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Sanjib Borah
Lakhimpur College of Veterinary Science, Assam Agricultural University, North Lakhimpur, Assam, India

Simson Soren
Lakhimpur College of Veterinary Science, Assam Agricultural University, North Lakhimpur, Assam, India

Gautam Bordoloi
Lakhimpur College of Veterinary Science, Assam Agricultural University, North Lakhimpur, Assam, India

Anajn Jyoti Nath
Lakhimpur College of Veterinary Science, Assam Agricultural University, North Lakhimpur, Assam, India

L Sanathoi Khuman
Lakhimpur College of Veterinary Science, Assam Agricultural University, North Lakhimpur, Assam, India

Uma Ram Tamuli
Lakhimpur College of Veterinary Science, Assam Agricultural University, North Lakhimpur, Assam, India

Correspondence
Sanjib Borah
Lakhimpur College of Veterinary Science, Assam Agricultural University, North Lakhimpur, Assam, India

Study on the efficacy of herbal liver stimulants as adjunct therapy in bovine

Sanjib Borah, Simson Soren, Gautam Bordoloi, Anajn Jyoti Nath, L Sanathoi Khuman and Uma Ram Tamuli

Abstract
The preference for use of herbal medicines is increasing as the products are considered to have no or minimum side effects as well as a residual effect in meat or milk. Therefore a trial is designed to study the effect of different poly herbal preparations as adjunct liver stimulants with anthelmintic treatment in cattle. AV/SYL/12, AV/SYB/12, Yakrifit Liquid and Yakrifit Bolus (Ayurvet Limited, India) were fed orally along with anthelmintic in seven treatment group (n=5/ group) of cattle. An improvement of hemato-biochemical profile was observed in all the groups supplemented with adjunct herbal liver stimulants. Nevertheless, significant reduction of SGPT (liver function test) was recorded with AV/SYL/12 indicating the beneficial effects of AV/SYL/12 during deworming compared to other liver stimulants used in the present study. Further, no any negative effect on kidney function was observed during medication.

Keywords: Herbal liver stimulants, adjunct therapy, bovine

Introduction
Livestock farming is central to the sustainability of rural communities worldwide. All grazing animals are exposed to helminth infections at pasture and any future intensification of pasture-based systems will likely increase the risk of helminth disease. Gastrointestinal nematodes and liver fluke are the two major causes of lost productivity in ruminants. The economic loss due to parasitic disease is currently difficult to quantify; however, some estimates exist within the scientific literature.

Helminthiasis is the gastrointestinal parasitic infestation and more prevalent in the very young and the very old or immuno-deficient animals. Helminth infections lead to production and reproductive losses through a reduction of feed intake or feed conversion efficiency, haematological disorders and even death of livestock worldwide which result in serious economic losses to the farmer. It underlies the need of appropriate treatment for the farm animals with suitable anthelminthic to reduce economic losses to the farmer.

The liver is one of the vital organs of the body, susceptible to various parasites and disease conditions which affect the total health status of the animal. Good nutritional status may make the host cattle more resilient to the pathogenic effects of helminth infections. Good managemental practices and balanced nutrition helps in reducing potential health risks to infection and diseases; improving appetite, feed efficiency, better body weight gain and general appearance of animals. In the absence of reliable liver protecting drugs in modern medicine, there are a number of herbal based medicinal preparations in Ayurveda, recommended for the treatment of liver disorders as an adjunct therapy

AV/SYL/12, AV/SYB/12, Yakrifit Liquid and Yakrifit Bolus (Ayurvet Limited, India) have been used for their hepatoprotective, hepatoregenrative, hepatostimulant properties and well evident as growth promoter. Hence a trial is designed to study the antihelminthic activity of different poly herbal preparations with or without adjunct liver stimulants.

Materials and Methods
Fecal samples from a herd of Lakhimi cattle of Assam reared under semi intensive system were examined for most common helminth species prevalent in the area. The helminth species considered for the study was *Haemonchus contortus*, *Oesophagostomum spp*, *Bunostomum spp*, *Trichuris spp*, *Toxocara vitulorum*, *Fasciola gigantica*, and *Paramphistomum spp* infestation. Thirty five (35) numbers of cattle found positive and or moderately positive for

naturally acquired helminthes through coprological examination were randomly assigned to 7 groups (n=5/ group) to constitute the experimental animal groups. All the experimental animals were housed in stall feeding system on the day 0 of the experiment and kept confined upto day 14th of the experiment.

