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Effect of giloy (*Tinospora cordifolia*) and neem (*Azadirachta indica*) on haematological parameters and gastrointestinal parasitic load in Marwari lambs under arid zone

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Abstract
Haematological parameters are useful tools for assessing health status of an animal. In Arid region of Rajasthan where sheep rearing is commonly practiced as pasture graze system, gastrointestinal parasitic infections are more frequent. So the present study was designed to evaluate the effect of Giloy and Neem alone and in combination on harmatological parameters of Marwari lambs and gastrointestinal parasitic load in treated animals compare to control group under arid zone. An experiment was conducted for 12 weeks on 42 Marwari male lambs and divided into seven groups, six lambs in each group in a randomized block design. The lambs of T_1 group were provided only basal diet (grazing + *ad lib.* fodder + 400 g concentrate per lamb per day) and were kept as control group and lambs of all other treatment groups were provided basal diet with herbal supplementation i.e. 0.5 per cent Giloy stem powder in group T_2, 1.0 per cent Giloy stem powder in group T_3, 0.5 per cent Neem leaf powder in group T_4, 1.0 per cent Neem leaf powder in group T_5, 0.25 per cent Giloy stem powder along with 0.25 per cent Neem leaf powder in group T_6 and 0.5 per cent Giloy stem powder along with 0.5 per cent Neem leaf powder in group T_7. Higher level of Hb and PCV were recorded in different treatment groups than control group though, they were within normal physiological range but the effect was non-significant. Results of parasitic infection showed significant difference in the infection rates among the treatment groups and group T_7 have minimum parasitic infection compared to other treatment groups. At the end of experiment it appears that incorporation of 0.5 per cent Giloy stem powder along with 0.5 per cent Neem leaves powder can be used as a part of strategy as they are effective to control gastrointestinal parasitic infection and maintain the value of Hb and PCV which indicate good health status of lambs in arid zone of Rajasthan.

Keywords: Giloy, neem, haemoglobin, packed cell volume, gastrointestinal parasitic infection

1. Introduction
In developing countries, the quality of pasture being grazed substantially deteriorates due to seasonal influences, consequently reducing productivity as experienced in small ruminant production systems in Rajasthan where the animals depend extensively on pasture. Low quality feed also act as a stressor for animals. Therefore, improvement in nutrition is the key factor to increase productivity of animals. Gastrointestinal Parasitic infection is one of the most important cause of decreased production of small ruminants especially in pasture grazed animals as they are adversely affected by parasitic infection. Some Gastrointestinal parasites suck blood and where there is a heavy parasitic burden, they may cause anemia (Ahmed *et al*., 2015) [1] as well as other hematological and biochemical disturbances (Moudgil *et al*., 2017 [2], Wamboi *et al*., 2020) [3]. Anemia is not a disease entity, but rather is functionally defined as decreased oxygen-carrying capability of blood (Goklaney *et al*., 2012) [4]. Clinically, anemia is characterized by an absolute decrease in the packed cell volume (PCV) and hemoglobin concentration (Hb) (Radostitis *et al*., 1994) [5]. 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) [6]. The interest in herbal feed additive in livestock production is being increased due to development of microbial resistance to drugs and their consequences on human and animal health. Meat consumers are becoming more demanding in terms of product quality.

