



Effects of Dietary Silymarin and Selenium-yeast on Energy Metabolism, Growth, Hematological Indices and Immune Status of Barbari Goat during Cold Stress

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10.18805/IJAR.B-5356

ABSTRACT

Background: The aim of the present study was to assess the effects of silymarin (SM) and selenium-yeast (SY) on energy metabolism, growth, hematological indices and immune status of Barbari goat during cold stress.

Methods: Thirty two Barbari goats (age: 6-8 months) were selected from ILFC- II, SVPUAT, Meerut (Uttar Pradesh) India. Experimental animals were randomly divided into four groups ($n=8$) according to body weight and age. First group (CON/C) was kept as control group giving only basal diet, second group (T1 /SM600) supplemented with 600 mg Silymarin/kg DM, third group (T2 /SeY) supplemented with 0.3 mg Selenium as Se-Yeast/kg DM and fourth group (T3 /SM600+SeY) supplemented with 600 mg Silymarin + 0.3 mg Selenium as Se-Yeast/kg DM along with basal diet for 90 days of the experimental periods.

Result: Mean body weight gain (BWG) and dry matter intake (DMI) of the supplemented group was found significantly ($P<0.01$) more than control group where as feed conversion ratio (FCR) was lower ($P<0.01$) in the supplemented group. Plasma glucose concentration was found significantly ($P<0.01$) lower where as plasma concentration of NEFA had no effect in the supplemented group as compare to control group. Haemoglobin and total immunoglobulin level was found significantly ($P<0.01$) higher in supplemented group as compare to control. The results of this study indicated that supplementation of SM and SY have important role in improving growth performance, feed intake and immune status of Barbari goat during cold stress.

Key words: Body weight gain, Cold stress, Energy metabolism, Goat, Immunity.

INTRODUCTION

Goat plays an important role in the economy of the poor livestock owners in earning their livelihood and popularly known as "poor man's cow". Goat has a very valuable contribution for the small, marginal farmers and landless laborers especially in the developing countries. Extreme cold during winter season cause cold stress which has a negative impact on the growth, production and immune response of the animals. Some feed supplements from herbal source and also minerals are important in reducing the impact of cold stress in the goats.

Silymarin (SM) is a flavonoid obtained from a spiny herb, milk thistle (*Silybum marianum*) belongs to *Asteraceae* family. Milk thistle seeds contain approximately 60% SM. SM is composed of silybin A and B, isosilybin A and B, silychristin and silydianin (Anthony and Saleh 2012). SM has been used as medicinal purposed particularly in the liver and kidney dysfunctions. SM plays an important function in the cell membrane stabilization, scavenging free radicals, synthesizing functional proteins (Saeed *et al.*, 2017) and in improving immune status (Bagno *et al.*, 2021). SM also plays an important role in improving feed consumption, ADG and FCR in finishing pigs (Koo *et al.*, 2022).

Selenium (Se) is an essential trace element and an integral component of several antioxidant enzymes such

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How to cite this article: Maurya, P.K., Singh, R.K., Chandra, G., Ramakant, Gautam, S., Singh, M.K. and Kumar, P. (2024). Effects of Dietary Silymarin and Selenium-yeast on Energy Metabolism, Growth, Hematological Indices and Immune Status of Barbari Goat during Cold Stress. Indian Journal of Animal Research. 1-7. doi:10.18805/IJAR.B-5356

Submitted: 18-03-2024 **Accepted:** 07-05-2024 **Online:** 24-12-2024

as glutathione peroxidase. Se functions as antioxidants, prevents oxidative damages (Saha *et al.*, 2016) role in immune functions (Baum *et al.*, 2001), reducing lipid peroxidation and decreasing toxicity of other elements. Organic form of Se in the feed could be transferred to the muscle and organs, and has better bioavailability than inorganic Se in fattening lambs (Antunovic *et al.*, 2009). Selenium-yeast (SY) is the source of organic Se mainly selenomethionine which can be absorbed and retained more readily than inorganic Se (Sevcikova *et al.*, 2011). SY supplementation induces ruminal epithelial growth, improves ruminal fermentation pattern, and increases GSH-Px activity in ruminal epithelium in goats (Shahid *et al.*, 2020). Therefore the present study was conducted to assess the effects of SM and S Yon energy metabolism, growth, hematological indices and immune status of cold stressed Barbari goat.

