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Research Article

EFFECT OF FEEDING GILOY (*TINOSPORA CORDIFOLIA*) AND FENUGREEK (*TRIGONELLA FOENUM-GRAECUM*) ON LIPID PROFILE IN JERSEY CROSSBRED COWS

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ABSTRACT: The study was conducted on Jersey crossbred lactating cows maintained at the Instructional Livestock Farm, College of Veterinary and Animal Sciences, CSKHPKV, Palampur (Himachal Pradesh), India. The lactating cows were randomly divided into four groups, each group having six animals. T_1, T_2 and T_3 group cows received Giloy stem powder (150 g), or Fenugreek seed powder (150 g), and a combination of both the herbs (75 g of each) respectively, mixed with the concentrate feed for 60 days while the control group (T_0) received only the concentrate. Blood sampling was done fortnightly, from Day 0 to Day 75. Blood plasma samples were analyzed for Triacylglycerol (TAG), Total Cholesterol, High Density lipoproteins (HDL) and Low density lipoproteins (LDL) plasma levels. It was found that fenugreek alone or in combination with giloy was effective in lowering the Total Cholesterol and LDL levels ($p<0.05$) in the blood of lactating dairy cows. TAG and HDL levels remained unchanged in control and treated animals. Feeding of giloy alone did not bring about any reduction in lipid profile of the treated animals.

Key words: Giloy (*Tinospora cordifolia*), Fenugreek (*Trigonella foenum-graecum*), Lipid lowering, Jersey crossbred.

INTRODUCTION

Farm animals are reared for production to meet up the demand for animal protein in human. Various modern medicines are extensively used for production as well as treatment and prevention of diseases of animals, which can ultimately reach us through food chain (Pattanayak *et al.* 2013). The use of plants as medicine is widespread throughout the world. It is estimated that more than 35,000 plant species are being used around the world for medicinal purposes (Dandotiya *et al*. 2013). The modern developments in the therapeutic field brought about a rapid decline in traditional medicine, the plant-based remedies are still having a crucial role as the potential source of therapeutic aids in health systems all over the world for both humans and animals (Chakraborty and Pal 2012). The present day allopathic system of medicine suffers many obstacles in the way of achieving its target. Discovery and development of newer drugs to replace ineffective or less effective drugs is a continuous phenomenon in modern medicine (Pattanayak *et al.* 2016). Giloy (*Tinospora cordifolia*), also known as *guduchi*, occupies the top spot in "*Ayurvedic Materia Medica*" and it has been designated as "*Rasayana*" (Bhattacharyya and Bhattacharya, 2013). This plant finds mention in ancient Sanskrit literature like *Charak Samhita* and *Sushruta Samhita,* as a potential healer of many diseases. The root extract of *T. cordifolia* showed the hypolipidemic effect in alloxan diabetic rats (Stanley *et al.*1999). The root extract which is reported to have the hypoglycaemic and hypolipidemic effect was found to reduce serum and tissue cholesterol, phospholipids and free fatty acids (Stanley *et al.* 2000).

Oral administration of the alcoholic extract of *T. cordifolia* roots for 6 weeks resulted in a significant reduction in blood and urine glucose and lipids in serum and tissues in alloxan diabetic rats. The extracts, however, had no significant effect on total lipid levels in normal as well as in alloxan-treated diabetic rabbits (Stanley *et al.* 2003). It has been reported that extracts of the leaves of *T. cordfolia* have insulin-like action and can significantly reduce the blood glucose but not the total lipid levels in normal rabbits and in alloxan-induced diabetic rabbits (Wadood *et al.* 1991). Fenugreek (*Trigonella foenum-graecum*) is a leguminous herb cultivated in India. The endosperm of the seed is rich in galactomannan and the young seeds mainly contain carbohydrates and sugar. Mature seeds contain the amino acid, fatty acid, vitamins and saponins. The main chemical constituents of

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fenugreek are fibres, flavonoids, polysaccharides, saponins and polysaccharides and some identified alkaloids such as trigonelline and choline (Toppo *et al.* 2009). Fenugreek seed extract has been reported to decrease total cholesterol, VLDL and LDL levels in the blood of rats (Petit *et al.* 1993). It is in agreement with the studies in later years that fenugreek treatment selectively reduces the LDL and VLDL fractions of total cholesterol without any toxicological effects (Al-Habori and Raman 1998). HDL fractions showed an opposite trend (p=0.024) in rats fed with fenugreek-soluble dietary fibre whereas triglycerides, cholesterol and LDL were found to decrease significantly (Hannan *et al.* 2003). Fenugreek saponins and galactomannan induce a notable delay in the absorption of LDL and triglycerides along with a remarkable increase in levels of HDL. Saponin fraction significantly increased the hepatic glycogen content and suppressed the blood glucose level (Hamden *et al.* 2010). Saponins have been indicated to be the hypocholesterolemic component in fenugreek seeds that interact with bile salts in the digestive tract (Stark and Madar 1993).