Products used to control parasite infections in ruminants, in general, leave residues in meat and milk, as well as are eliminated in the faces into the environment. The use of natural products may be an alternative to reduce the use of chemical drugs for the control of parasites. A larger number of plants are naturally available in the Indian subcontinent, which possess specific or broad spectrum anthelmintic activities (Sujon *et al.*, 2008) [7]. According to Shaalana *et al.* (2005) [8] botanical insecticides are relatively safe and easily degradable in the environment, thereby becoming possible sources of biopesticides. 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) [9]. This plant as a potential healer of many diseases, finds mention in ancient Sanskrit literature like *Charak Samhita* and *Sushruta Samhita.* Pharmaceutical properties are present in this plant so supplementation is done in powder or dried form in diet of animals with the intention of preventing diseases and improving quality of life. A variety of constituents related to different classes such as alkaloids, sesquiterpenoids, diterpenoid lactones, glycosides, ecdysteroids, phenolics, aliphatic compounds and polysaccharides have been extracted from *T. cordifolia* (Singh *et al*., 2003) [10]. It is claimed that the plant climbing up the Neem tree is said to be the best as synergy between these two bitter plants enhances Giloy`s efficacy. Neem (*Azadirachta indica*) is a plant from the Meliaceae family, of Asiatic origin and the arid regions of the Indian sub-continent. It has been indicated for use as a possible alternative phytochemical within the nutritional, sanitary management and a potential phytochemical pesticide. Results of two studies done by Chandrawathani *et al.* (2000 & 2002) [11, 12] showed an anthelmintic effect of Neem against nematode parasites of small ruminants. As one of the most researched tree in the world Neem tree has attracted world-wide prominence due to its vast range of medicinal properties like antibacterial, antifungal, antiviral, antiprotozoal, anthelmintic, hepatoprotective and several other qualities without showing any adverse effects (Kale *et al*., 2003) [13].
Despite the fact that *T. cordifolia* is an important medicinal plant, so far to our knowledge, no attempt was made to study their effect on haematological parameters in ruminants. So, the present investigation was planned to study the effect of Giloy (*Tinospora cordifolia*) and Neem (*Azadirachta indica*) on haematological parameters of Marwari lambs and compare the gastrointestinal parasitic load in treated animals to control group under arid zone.

2. Materials and Methods

2.1 Experimental design

The present study was conducted on Marwari lambs maintained at Arid Region Campus of Central Sheep and Wool Research Institute (ICAR-ARC-CSWRI), Bikaner. The experiment was conducted from May to July, 2019. Forty-two growing male Marwari lambs (3-4 months old) were divided into seven groups T_1, T_2, T_3, T_4, T_5, T_6 and T_7 in a randomized block design having six lambs in each group on body weight basis. The lambs of T_1 group were provided only basal diet (grazing for a period of 8 hours + *ad lib*. fodder + 400 g concentrate per lamb per day) and were kept as control group. The lambs of T_2 group were provided basal diet supplemented with 0.5 per cent Giloy stem powder. The lambs of T_3 group were provided basal diet supplemented with 1.0 per cent Giloy stem powder. The lambs of T_4 group were provided basal diet supplemented with 0.5 per cent Neem leaf powder. The lambs of T_5 group were provided basal diet supplemented with 1.0 per cent Neem leaf powder. The lambs of T_6 group were provided basal diet supplemented with 0.25 per cent Giloy stem powder and 0.25 per cent Neem leaf powder. The lambs of T_7 group were provided basal diet supplemented with 0.5 per cent Giloy stem powder and 0.5 per cent Neem leaf powder.

2.2 Haematological parameters

Blood samples from experimental lambs were collected in the morning hours before the feeding and watering of lambs. Samples were collected at monthly interval by puncturing jugular vein following aseptic measures. The blood, so drawn was collected in sterilized test tubes containing adequate amount of anticoagulant. Haematological studies were performed soon after collection of blood. Haemoglobin (Hb) and packed cell volume were determined by Sahli-Hellige haemoglobinometer and microhaematocrit method, respectively. The data obtained in the study was analyzed in one-way ANOVA using SPSS 20.00 statistical analysis software.

2.3 Gastrointestinal parasitic infection

Total 420 faecal samples, 60 samples from each group were collected during the period of experiment of 12 weeks. The samples were placed in sterile polythene bags after labelling group and Tag no. of lamb, kept in cool transport box and brought to the Postgraduate laboratory, Department of Veterinary Parasitology, CVAS, Bikaner, for further examination.

2.3.1 Qualitative examination of faecal samples

Qualitative examination was conducted to record the presence/ absence of gastrointestinal parasitic eggs/ cyst/ oocysts in the faeces of experimental lambs. The faecal samples were qualitatively examined by using centrifugal floatation and sedimentation techniques.