MATERIALS AND METHODS

The experiment was conducted at ILFC- II, SVPUAT, Meerut, Uttar Pradesh, India. In Meerut highest temperature rises up to 45°C in summer and there is remarkable fall of temperature sometimes up to freezing point in winter.

Ethical approval

The research protocol was duly permitted by Institutional Animal Ethics Committee (IAEC), constituted under control of the committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (Approval no. IAEC/SVPUAT/2022/102).

Experimental animals

For the present study 32 Barbari goats of 6-8 month age group has been selected and randomly divided into four groups ($n=8$) according to age and body weight. First group (CON) was kept as control group giving only basal diet, second group (SM_{600}) supplemented with 600 mg SM/kg DM, third group (SeY) supplemented with 0.3 mg Se as SY/kg DM and fourth group ($SM_{600}+SeY$) supplemented with 600 mg SM+0.3 mg Se as SY/kg DM along with basal diet for 90 days of experimental periods. SY (purity 80%) was purchased from Alenit Chemicals LLP, Delhi. SY (2000 ppm) was provided by Chaitanya Agro-Biotech. Pvt. Ltd., 120/2 Laxmi Nagar, Malkapur, Buldana (Maharashtra) India. The computed and weighed doses of SM, SY alone or both mixed in small amount of concentrate and fed individually to each animal for 90 days of study period. Clean and fresh tap water was offered *ad-lib*.

Ambient temperature and relative humidity for the entire study period have been recorded and calculated mean values which is given in Table 1. Temperature-humidity index (THI) values were calculated as per the method described by LPHSI (1990). Accordingly the formula used was-

$$THI = DB - ([0.55 - 0.55 \times RH][DB - 58])$$

Where,

DB= Dry bulb temperature, in Fahrenheit.

RH= Relative humidity (%/100).

Growth parameters

Body weight (BW) and feed consumption was recorded before start of experiment and thereafter repeated at fortnightly intervals with digital electronic balance. Then body weight gain (BWG) and feed conversion ratio (FCR) was calculated as:

$$FCR = \frac{FC (g)}{BWG (g)}$$

Blood collection

About 5 ml blood was collected at 7.30 a.m. in the EDTA coated Vacutainer tube before feeding and watering aseptically from jugular vein with the help of sterilized needles at 0, 15, 30, 45, 60, 75 and 90 days of experimental feeding. The blood was used for the analysis of hemoglobin (Hb), packed cells volume (PCV), total leukocyte counts (TLC), neutrophils and lymphocytes. Blood plasma was further analyzed for glucose and non-esterified fatty acid (NEFA).

Blood biochemical variables

Plasma glucose concentration was estimated by O-toluidine method (Cooper and Mc Daniel 1970) using kits from ERBA diagnostics Mannheim, Germany. The NEFA was analyzed in plasma by modified copper soap extraction method (Shipe *et al.*, 1980).

Statistical analysis

Data was analyzed using MIXED Models of statistical software package SPSS version 20 (SPSS for Windows, V 20.0., SPSS, Inc., Chicago, IL, USA). The model was used to estimate the effects of SM and SY on energy metabolism, growth, hematological indices and immune status of Barbari goat during cold stress as follows:

$$Y_{ij} = \mu + T_i + F_j + (T \times F)_{ij} + e_{ij}$$

Where,

Y_{ij} = Dependent variable.

μ = Overall mean of a population.

T_i = Effect of the treatment (SM and SY) ($i= 1....4$).

F_j = Effect of days ($j= 1....7$).

$(T \times F)_{ij}$ = Effect of the interaction between effect of treatments and days.

e_{ij} = Random error.

RESULTS AND DISCUSSION

Meteorological variables

Fortnightly mean AT, RH and THI of 90 d of the study period during winter season are presented in the Table 1.

Growth performance

The result of growth performance; BW, BWG, DMI and FCR of Barbari goat during winter season are presented in the

Table 1: Fortnightly mean ambient temperature (AT), relative humidity (RH) and THI during experimental period.