The treatment groups designated as T0, T1, T2, T3, T4, T5 and T6 were subjected to the following treatments with the ayurvedic preparations supplied from Ayurvet Limited, India. T0 group was orally administered with anitparasitic drug alone (APD) and rest of the experimental animal groups were administered ADP orally on day 0 of treatment followed by herbal liver stimulant as per following dose and duration-

Group	Treatment	Dose
T0:	Anitparasitic drug alone (APD)	Albendazol @7.5mg/ Kg Bwt
T1:	APD+ AV/SYL/12	AV/SYL/12@50ml per day bid for 5 days
T2:	APD + AV/SYB/12	AV/SYB/12@ 2bolus per day bid for 5 days
T3:	APD+ Yakrifit Liquid	Yakrifit Liquid @50ml per day bid for 5 days
T4:	APD+ Yakrifit Bolus	Yakrifit Bolus @ 2 bolus per day bid for 5 days
T5:	APD+ Liver tonic Brand A	Liver tonic Brand A @ 50ml per day bid for 5 days
T6:	APD+ Liver tonic Brand B	Liver tonic Brand B @ 2 bolus per day bid for 5 days

On day 14th of experimentation the animals were allowed for free grazing till day 21st of experimentation. On day 21st of the experimentation the faecal as well as blood/ serum samples were collected and examined for the same parameters as describe underneath.

Faecal as well as blood/ serum samples were examined on day 0, 7th, 14th and 21st of experiments to study the mean faecal egg count (EPG: eggs per gram of faeces) as well as haemato-biochemical parameters *viz*- packed cell volume, total erythrocyte counts, total leucocytes count, serum total protein, albumin: globulin ratio, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), Blood urea nitrogen (BUN), creatinine and serum bilirubin.

Faecal samples were collected per rectum and put into faecal pots, labelled and kept cool prior to transportation to the laboratory where they were examined immediately or stored in refrigerator for a maximum of 4 hours before processing. The samples were processed by Standard Floatation and Sedimentation techniques to investigate the eggs of helminth parasites. The ova of parasites were identified from their morphological features [1]. Quantitative examination of faeces was conducted to record the intensity of parasitic infestation (EPG; egg per gram of faeces) by Modified McMaster method were performed as described by [2, 3].

Blood samples were collected in sterile tubes containing anti-coagulant by jugular vein-puncture following all aseptic precautions, for generating haematological parameters using standard techniques. To study the blood biochemical parameters, the blood samples were collected in other sterile tubes having no anticoagulant. The blood slants were incubated following standard methods and blood clots were broken and tubes were centrifuged at 2,500 rpm for 30 min to separate the serum. The serums thus obtained were stored for further estimation of biochemical parameters.

Haemato-biochemical parameters were studied following standard protocol as well as using available commercial kits as follows:

Hematological profile *viz*Packed cell volume (PCV), haemoglobin (Hb) concentration determination followed the procedures outlined by [4] and total erythrocyte counts (TEC), total leucocytes count (TLC) were determined using the Neubauer haemocytometer after appropriate dilution.

Biochemical parameters *viz* total serum protein [5], albumin: globulin ratio [6], SGOT [7], SGPT [7], BUN [8], Creatinine [5] and serum bilirubin [8] were estimated using commercial kits procured from Siemens Healthcare Private Limited, Gujrat, India, in an automated visible spectrophotometer (Visible spectro 105, Systronics, India Ltd.).

The data generated was statistically analyzed and compared with standard values following statistical procedures[9] and significance of mean difference will be tested by Duncan's new multiple range.

Results and Discussion

Fecal egg count

The fecal egg count (EPG) was found positive only for Strongyle eggs (Table 2) on day 0 of experiment, however, no other helminth egg was detected either on qualitative or quantitative methods of fecal sample examination. On day14th and21st post treatment no helminth eggs were found (Table 2) in the fecal samples of the experimental animal. The absence of helminth eggs in the fecal sample of experimental animals indicate positive effect of anthelmintic with adjunct herbal liver tonic preparation AV/SYL/12, Yakrifit Liquid/ bolus and liver tonic brand A/B might contain herbs that might have negative effect on worms [10, 11].