MATERIALS AND METHODS

The study trial was conducted on Jersey crossbred, healthy cows in various stages of lactation, maintained at the Instructional Livestock Farm, College of Veterinary and Animal Sciences, CSKHPKV, Palampur (Himachal Pradesh) after approval of study from Directorate of Research, CSKHPKV. Necessary permission for conducting the trial was obtained from Incharge, Instructional Livestock Farm. The experimental animals were maintained in loose housing system, under standard feeding and management conditions being followed at Livestock farm. The animals were fed twice daily and watered *ad libitum*. The major fodder provided to the cows during entire study consisted of Setaria grass, Maize fodder, Sorghum, local grass. In addition, the animals were also offered concentrate during milking time (Table 5).

The Jersey crossbred lactating cows randomly divided into four groups, each group having six animals. Each group had two animals each for early, mid and late lactation. T_1, T_2 and T_3 group cows received Giloy stem powder (150 g), or Fenugreek seed powder (150 g), and a combination of both the herbs (75 g of each) respectively, mixed with the concentrate feed for 60 days while the control group (T_0) received only the concentrate. The concentrate fed was same for all the groups and in an amount as per the managemental practices of farm. The herbal treatment was administered at a fixed time daily *i.e.* afternoon milking hours to all the animals till day 60 of the experimental trial mixed in the concentrate.

Table 1. TAG (mg/dL) in the blood plasma of lactating cows treated with herbal supplements and control group (Mean±S.E.).

Group	Day 0	Day 15	Day 30	Day 45	Day 60	Day 75
T_0	10.75^a ±0.69	10.95^a ±0.56	10.43^a ±0.39	10.80^a ±1.08	10.95^a ±0.39	11.00^a ±0.49
T_1	11.80^a ±1.17	9.87^a ±1.06	9.73^a ±0.87	10.20^a ±0.94	9.13^a ±0.96	12.30^a ±1.14
T_2	10.13^a ±0.87	9.65^a ±1.01	8.82^a ±0.93	9.10^a ±1.12	8.88^a ±1.36	9.87^a ±1.24
T_3	10.95^a 1.16	10.73^a ±0.57	8.30^a ±0.84	8.78^a ±0.69	9.88^a ±0.86	10.52^a ±0.78

Figures with different superscripts (a, b, c) differ significantly ($p<0.05$) between rows. T_0-Control, T_1- Giloy, T_2-Fenugreek, T_3- Both (Fenugreek+Giloy).

Blood samples from experimental dairy cows were collected at regular intervals of 15 days till Day 75 of the experiment. First sampling was done a day before the start (Day 0) of feeding trial. Blood samples were collected in clean screw capped heparinised centrifuge tubes aseptic venipuncture technique in the morning working hours of the farm. All necessary precautions were taken to avoid any contamination and deterioration to blood collected. The harvested blood plasma was then stored at -20°C till further analysis. The blood plasma lipid profile (TAG, Cholesterol, HDL and LDL) was determined using biochemical estimation kits (*Manufactured by Agappe Diagnostics Ltd., Agappe Hills, Distt. Ernakulam Kerala, India*) on automatic blood biochemistry analyzer (*Mispa Nano, Agappe*). The results so obtained in the study were analyzed using computer software 'SAS Enterprise Guide'. The data was analyzed by using ANOVA at 5 per cent level of significance.

RESULTS AND DISCUSSION

Total Plasma Lipid Profile

i) Triacylglycerol (TAG)

The TAG (mg/dL) in the blood plasma of lactating cows treated with herbal supplements and control are tabulated in Table 1.

TAG values were found to be in the normal physiological limits of 0-14mg/dL (Kaneko *et al.*2008). The values observed in this study varied between 8.30±0.84 and 12.30±1.14 mg/dL in lactating dairy cows.