Fortnight	1 st	2 nd	3 rd	4 th	5 th	6 th
AT (°C)	15.97±0.18	10.27±0.32	10.09±0.30	7.93±0.23	6.29±0.51	7.46±0.93
RH (%)	80.12±1.61	72.20±0.95	68.79±1.24	76.08±1.56	82.58±1.47	70.70±2.62
THI	60.98±0.29	52.15±0.52	61.91±0.25	56.73±0.63	52.24±0.75	57.24±0.86

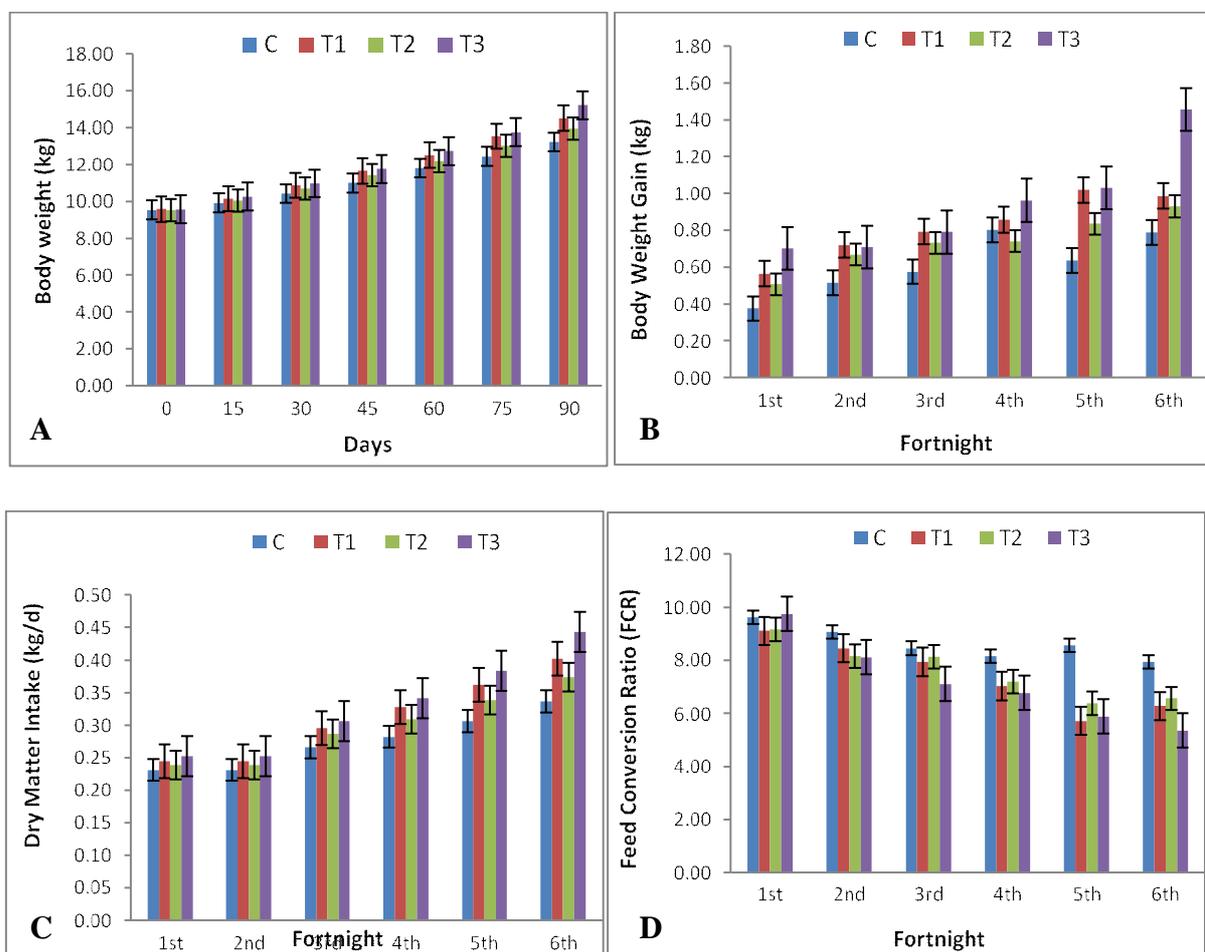
**Fig 1:** Effect of SM and SY supplementation on BW (A), BWG (B), DMI (C) and FCR (D).