Hematological parameters

The improvement of hematological parameters were observed in all the groups with significant differences ($p<0.05$) between days (table 1). However, the values of Hb were higher on day 7 in all the group treated with anti-parasitic plus adjunct liver stimulant (T1, T2, T3, T4, T5 and T6) as compared to anti-parasitic drug alone (T0). The percentage of PCV was improved on day 14 and 21 in all groups, higher values were observed in T2, T3 and T4 on day 21 as compared to T0 (Figure 1 a). The TLC was decreased significantly on day 14and 21 in group T0, T1, T2 and T3 as compared to other groups (Table 1). Non-significant impact was noticed in group T2 on day 14 where the mean value of T2 was declined faster than T0 (Figure 1 b). Improvement of TEC was observed in all the groups significantly between the days. The improved levels of hematological values might be due to effect of given herbal liver stimulant. Because the herbs present in herbal liver stimulants used in the present study might having hepatoprotective effect [12, 13] apart from causing elimination of worms from intestinal tract [12, 13]. The hepatoprotective effect of herbal liver stimulants used in the present study is also supported by SGOT and SGPT concentration (Figure 2).

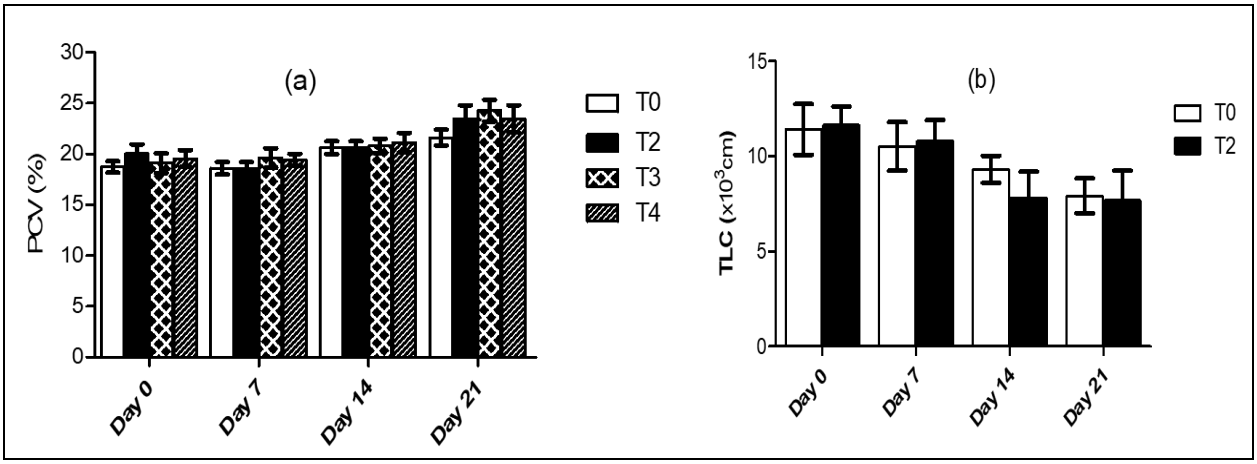

Fig 1: The percentage of PCV (a) and the concentration of TLC (b) in anti-parasitic drug with or without adjunct liver stimulants

Blood biochemical parameters

The concentration of creatinine was declining significantly between days 0, 7 and 21 in all the groups after treatment, however, no significant difference was observed between the treatments (Table 1). It indicates that the herbal liver stimulant used in the present experiment did not have negative impact on metabolic process on the experimental animals. Similarly it was also reported no significant effect [14] on plasma creatinine, urea nitrogen, total protein, albumin, globulin, albumin /globulin following oral administration of medicinal plant.

The SGOT, SGPT and ALP showed significant improvement in all the groups (Table 1) after treatment with anti-parasitic with or without adjunct liver stimulant. This might be due to hepato protective effect [12, 13] of the herbs present in the herbal liver stimulants. However, T1 showed significant reduction of SGPT on day 14 as compared to T0 (Figure 2 A). The T2 group showed faster declining rate of SGOT on day 7 and14 (Figure 2B). The total serum protein, serum albumin: globulin ratio did not differ significantly between the treatments or the days (table 1). Although the concentration of BUN and serum bilirubin did not differ significantly between treatments nevertheless they differ significantly between days (Figure 3 A, B).