TAG values in the case of control (T_0) group were found to be in normal range and slight variation was observed during the study. However, no specific pattern could be identified. The treatment groups (T_1, T_2 and T_3) followed a similar trend in plasma TAG values. As this

Table 2. Cholesterol (mg/dL) in the blood plasma of lactating cows treated with herbal supplements and control group (Mean±S.E.).

Group	Day 0	Day 15	Day 30	Day 45	Day 60	Day 75
T_0	163.48^{ab} ±2.65	159.00^{bx} ±3.26	162.43^{ab} ±7.59	167.43^{ab} ±4.59	175.80^{ax} ±2.95	177.23^{axy} ±6.12
T_1	170.52^{a} ±5.70	156.82^{axy} ±11.99	163.35^{a} ±11.86	156.22^{a} ±9.07	158.30^{ax} ±11.29	182.50^{ax} ±11.15
T_2	156.62^{a} ±12.79	136.36^{axy} ±7.36	147.47^{a} ±10.59	148.54^{a} ±10.15	129.63^{ay} ±8.82	148.73^{ay} ±11.11
T_3	152.45^{ab} ±8.04	132.98^{by} ±7.42	149.95^{ab} ±7.64	152.32^{ab} ±3.29	129.88^{by} ±2.25	161.68^{axy} ±11.52

Figures with different superscripts (a, b, c) differ significantly ($p<0.05$) between rows.
Figures with different superscripts (x, y, z) differ significantly ($p<0.05$) between columns.
T_0-Control, T_1- Giloy, T_2-Fenugreek, T_3- Both (Fenugreek+Giloy).

Table 3. HDL (mg/dL) in the blood plasma of lactating cows treated with herbal supplements and control group (Mean±S.E.).

Group	Day 0	Day 15	Day 30	Day 45	Day 60	Day 75
T_0	117.70^{ab} ±2.15	115.05^{b} ±3.35	122.03^{ab} ±3.82	116.85^{ab} ±1.13	124.65^{a} ±2.47	122.07^{ab} ±0.58
T_1	112.38^{a} ±5.07	106.82^{a} ±7.72	114.30^{a} ±10.16	114.60^{a} ±7.06	118.58^{a} ±7.17	121.33^{a} ±6.69
T_2	112.37^{a} ±8.41	104.75^{a} ±8.91	109.55^{a} ±8.91	108.08^{a} ±7.32	110.72^{a} ±8.32	114.10^{a} ±6.54
T_3	109.65^{b} ±4.23	102.68^{b} ±1.89	109.13^{b} ±4.14	114.73^{ab} ±3.45	114.73^{ab} ±5.71	126.33^{a} ±6.18

Figures with different superscripts (a, b, c) differ significantly ($p<0.05$) between rows.
T_0-Control, T_1- Giloy, T_2-Fenugreek, T_3-Both (Fenugreek+Giloy).

pattern was seen in control as well as treatment groups, hence the variation cannot be attributed to herbal supplementation. No variation among and between the groups was observed statistically, thus herbal supplementation was observed to exert no particular effect. The depression in TAG values towards day 30 could possibly be due to side effect of vaccination. Maher and N.M.B (2013) found no significant effect on TAG level in fenugreek fed Friesian cows as compared to control animals. However, Hannan *et al.*(2003) and Hamden *et al.*(2010) documented a decline in plasma TAG values on fenugreek extract supplementation in diabetic rats. Dhingra *et al.*(2011) reported lower TAG levels in rats treated with *T. cordifolia* stem extract.

Table 4. LDL (mg/dL) in the blood plasma of lactating cows treated with herbal supplements and control group (Mean±S.E.).

Group	Day 0	Day 15	Day 30	Day 45	Day 60	Day 75
T_0	53.63^{b} ±2.02	48.28^{b} ±4.61	48.48^{b} ±4.35	51.15^{b} ±2.88	54.88^{abx} ±1.90	63.78^{a} ±1.53
T_1	59.60^{a} ±2.66	54.73^{a} ±5.81	54.63^{a} ±6.50	56.67^{a} ±5.98	53.50^{ax} ±5.19	67.77^{a} ±6.96
T_2	56.41^{a} ±4.89	50.43^{ab} ±4.54	51.04^{ab} ±3.40	48.52^{ab} ±2.09	42.72^{by} ±2.68	55.89^{a} ±3.52
T_3	51.38^{ab} ±4.96	44.09^{b} ±4.19	46.13^{ab} ±3.90	46.09^{ab} ±1.99	40.68^{by} ±1.68	55.36^{a} ±2.02

Figures with different superscripts (a, b, c) differ significantly ($p<0.05$) between rows.
Figures with different superscripts (x, y, z) differ significantly ($p<0.05$) between columns.
T_0-Control, T_1- Giloy, T_2-Fenugreek, T_3-Both (Fenugreek+Giloy).