Fig 1. BWG and DMI (kg/d) were observed significantly higher ($P < 0.05$) in the treatment groups fed SM, SY alone, or both in their diet compared to the non-supplemented group. Moreover, FCR was found significantly ($P < 0.05$) lower in SM₆₀₀, SeY and SM₆₀₀+ SeY groups as compare to CON group. In agreement with our findings Belvins *et al.*, (2010) reported that supplementation of SM @ 1000 mg/kg increased the BW and feed intake in broilers. Similar findings were also reported by Singh (2022) in Murrah buffalo calves supplemented with SM @ 600 mg/kg DM and Dubey (2022) in Sahiwal calves supplemented with SM @ 500 mg/kg DM. Abdalla *et al.* (2018) reported an increased BW of chickens supplemented with SM @ 25 g/kg of diet. Further, the supplementation of 100 and 200 g SM/kg feed increased BW and found higher BWG in fishes (Wang *et al.*, 2019). In

accordance with our findings, SM supplementation showed an improvement in BWG and FCR in finishing pigs (Koo *et al.*, 2022). This growth-promoting effect of SM might be attributed to its hepatoprotective and immune-enhancing properties. Moreover, it is possible to improve digestibility by changing intestinal microflora.

From the present study SeY also has role in growth performance in goats as Se can improve the antioxidant defense system being critically important for the ruminant's adaptation to nutritional stress (Surai *et al.*, 2019) and increase microbial count and improved fermentation rate in goat-rumen (Abbasi *et al.*, 2018). Further, dietary supplementation of SeY increases DMI and feed conversion in the animals as it improves fermentation pattern in rumen, induced ruminal epithelial growth and

Table 2: Effect of SM and SY supplementation on plasma glucose and NEFA level of cold stressed Barbari goat.

Variable	Days	Treatment				SEM	P- value		
		CON	SM ₆₀₀	SeY	SM ₆₀₀ +SeY		T	D	TxD
Glucose (mM/L)	0	2.98	2.94	2.97	2.94	0.13			
	15	3.23	2.84	3.11	3.09	0.18			
	30	3.44	3.22	3.47	3.05	0.17			
	45	3.41	3.29	3.51	3.29	0.21			
	60	3.34	3.25	3.48	3.17	0.16			
	75	3.52	3.13	3.52	2.99	0.14			
	90	3.46	3.11	3.50	3.11	0.17			
	Mean	3.34 ^b	3.11 ^a	3.37 ^b	3.09 ^a	0.17	0.033	0.062	0.998
NEFA (µM/L)	0	121.58	120.85	118.41	115.97	6.72			
	15	118.41	119.63	112.06	116.21	6.42			
	30	130.37	130.62	131.35	127.20	6.83			
	45	147.95	142.58	138.67	138.92	6.12			
	60	140.87	136.47	135.74	135.25	6.31			
	75	143.31	135.25	132.57	132.57	4.48			
	90	137.21	126.95	127.44	121.09	4.65			
	Mean	134.24	130.34	128.03	126.74	5.93	0.134	0.000	1.000

CON: control. SM₆₀₀: Silymarin (600 mg/kg DM/d) supplemented group. SeY: Se as selenium-yeast (0.3 mg/kg DM/d) supplemented group. SM₆₀₀+SeY: Silymarin (600 mg/kg DM/d) and Se as selenium-yeast (0.3 mg/kg DM/d) supplemented group. SEM: Standard error mean. T: Effect of treatment, D: Effect of day. TxD: Interaction between treatment and day. ^{a, b}Mean bearing different superscripts in a row showed a statistical difference at P<0.05. NEFA: Non-esterified fatty acid.

