 ml/animal/day of sunflower oil supplementation and $T_3$: Basal diet + 250 ml/animal/day of sunflower oil supplementation. In the group $T_2$ and $T_3$, sunflower oil was mixed into a portion of the concentrate thoroughly and provided once daily during the experimental period. The duration of experiment was of 90 days. The experimental animals were fed as per ICAR (2013) [9] to the nutrient requirements.

**Collection of blood samples**

The blood samples were collected at the start (0 day) and end (90th day) of experimental feeding. The blood samples from external jugular vein were collected from each experimental animal in two sterilized vials one with anti-coagulant for hematological parameters and other without anti-coagulant for analysis of blood biochemical parameters.

**Analysis of haematological parameters**

The fresh blood samples the one with anti-coagulantwere analysed for haematological parameters *viz*., haemoglobin, haematocrit, erythrocytes, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), leucocytes, neutrophils, lymphocytes and monocytes using automatic analyser.

**Analysis of blood biochemical parameters**

The serum was harvested from the blood without anti-coagulant. The serum samples were analysed for glucose, total proteins, albumin, urea, creatinine, triglycerides, cholesterol, alanine aminotransferase (ALT) andaspartate aminotransferase (AST) concentrations using commercial diagnostic kits.

**Statistical Analysis**

All the experimental data obtained were statistically analyzed using SPSS v.16.0 (SPSS Inc., Chicago IL) as per the standard statistical method (Snedecor and Cochran, 1994) [20]. Significant differences between means of treatments were assessed by Duncun's test, and differences between treatments were declared significant at $p<0.05$.

## Results and Discussion

**Haematological parameters**

The effect of supplementation of sunflower oil on haematological parameters of lactating Kankrej cows estimated at 0th day and 90th day of experimental feeding are presented in Table 1. The mean hemoglobin (Hb) and hematocrit values were comparable ($p>0.05$) among the treatment groups at d0 and d90 of experimental feeding. The obtained values of Hb and hematocrit were within normal range of 8-15 g/dL and 26-37% (Kaneko *et al*., 2008) [12]. There was no effect ($p>0.05$) on red blood cells count (erythrocytes, MCV, MCH and MCHC) caused by the dietary addition of sunflower oil at both the supplemental doses in lactating Kankrej cows at d0 and d90 of experimental feeding. Rate variables for red blood cells observed in this study agreed with the reference described by Jones and Allison (2007) [10]. Therefore, no inflammatory process was reported in the lactating cows during the experimental period due to sunflower oil supplementation. The red blood cells series reflect the animals' health and nutrition (Jones and Allison, 2007) [10]. The white blood cell count (leucocytes, neutrophils, lymphocytes, monocytes and eosinophils) were not influenced ($p>0.05$) by the dietary addition of sunflower oil at both the supplemental doses in lactating Kankrej cows at d0 and d90 of experimental feeding. Results for all dietary treatments groups agreed with reference for cattle (Jones and Allison, 2007) [10]. In accordance with present findings, Bodas and Richardson (2011) [3] observed no difference ($p>0.05$) haematological parameters due to supplementation of sunflower oil (28 g) plus cholesterol (2 g/kg concentrate) in Merino-cross lambs. Similarly, Diapari *et al*. (2018) [7] observed that addition of black tea extract and sunflower seed oil in the diet did not affect ($p>0.05$) haematology of ewes except for number of erythrocytes. Concentrate with 6% sunflower seed oil showed higher erythrocytes number compared with concentrate with 4% sunflower oil ($P<0.05$). This results suggested that higher amount of oil diet could increase number of erythrocytes. The results obtained for the counts of red and white blood cells this trial clearly show that sunflower oil at the dose rate of 125 and 250 ml/d is safe for use in dairy cattle without compromising the health and welfare of animals.

**Table 1:** Effect of supplementation of sunflower oil on haematological parameters of lactating Kankrej cows (n=30)

| Parameters | Dietary Treatments¥ | | | SEM | P value |
|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | | |
| **Initial (0d)** | | | | | |
| Haemoglobin (g/dL) | 9.66 | 10.55 | 10.17 | 0.175 | 0.113 |
| Haematocrit (%) | 27.89 | 28.14 | 27.41 | 0.313 | 0.642 |
| Erythrocytes ($10^6$/ µL) | 5.76 | 5.70 | 5.66 | 0.119 | 0.949 |
| MCV (fL) | 49.76 | 49.67 | 49.69 | 0.445 | 0.997 |
| MCH (pg) | 18.45 | 18.46 | 18.48 | 0.221 | 0.999 |
| MCHC (g/dL) | 36.74 | 36.75 | 36.78 | 0.318 | 0.999 |
| Leukocytes($10^3$/ µL) | 8.53 | 8.51 | 8.50 | 0.148 | 0.997 |
| Neutrophils($10^3$/ µL) | 37.50 | 37.60 | 37.60 | 0.469 | 0.995 |
| Lymphocytes($10^3$/ µL) | 53.60 | 53.70 | 53.70 | 0.615 | 0.997 |
| Monocytes($10^3$/ µL) | 5.70 | 5.80 | 5.80 | 0.243 | 0.983 |
| Eosinophils($10^3$/ µL) | 4.30 | 4.50 | 4.40 | 0.278 | 0.961 |
| **At 90d** | | | | | |
| Haemoglobin (g/dL) | 9.82 | 10.41 | 10.07 | 0.224 | 0.575 |
| Haematocrit (%) | 26.85 | 28.31 | 26.78 | 0.388 | 0.196 |
| Erythrocytes ($10^6$/ µL) | 5.47 | 5.48 | 5.47 | 0.105 | 0.998 |
| MCV (fL) | 49.38 | 49.34 | 49.36 | 0.731 | 1.000 |
| MCH (pg) | 18.62 | 18.63 | 18.61 | 0.321 | 1.000 |