Table 5. Composition of concentrate feed supplied to dairy cows (per 100kg).

Ingredient	Quantity (kg)	Ingredient	Quantity (kg)
Maize	30	Mineral Mixture	3
Wheat Bran	10	Urea	1
Deoiled Rice Bran	15	Cottonseed Cake	8
Ground Nut Cake	10	Soya flakes	5
Lime Powder	1	Mustard Cake	9
Molasses	6	Bypass fat	1
Salt	1	Total	100

ii) Cholesterol

The cholesterol (mg/dL) in the blood plasma of lactating cows treated with herbal supplements and control group are depicted below in Fig. 1 and tabulated in Table 2.

The plasma cholesterol values varied between 129.63±8.82 and 182.50±11.15 mg/dL in the present study. Very few references are available regarding normal value of parameters of lipid profile study. The value of total cholesterol was within normal range of 65 to 220 mg/dL (Jackson and Cockcroft 2002).

In the control (T_0) and the treatment groups (T_1, T_2 and T_3) a decline was observed on day 15. The cholesterol values in control group remained statistically similar as the trial progressed. The plasma cholesterol concentration in giloy fed group (T_1) group did not show much variation during herbal supplementation. The plasma cholesterol

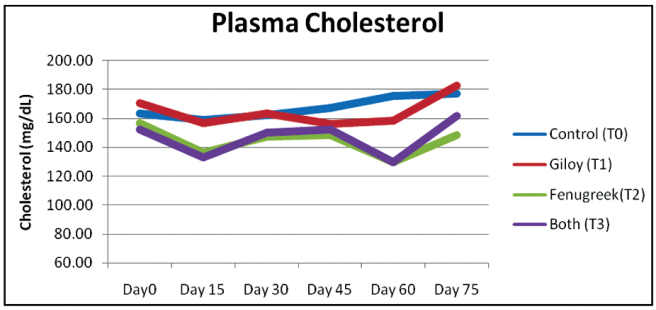

Fig. 1. Cholesterol (mg/dL) in the blood plasma of lactating cows treated with herbal supplements and control group.

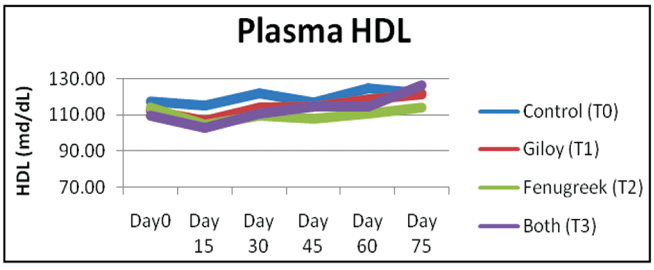

Fig. 2. HDL (mg/dL) in the blood plasma of lactating cows treated with herbal supplements and control group.

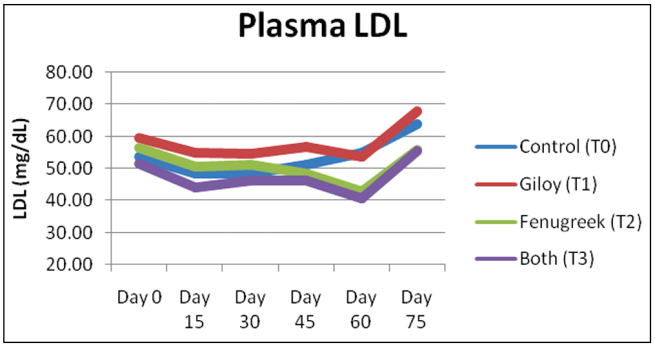

Fig. 3. LDL (mg/dL) in the blood plasma of lactating cows treated with herbal supplements and control group.