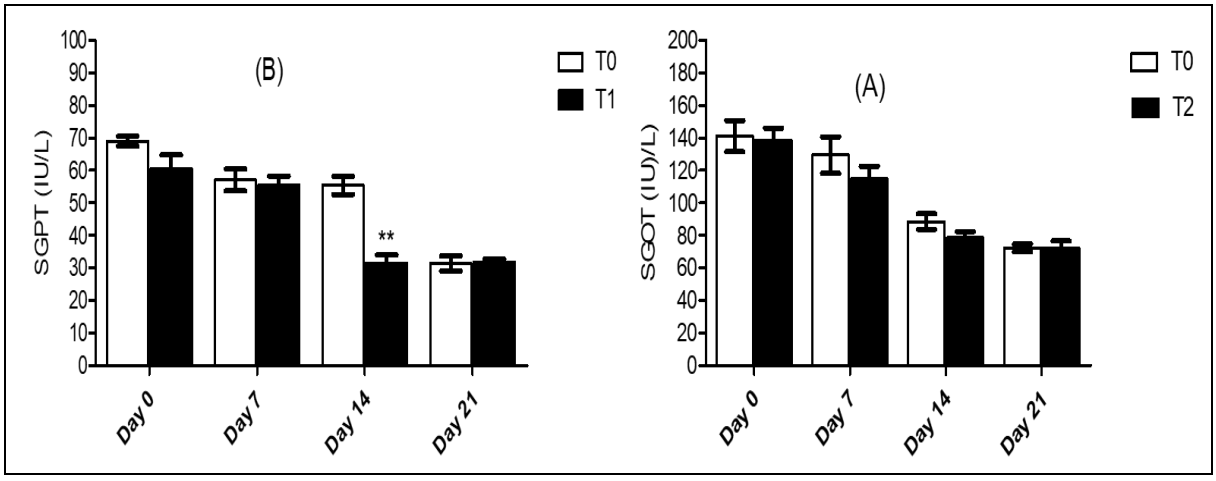

Fig 2: The concentration of SGOT and SGPT in herbal anti-parasitic with or without adjunct liver stimulant.

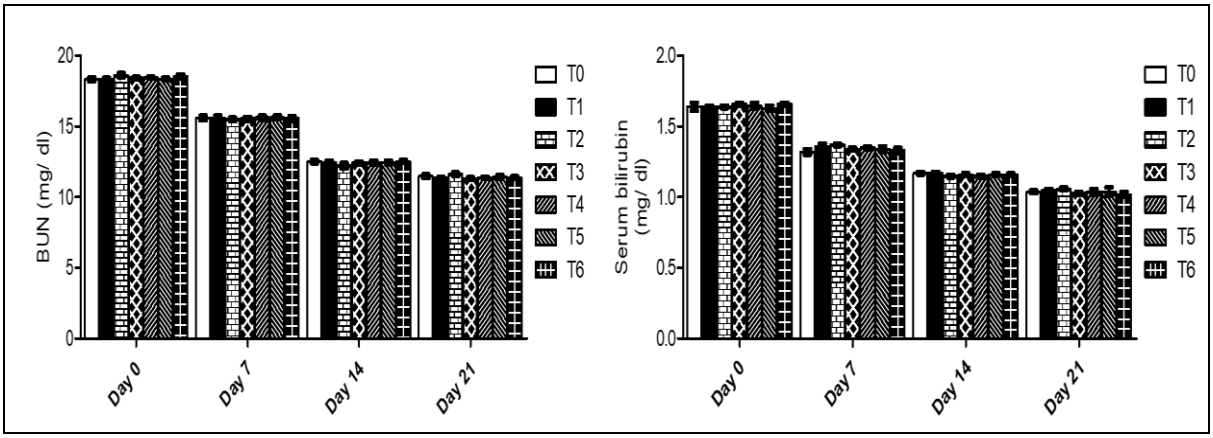

Fig 3: The concentration of BUN and serum bilirubin in herbal anti-parasitic with or without adjunct liver stimulant.