| | | | | | |
|---|---|---|---|---|---|
| MCHC (g/dL) | 36.61 | 36.60 | 37.58 | 0.511 | 0.683 |
| Leukocytes($10^3$/ µL) | 8.52 | 8.56 | 8.30 | 0.153 | 0.768 |
| Neutrophils($10^3$/ µL) | 36.20 | 35.00 | 36.60 | 0.809 | 0.717 |
| Lymphocytes($10^3$/ µL) | 52.00 | 51.90 | 51.90 | 0.896 | 0.999 |
| Monocytes($10^3$/ µL) | 7.50 | 7.80 | 7.60 | 0.411 | 0.958 |
| Eosinophils($10^3$/ µL) | 3.70 | 3.60 | 3.80 | 0.160 | 0.885 |

¥$T_1$: Control diet; $T_2$: Control diet + 125 ml of Sunflower oil; $T_3$: Control diet + 250 ml of Sunflower oil
MCV: mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration

**Blood biochemical parameters**

The effect of supplementation of sunflower oil on blood biochemical parameters of lactating Kankrej cows estimated at 0 day and 90 day of experimental feeding are presented in Table 2. The initial (d0) serum concentrations of glucose, total protein, albumin, globulin, urea, creatinine, triglycerides, cholesterol, ALT and AST were without any significant ($p$>0.05) difference across the treatment groups. Their levels were within the normal physiological limits for cattle (Kaneko *et al*., 2008) [12].

**Table 2:** Effect of supplementation of sunflower oil on blood biochemical parameters of lactating Kankrej cows (n=30)

| Parameters | Dietary Treatments¥ | | | SEM | P value |
|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | | |
| **Initial (0d)** | | | | | |
| Glucose (mg/dL) | 59.00 | 60.00 | 61.40 | 0.992 | 0.627 |
| Total protein (g/dL) | 7.83 | 7.80 | 7.81 | 0.091 | 0.994 |
| Albumin (g/dL) | 3.63 | 3.54 | 3.61 | 0.067 | 0.849 |
| Globulin (g/dL) | 4.20 | 4.26 | 4.20 | 0.100 | 0.957 |
| Urea (mg/dL) | 33.26 | 32.31 | 32.19 | 0.874 | 0.869 |
| Creatinine (mg/dL) | 1.19 | 1.21 | 1.22 | 0.033 | 0.942 |
| Triglycerides (mg/dL) | 18.12 | 18.44 | 18.60 | 0.605 | 0.951 |
| Cholesterol (mg /dL) | 221.53 | 218.52 | 228.61 | 4.878 | 0.701 |
| ALT (U/L) | 33.09 | 36.01 | 32.90 | 2.124 | 0.810 |
| AST (U/L) | 69.85 | 67.57 | 70.76 | 1.566 | 0.707 |
| **At 90d** | | | | | |
| Glucose (mg/dL) | 58.80 | 57.30 | 57.90 | 0.995 | 0.835 |
| Total protein (g/dL) | 7.63 | 7.96 | 7.68 | 0.100 | 0.373 |
| Albumin (g/dL) | 3.45 | 3.61 | 3.58 | 0.066 | 0.201 |
| Globulin (g/dL) | 4.18 | 4.35 | 4.11 | 0.130 | 0.453 |
| Urea (mg/dL) | 36.82 | 33.70 | 33.68 | 0.820 | 0.203 |
| Creatinine (mg/dL) | 1.36 | 1.34 | 1.35 | 0.037 | 0.980 |
| Triglycerides (mg/dL) | 25.93[a] | 27.07[a] | 34.52[b] | 0.976 | 0.001 |
| Cholesterol (mg /dL) | 246.21 | 247.38 | 284.08 | 10.538 | 0.256 |
| ALT (U/L) | 35.10 | 38.80 | 34.75 | 1.880 | 0.638 |
| AST (U/L) | 72.72 | 74.66 | 70.59 | 1.571 | 0.588 |