concentration in the cows receiving fenugreek seed powder or a combination of fenugreek and giloy (T_2 and T_3 groups) was significantly lower on day 60. The elevation in plasma cholesterol levels on day 75 was observed in control as well as the treatment group. Thus, it cannot be attributed to herbal supplementation. This could probably due to a longer withdrawal period *i.e* 15 days of herbal supplementation. A slight variation was observed in the control group from day 60 to 75 of trial. The decline in T_1 group on day 60 when giloy was being fed to the animals was statistically non-significant in comparison to control group. Hypolipidemic effect of Giloy supplementation has been reported by many workers (Stanley *et al.*1999 and Stanley *et al.*2003). The researchers documented the significant reduction in blood lipid levels in alloxan diabetic rats. In fenugreek fed cows of group T_2, the reduction in plasma cholesterol levels was significant on day 60. Hypocholesterolaemic effects of Fenugreek are well documented. Nasser (2013) and Maher and Nadya (2013) reported a decline in plasma cholesterol levels on fenugreek supplementation in dairy cows. In group T_3 the decline on day 60 in plasma cholesterol values was significant. As these animals received both fenugreek and giloy, the cumulative effect of both the herbs probably lowered the plasma cholesterol levels in this group.

iii) HDL

The HDL (mg/dL) in the blood plasma of lactating cows treated with herbal supplements and control group are depicted below in Fig. 2 and tabulated in Table 3.

In a study, the normal level of HDL in cow was reported to be 111.75±3.48 mg/dL (Petkova *et al.* 2008). The plasma HDL values in the case of control (T_0) group and treatment groups(T_1, T_2 and T_3) with a slight decline on day 15 showed were found to be statistically similar during the trial. The HDL values in treatment groups followed an increasing trend till completion of the trial. The increasing trend in T3 group is statistically similar thus no change is attributable to the herbal supplementation and its withdrawal. The trend was similar in control and treatment groups.Various research workers documented an increase in plasma HDL levels on herbal supplementation. Vetrivadivelan *et al.*(2012) revealed that the aqueous extract of *T. cordifolia* was found to increase HDL concentrations in blood of streptozocin-induced diabetic rats. Hannan *et al.*(2003) and Hamden *et al.*(2010) reported an increase in blood HDL levels of the rats fed with fenugreek diet.

iv) LDL

The LDL (mg/dL) in the blood plasma of lactating cows treated with herbal supplements and control group are depicted below in Fig. 3 and tabulated in Table 4.

In a study, the normal level of LDL in cow was reported to be 82.75± 4.25 mg/dL (Petkova *et al.* 2008). No variation was observed in LDL values of T_0 group till day 60 but increased to a significantly higher value on day 75. In T_1 group the LDL values decreased non-significantly from day 0 to day 60 to a lower value as compared to control group. In T_2 and T_3 group a reduction in plasma LDL values was observed on day 60 in comparison to day 0 values within the group. The decline was also found significant in comparison with T_0 group. On day 75, mean plasma LDL levels were found to be higher as compared to day 60 values in all the treatment groups.

In group T_1, the decreasing trend in LDL levels during the period when giloy was being fed to the animals, was statistically non-significant. Hypolipidemic effect of giloy supplementation has been reported by many workers

(Stanley *et al.*1999 and Stanley *et al.*2003). The researchers documented the significant reduction in blood lipid levels in alloxan diabetic rats. In fenugreek fed cows of group T_2, the reduction in plasma LDL levels was significant. Hypocholesterolaemic effects of Fenugreek are well documented. Nasser (2013) and Maher and Nadya reported a decline in LDL levels on fenugreek supplementation in dairy cows. The Fenugreek saponins and galactomannans have been reported to delay absorption of LDL cholesterol by Hamden *et al.*(2010). In group T_3 also the decline in LDL values was significant. As these animals received both fenugreek and giloy, the cumulative effect of both the herbs probably lowered the plasma LDL levels in this group.

CONCLUSION

The plasma lipid profile (TAG, Total Cholesterol, HDL and LDL) values of giloy fed animals (T_1) remained lower in comparison to control group but the differences were non-significant. In T_2 and T_3 groups too, the plasma lipid profile values were lower than those in the control group and the differences for Total Plasma Cholesterol and LDL were significant on day 60. The decline was observed in individual lipid profile components on day 15. These changes may be attributed to the fact that the animals were subjected to vaccination (Raksha-Triovac (FMD-HS-BQ) during the study trial on day 5.

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