Table 1: Mean±SE of hematological and biochemical parameters at different days of treatment

Parameters	Group	Days of treatment			
		Day 0	Day 7	Day 14	Day 21
Hb(g %)	T_0	8.35±0.09^{a}	8.97±0.06^{ab}	10.36±0.08^{c}	11.45±0.11^{d}
	T_1	8.41±0.05^{a}	9.11±0.11^{b}	10.61±0.08^{c}	11.38±0.08^{d}
	T_2	8.35±0.05^{a}	9.01±0.11^{b}	10.56±0.05^{c}	11.36±0.10^{d}
	T_3	8.41±0.05^{a}	9.01±0.11^{b}	10.36±0.07^{c}	11.38±0.08^{d}
	T_4	8.41±0.06^{a}	9.12±0.11^{b}	10.58±0.11^{c}	11.40±0.09^{d}
	T5	8.39±0.03^{a}	9.12±0.11^{b}	10.60±0.06^{c}	11.39±0.08^{d}
	T6	8.44±0.05^{a}	9.06±0.11^{b}	10.52±0.07^{c}	11.33±0.08^{d}
PCV (%)	T_0	18.73±0.56^{a}	18.60±0.61^{ab}	20.62±0.63^{abc}	21.62±0.79^{bd}
	T_1	19.05±0.56^{a}	18.60±0.61^{ab}	20.62±0.63^{abc}	21.62±0.79^{bd}
	T_2	20.05±0.91^{a}	18.60±0.61^{ab}	20.62±0.63^{ac}	23.45±1.35^{bd}
	T_3	19.10±0.97^{a}	19.60±0.97^{ab}	20.78±0.73^{ac}	24.28±1.06^{bd}
	T_4	19.55±0.84^{a}	19.43±0.58^{ab}	21.11±0.98^{abc}	23.45 ±1.35^{bd}
	T5	19.88±0.88^{a}	18.93±0.45^{ab}	20.92±0.42^{bc}	21.95±0.60^{cd}
	T6	20.05±0.91^{a}	19.10±0.49^{ab}	20.92±0.42^{abc}	21.95±20.42^{cd}
TEC (10^6/µl)	T_0	4.22±0.02^{a}	4.48±0.03^{b}	5.23±0.07^{c}	5.79±0.03^{d}
	T_1	4.28±0.02^{a}	4.50±0.05^{b}	5.27±0.06^{c}	5.84±0.02^{d}
	T_2	4.28±0.03^{a}	4.49±0.03^{b}	5.23±0.07^{c}	5.82±0.02^{d}
	T_3	4.28±0.03^{a}	4.57±0.02^{b}	5.38±0.10^{c}	5.86±0.03^{d}
	T_4	4.25±0.04^{a}	4.55±0.03^{b}	5.48±0.05^{c}	5.84±0.03^{d}
	T5	4.28±0.02^{a}	4.56±0.04^{b}	5.35±0.06^{c}	5.86±0.04^{d}
	T6	4.23±0.03^{a}	4.53±0.01^{b}	5.36±0.06^{c}	5.81±0.02^{d}
TLC (x10^3cm)	T_0	11.41±1.33^{a}	10.52±1.27^{ab}	9.32±0.71^{c}	7.92±0.92^{d}
	T_1	12.56±1.48^{a}	12.11±1.22^{ab}	8.87±1.01^{c}	8.03±0.92^{d}
	T_2	11.65±0.97^{a}	10.79±1.12^{ab}	7.79±1.41^{c}	7.68±1.56^{cd}
	T_3	10.89±1.27^{a}	10.77±1.48^{ab}	9.88±1.21^{bc}	8.99±1.41^{d}
	T_4	11.10±1.37^{a}	11.21±1.23^{ab}	10.56±1.27^{abc}	8.10±0.97^{d}
	T5	13.11±1.07^{a}	11.21±1.15^{b}	10.62±1.23^{abc}	8.22±1.41^{d}
	T6	12.1.0 ±0.01^{a}	11.65±1.62^{ab}	10.22±1.13^{abc}	7.99±1.03^{d}
Total Serum Protein (g/dl)	T_0	6.33±0.02^{a}	6.44±0.07^{ab}	6.46±0.02^{abc}	6.39±0.08^{abcd}
	T_1	6.39±0.03^{a}	6.62±0.13^{ab}	6.60±0.08^{abc}	6.39±0.08^{abcd}
	T_2	6.39±0.03^{a}	6.56±0.11^{ab}	6.59±0.06^{abc}	6.39±0.06^{abcd}