2.3.1 (a) Centrifugal floatation technique

Two grams of strained faecal sample was mixed with ordinary water in 15 ml centrifuge tube and centrifuged for 1-2 minutes at 1500 RPM. The supernatant was removed and similarly two washings were given so that the colour of the faecal sample was removed. After last washing, the faecal decant at the bottom of the tube was mixed with Sheather's sugar solution and was filled up to its brim and was covered with clean coverslip and centrifuged at 1500 RPM for 2 minutes. After centrifugation, the coverslip was then picked up gently and put over a slide for examination under low power objective (10X).

2.3.1 (b) Sedimentation technique

Two grams of strained faecal sample was mixed with ordinary water in 15 ml centrifuge tube and centrifuged for 1-2 minutes at 1500 RPM. The supernatant was removed and similarly two washings were given so that the colour of faecal sample was removed. After last washing in water, the faecal decant at the bottom of the tube was mixed with 10 ml of 10% formalin. After that 3 ml of ether was added and tube was shaken vigorously. The tube was centrifuged for 3 min at 1200 RPM. There is a formation of plug between layers of two solutions, which is broken by glass rod. Supernatant was removed and a drop of sediment placed on a clean microglass slide, a micro coverslip was put over it and was examined under low power objective (10X) of the microscope.

The presence was recorded if parasitic infection was shown by any or all of these methods i.e., sedimentation and/ or floatation methods.

3. Results and Discussion

3.1 Haematological parameters

3.1.1 Haemoglobin (g/dl)

The mean values of heamoglobin (Hb) of lambs under different treatment groups at monthly intervals of experiment have been presented in Table 1. The mean values of Hb (g/dl) of lambs were recorded to be varied from 10.35 (initial) to 10.44 (2nd month) and 10.4 (3rd month) in T_1, 10.30 (zero day) to 10.79 (3rd month) in T_2, 10.32 (zero day) to 11.17 (3rd month) in T_3, 10.29 (zero day) to 10.72 (3rd month) in T_4, 10.35 (zero day) to 10.64 (3rd month) in T_5, 10.34 (zero day) to 11.35 (3rd month) in T_6 and 10.52 (zero day) to 11.57 (3rd month) in T_7 group. The statistical analysis of variance revealed highly significant ($P< 0.01$) effect on monthly mean Hb during 3rd month but remained non-significant during rest of the months of experimental period. Non-significant effect was also observed on average Hb values.

3.1.2 Packed cell volume (PCV)

The mean values of packed cell volume (PCV) of lambs under different treatment groups at monthly intervals of experiment have been presented in Table 2. The mean values of PCV (%) of lambs recorded to be varied from 31.02 (zero day) to 31.9 (3rd month) in T_1, 30.89 (zero day) to 32.37 (3rd month) in T_2, 30.94 (zero day) to 33.49 (3rd month) in T_3, 30.82 (zero day) to 32.10 (3rd month) in T_4, 31.02 (zero day) to 31.85 (3rd month) in T_5, 30.99 (zero day) to 34.05 (3rd month) in T_6 and 31.49 (zero day) to 34.67 (3rd month) in T_7 group. The statistical analysis of variance revealed highly significant ($P< 0.01$) effect on monthly mean percent PCV during 3rd month, but remained non-significant during rest of the months of experimental period. Non-significant effect was also observed on average PCV values.

The observed results of Hb and PCV indicate that there was higher level of Hb and PCV in different treatment groups than control group though, they were within normal physiological range. The results are in agreement with Rahman *et al*. (2009) [14] who observed increased value of Hb and PCV in calves supplemented with Neem. Further, Habeeb and El-Tarabany (2012) [15] also reported increased value of Hb in growing goats supplemented with *Nigella sativa* or Curcumin. However, it was contrary to the findings of Kareem (2017) [16] who reported no significant effect on Hb and PCV in sheep when supplemented with Neem leaf meal.