¥$T_1$: Control diet; $T_2$: Control diet + 125 ml of Sunflower oil; $T_3$: Control diet + 250 ml of Sunflower oil
[ab]Means with different superscripts in a row differed significantly ($p$<0.05).
ALT: alanine aminotransferase; AST: aspartate aminotransferase

At end of experimental feeding (90d), serum concentrations of glucose (58.80, 57.30 and 57.90 mg/dL), total protein (7.63, 7.96 and 7.68 g/dL), albumin (3.45, 3.61 and 3.58 g/dL), globulin (4.18, 4.35 and 4.11 g/dL), urea (36.82, 33.70 and 33.68mg/dL), creatinine (1.36, 1.34 and 1.35 mg/dL), cholesterol (246.21, 247.38 and 284.08 mg /dL), ALT (35.10, 38.80 and 34.75 U/L) and AST (72.72, 74.66 and 70.59 U/L) were not affected ($p$>0.05) by the dietary supplementation of sunflower oil in Kankrej cows. No effect on serum concentrations of total protein, albumin and globulin in lactating Kankrej cows suggesting that feeding of sunflower oil unchanged protein catabolism in the muscles of cows. Moreover, lack of effect on liver enzymes ALT and AST due to feeding sunflower oil in lactating Kankrej cows indicates that supplementation did not have any adverse effect on liver function. Though, there was non-significant (P=0.203) variation in the mean urea levels among the treatment groups, numerically serum urea concentrations were reduced ($T_1$ - 36.82 *vs.* $T_2$ - 33.70 and $T_3$ - 33.68 mg/dL) by the supplementation of sunflower oil. The serum urea levels in ruminants are known to be dependent on the amount of ammonia nitrogen concentration in rumen, which is formed from deamination of proteins. The lower serum urea value was indication of decreased deamination and lower nitrogen concentrations in rumen as well as efficient use of nitrogen (Pawar *et al*., 2019) [15]. Supplementations of lipids/oils in ruminant's diet improve protein/nitrogen utilisation due to decrease in population of ciliated protozoa (Joshi *et al*., 2021) [11].

However, supplementation of sunflower oil in the diet at the dose rate of 250 ml/h/d leads to increased ($p$<0.001) triglycerides concentration as compared to the control and 125 ml/h/d supplemented group. The serum triglycerides levels were 25.93, 27.07 and 34.52 mg/dL in $T_1$, $T_2$ and $T_3$ groups, respectively. In agreement with the present findings, past studies have reported that sunflower oil supplementation increased concentrations of serum triglycerides. Fats in the diet encourage the production of lipoproteins in the intestine which is the major site of de novo triglycerides and cholesterol synthesis in ruminants. In addition some

researchers have reported higher serum cholesterol levels after the supplementation of sunflower oil. Roy *et al*. (2013) [17] reported that sunflower oil supplementation at a rate of 67 g/kg of the concentrate mixture in Black Bengal goats significantly increased serum cholesterol (P=0.071) and triglyceride (P=0.014) concentrations as compared to the control. Abdel-Gawad and El-Emam (2018) [1] observed that concentrations of serum glucose, total protein, albumin, globulin, triglycerides and cholesterol did not affected by dietary sunflower oil (3% of DM) in Zaraibi kids. However, supplementation of sunflower oil in the diet resulted in higher ($p$<0.05) HDL and lower ($p$<0.05) LDL than the control. Rufino *et al*. (2018) [18] observed that sunflower oil supplementation (59.6 g/kg DM) in Jersey cows increased (P=0.04) plasma cholesterol concentrations as compared to the control. The other metabolite concentrations were similar across the treatments. Similarly, supplementation of sunflower oils (33, and 66 g/ kg of DM) in crossbreds (Macheng Black x Boer) female goats did not show any significant ($p$>0.05) changes in serum concentrations of glucose, triglyceride, total protein, BUN, AST and ALT. However, the cholesterol was increased significantly ($p$<0.001) due to supplementation of sunflower oil (Hartanto *et al*., 2019) [8]. De Souza *et al*. (2019) [6] reported that there was linear increases ($p$<0.01) in the plasma concentrations of NEFA, cholesterol, and triglycerides in response to increased sunflower oil (1.5, 3.0 and 4.5%) dose in Holstein x Gyr dairy cows. There was no effect ($p$>0.05) of sunflower oil (15, 30, and 45 g/kg of DM) supplementation in Holstein x Gyr cows on the serum concentrations of glucose, NEFA, triglycerides and urea nitrogen, but there was a quadratic increase ($p$<0.05) in serum cholesterol content (Lopes *et al*., 2020) [14].

## Conclusion

Based on the results, it may be concluded that sunflower oil supplementation at 125 or 250 ml/d in the diet of lactating Kankrej cows did not have any adverse effect on haemato-biochemical parameters except elevated triglycerides levels in lactating Kankrej cows.

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