	T_3	6.39±0.04^{a}	6.57±0.11^{ab}	6.65±0.08^{ab}	6.38±0.08^{ad}
	T_4	6.37±0.06^{a}	6.62±0.13^{ab}	6.55±0.08^{abc}	6.39±0.08^{abcd}
	T5	6.37±0.03^{a}	6.61±0.12^{ab}	6.65±0.08^{ab}	6.37±0.09^{ad}
	T6	6.44±0.02^{a}	6.60±0.12^{ab}	6.58±0.07^{abc}	6.41±0/07^{abcd}
Serum Albumin: Globulin	T_0	0.93±0.01^{a}	1.01±0.01^{ab}	0.89±0.01^{ac}	1.18±0.03^{cd}
	T_1	0.99±0.01^{a}	1.04±0.01^{ab}	0.95±0.01^{ac}	1.10±0.02^{cd}
	T_2	1.00±0.01^{a}	1.04±0.01^{abc}	0.96±0.02^{ac}	1.09±0.03^{cd}
	T_3	1.00±0.004^{ab}	1.04±0.01^{ab}	0.96±0.01^{ac}	1.09±0.03^{cd}
	T_4	0.99±0.01^{a}	1.04±0.01	0.96±0.01^{ac}	1.08±0.02^{cd}
	T5	1.00±0.003^{ab}	1.03±0.01^{ab}	0.97±0.01^{ac}	1.07±0.02^{d}
	T6	0.98±0.01^{a}	0.96±0.01^{ab}	1.03±0.03^{ac}	1.18±0.03^{bd}
BUN (mg/ dl)	T_0	18.33±0.14^{a}	15.60±0.17^{b}	12.53±0.12^{c}	11.49±0.13^{d}
	T_1	18.35±0.11^{a}	15.60±0.18^{b}	12.46±0.13^{c}	11.36±0.09^{d}
	T_2	18.62±0.15^{a}	15.50±0.12^{b}	12.22±0.20^{c}	11.62±0.12^{d}
	T_3	18.44±0.08^{a}	15.55±0.15^{b}	12.43±0.11^{c}	11.32±0.09^{d}
	T_4	18.46±0.09^{a}	15.62±0.16^{b}	12.45±0.13^{c}	11.37±0.09^{d}
	T5	18.36±0.09^{a}	15.65±0.14^{b}	12.46±0.13^{c}	11.43±0.12^{d}
	T6	18.55±0.15^{a}	15.58±0.18^{b}	12.53±0.11^{c}	11.37±0.10^{d}
Serum bilirubin (mg/ dl)	T_0	1.64±0.03^{a}	1.32±0.02^{b}	1.17±0.01^{c}	1.04±0.01^{d}
	T_1	1.64±0.01^{a}	1.36±0.02^{b}	1.17±0.01^{c}	1.05±0.01^{d}
	T_2	1.64±0.01^{a}	1.37±0.01^{b}	1.15±0.01^{c}	1.06±0.02^{d}
	T_3	1.66±0.01^{a}	1.34±0.01^{b}	1.16±0.01^{c}	1.03±0.01^{d}
	T_4	1.65±0.02^{a}	1.35±0.01^{b}	1.15±0.01^{c}	1.04±0.02^{d}
	T5	1.63±0.02^{a}	1.34±0.02^{b}	1.16±0.01^{c}	1.04±0.03^{d}
	T6	1.66±0.01^{a}	1.33±0.02^{b}	1.16±0.01^{c}	1.02±0.02^{d}
Creatinine (mg/dl)	T_0	2.26±0.51^{a}	1.35±0.18^{ab}	1.51±0.21^{bc}	0.97±0.15^{bcd}
	T_1	2.23±0.39^{a}	1.26±0.13^{b}	1.54±0.22^{bc}	1.03±0.17^{bcd}
	T_2	2.36±0.38^{a}	1.54±0.20^{b}	1.66±0.10^{bc}	1.12±0.08^{bcd}
	T_3	2.28±0.12^{a}	1.89±0.18^{ab}	1.62±0.12^{bc}	1.23±0.14^{bcd}
	T_4	2.81±0.47^{a}	1.54±0.21^{b}	1.70±0.13^{bc}	0.91±0.11^{bcd}
	T5	2.50±0.21^{a}	1.42±0.18^{b}	1.47±0.21^{bc}	1.14±0.12^{bcd}
	T6	2.52±0.56^{a}	1.91±0.49^{ab}	1.62±0.36^{bc}	1.19±0.32^{bcd}
SGOT (IU/L)	T_0	141.18±9.58^{a}	129.54±11.21^{ab}	88.56±4.78^{c}	72.35±2.48^{cd}
	T_1	148.85±9.88^{a}	121.62±6.35^{b}	103.02±12.28^{bc}	68.41±2.86^{d}