3.2 Infection of gastrointestinal parasites

The mean values of gastrointestinal parasitic infection in lambs under different treatment groups of experiment have been presented in table 3. Out of 420 samples, 140 samples were found positive with an overall infection rate of 33.33% for gastrointestinal parasites. Group wise overall parasitic infection revealed maximum infection in group T_1 (38.33%) followed by group T_2, T_4 and T_5 (31.67%), group T_3 (16.67%), group T_6 (13.33%) and group T_7 (10.00%). *Trichuris sp.* infection showed higher infection (19.2%) followed by *Eimeria sp.* infection (10.47%). Statistical analysis using chi-suare test revealed highly significant difference in the infection rates among the treatment groups. A highly significant difference in the infection of *Trichuris sp.* was reported while a non-significant difference in *Eimeria sp.* infection was reported. Statistical analysis using binary logistic regression for gastrointestinal parasitic infection revealed a negative association between groups i.e. odds ratio was 0.745 in group T_2, T_4 and T_5, 0.322 in group T_3, 0.247 in group T_6 and 0.179 in group T_7 compare to group T_1. Looking into the results of present study group T_7 having supplementation of 0.5 per cent Giloy stem powder and 0.5 per cent Neem leaf powder showed minimum parasitic infection as compared to other treatment groups which may be due to anthelmintic activity of neem leaves (Amin *et al*., 2008) [17] along with antistress activity of Giloy (Devasena and Adilaxmamma, 2014) [18]. The findings of the present study are similar to the findings of Sarker *et al*. (2016) [19] who reported significant effect ($p< 0.01$) on parasitic load in zebu cattle supplemented with Neem leaf and Ata leaf. However, on contrary Macedo *et al*. (2007) [20] reported that increasing doses of Neem leaves did not improve the control of parasitic infestation in sheep.

Table 1: Haemoglobin (gm/dl) of lambs at monthly intervals in different treatment groups

Treatment Groups	Period (Monthly)				
	0	I	II	III	Average
T_1	10.35	10.39	10.44	10.40^{a}	10.40
T_2	10.30	10.34	10.72	10.79^{bc}	10.54
T_3	10.32	10.44	10.77	11.17^{bcd}	10.68
T_4	10.29	10.35	10.50	10.72^{ab}	10.47
T_5	10.35	10.47	10.54	10.64^{ab}	10.50
T_6	10.34	10.54	11.00	11.35^{cd}	10.81
T_7	10.52	10.79	11.05	11.57^{d}	10.98
SEM	0.23	0.21	0.16	0.19	0.18

Note: Means with different superscripts in a column differ significantly.

Table 2: Packed cell volume (%) of lambs at monthly intervals in different treatment groups

Treatment Groups	Period (Monthly)				
	0	I	II	III	Average
T_1	31.02	31.09	31.29	31.19^{a}	31.15
T_2	30.89	31.02	32.09	32.37^{ab}	31.59
T_3	30.94	31.29	32.25	33.49^{bc}	31.99
T_4	30.82	31.02	31.49	32.10^{ab}	31.36
T_5	31.02	31.35	31.59	31.85^{ab}	31.45
T_6	30.99	31.55	32.95	34.05^{c}	32.39
T_7	31.49	32.30	33.14	34.67^{c}	32.90
SEM	0.68	0.62	0.49	0.55	0.55

Note: Means with different superscripts in a column differ significantly.

Table 3: Overall infection of gastrointestinal parasites of lambs

Treatment Groups	Examined	Infected	*Trichuris*	*Eimeria*
T_1	60	23 (38.3)	18 (30)	8 (13.33)
T_2	60	19 (31.67)	15 (25)	7 (11.67)
T_3	60	10 (16.67)	8 (13.33)	6 (10)
T_4	60	19 (31.67)	15 (25)	7 (11.67)
T_5	60	19 (31.67)	14 (23.33)	6 (10)
T_6	60	8 (13.33)	6 (10)	5 (8.33)
T_7	60	6 (10)	5 (18.33)	5 (8.33)
χ^2 value	-	23.873^{**}	16.886^{**}	1.320
Total	420	140 (33.33)	81 (19.28)	44 (10.48)

Note: Figures in parenthesis are percentage.
NS = non-significant and ** = highly significant ($p \leq 0.01$).

4. Conclusion

Supplementation of Giloy and Neem positively influence the health status of lambs under arid zone of Rajasthan as they are combinedly effective to control gastrointestinal parasitic

infection and maintain the value of Hb and PCV at higher level within normal physiological rage. At the end of experiment it appears that incorporation of 0.5 per cent Giloy stem powder along with 0.5 per cent Neem leaves powder can be used as a part of strategy in lambs under arid zone.

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