	T_2	138.40±7.62^{a}	114.93±7.68^{b}	78.55±3.87^{c}	72.29±4.21^{cd}
	T_3	151.11±5.73^{a}	120.07±7.31^{b}	103.02±12.88^{bc}	73.69±4.12^{d}
	T_4	135.35±8.29^{a}	114.33±9.17^{b}	85.88±4.50^{c}	73.55±5.14^{cd}
	T5	133.07±10.97^{a}	118.35±5.45^{ab}	86.30±4.79^{c}	76.23±3.56^{cd}
	T6	156.65±5.07^{a}	114.06±8.45^{b}	83.98±4.44^{c}	73.20±4.41^{cd}
SGPT (IU/L)	T_0	69.01±1.53^{a}	57.09±3.40^{b}	55.46±2.82^{bc}	31.42±2.26^{d}
	T_1	60.57±4.19^{a}	55.46±2.82^{ab}	31.42±2.59^{c}	31.81±0.98^{cd}
	T_2	66.23±2.14^{a}	55.46±2.82^{b}	55.46±2.82^{bc}	35.13±3.96^{d}
	T_3	73.69±4.12^{a}	64.17±3.14^{ab}	48.25±5.62^{c}	33.18±2.10^{d}
	T_4	63.88±3.47^{a}	60.36±1.75^{ab}	41.69±4.23^{c}	36.98±4.57^{cd}
	T5	66.06±2.13^{a}	66.29±2.14^{ab}	55.46±2.82^{c}	28.09±1.80^{d}
	T6	69.87±4.36^{a}	63.88±3.47^{ab}	41.69±4.23^{c}	36.80±3.49^{cd}
ALP (IU/L)	T_0	16.36±2.49	11.23±3.11^{b}	8.34±2.57^{c}	5.23±1.17^{d}
	T_1	17.62±1.86^{a}	12.66±1.21^{b}	10.22±1.01^{c}	4.95±1.49^{d}
	T_2	16.97±1.48^{a}	11.21±1.22^{b}	7.77±1.11^{c}	7.14±1.07^{d}
	T_3	18.11±2.49^{a}	16.22±2.03^{b}	10.88±1.78^{c}	8.01±1.21^{d}
	T_4	14.98±1.27^{a}	10.68±1.22^{b}	10.21±1.49^{c}	7.98±1.42^{d}
	T5	16.77±0.88^{a}	14.82±0.32^{b}	8.88±1.21^{c}	6.98±1.41^{d}
	T6	15.87±1.41^{a}	14.12±1.23^{b}	10.42±1.25^{c}	7.11±0.98^{d}

Different superscripts (a, b, c & d) differ significantly at 5% level of significant or at $p<0.05$ (row wirse).

Table 2: Mean faecal egg count (EPG: eggs per gram of faeces) before and after treatment

Parameters	Group						
	T_0	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**
EPG before treatment	475±21.41	491.67±23.86	483.33±27.89	525.00±21.41	491.67±23.86	491.67±15.37	500.00±18.26
EPG after treatment	0	0	0	0	0	0	0

Conclusion

The improvement of hemato-biochemical profile was observed in all the groups supplemented with adjunct herbal liver stimulants. Nevertheless, significant reduction of SGPT (liver function test) was recorded with AV/SYL/12 indicating the beneficial effects of AV/SYL/12 during deworming. We did not find any negative effect on kidney function during medication. The endo-parasitic infestation of livestock in Assam is very common owing to its favorable geo climatic conditions. However, the Lakhimi cattle of Assam might have acquired some degree of adaptation to parasitic infestation as indicated by the outcome of the present study. The present study showed the beneficial effect of AV/SYL/12 liver stimulant compared to other liver stimulants with or without anti parasitic drug on SGPT on day 14 of the experiment.

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