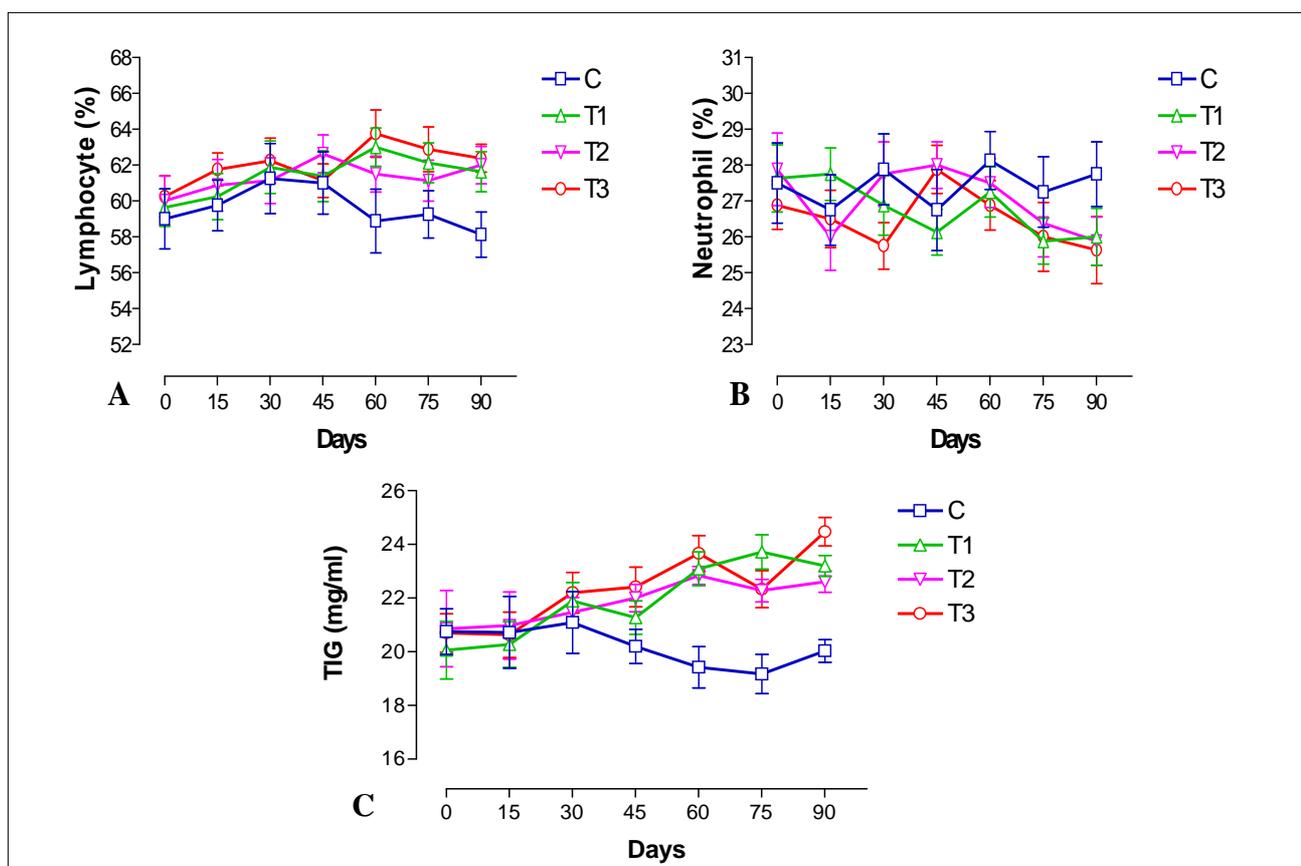


Fig 2: Effect of SM and SY supplementation on Lymphocyte (A), Neutrophil (B) and TIG (C) in cold stressed Barbari goat.

increased enzyme activities in ruminal epithelium of goats (Shahid *et al.*, 2020).

Energy metabolism

The effects of SM and SY supplementation on energy metabolism of Barbari goat during 90 days experimental period are presented in the Table 2. The mean concentration of plasma glucose was found significantly ($P < 0.05$) lower in SM₆₀₀ and SM₆₀₀+ SeY groups as compare to CON group. There was found relatively lower NEFA values in supplemented group but statistically non-significant ($P < 0.05$) variation in the mean values of NEFA among the groups. Similar findings were also reported by Singh (2022) in buffalo calves and Dubey (2022) in Sahiwal calves supplemented with SM. As per the other reports, SM supplementation decreased serum glucose levels in diabetic rats and human (Soto *et al.*, 2004) and in fishes supplemented with 400 mg SM/kg (Banaee *et al.*, 2011). Silymarin can have the ability to increase insulin sensitivity and reduce elevated insulin levels and may protect the pancreas from toxic effects (Mendelson 2008). In present study SeY supplementation increases plasma glucose

level but no significant difference between the groups whereas Shi *et al.* (2018) reported significant increase in the plasma glucose level in Taihang Black Goats supplemented with different level of Se-enriched yeast.

Immune status

The results of SM and SY supplementation on immune status of Barbari goat during 90 days experimental period are presented in the Fig 2. In the present study blood concentrations of lymphocyte and TIG were observed significantly ($P < 0.05$) higher in the treatment groups as compare to control group. Whereas, no significant ($P < 0.05$) difference in the neutrophils (%) in supplemented groups as compare to control group. No effect of SM supplementation on neutrophils in fish was observed by Ahmadi *et al.* (2012) is consistent with the present findings. Samadi *et al.* (2017) reported that SM increased the concentrations of IgG and total antibodies in the blood of Japanese quails. Bagno *et al.* (2021) reported milk thistle feeding improves immune status in broilers. Long-term administration of SM could improve the immune response by increasing the production of T-lymphocytes (Das *et al.*,

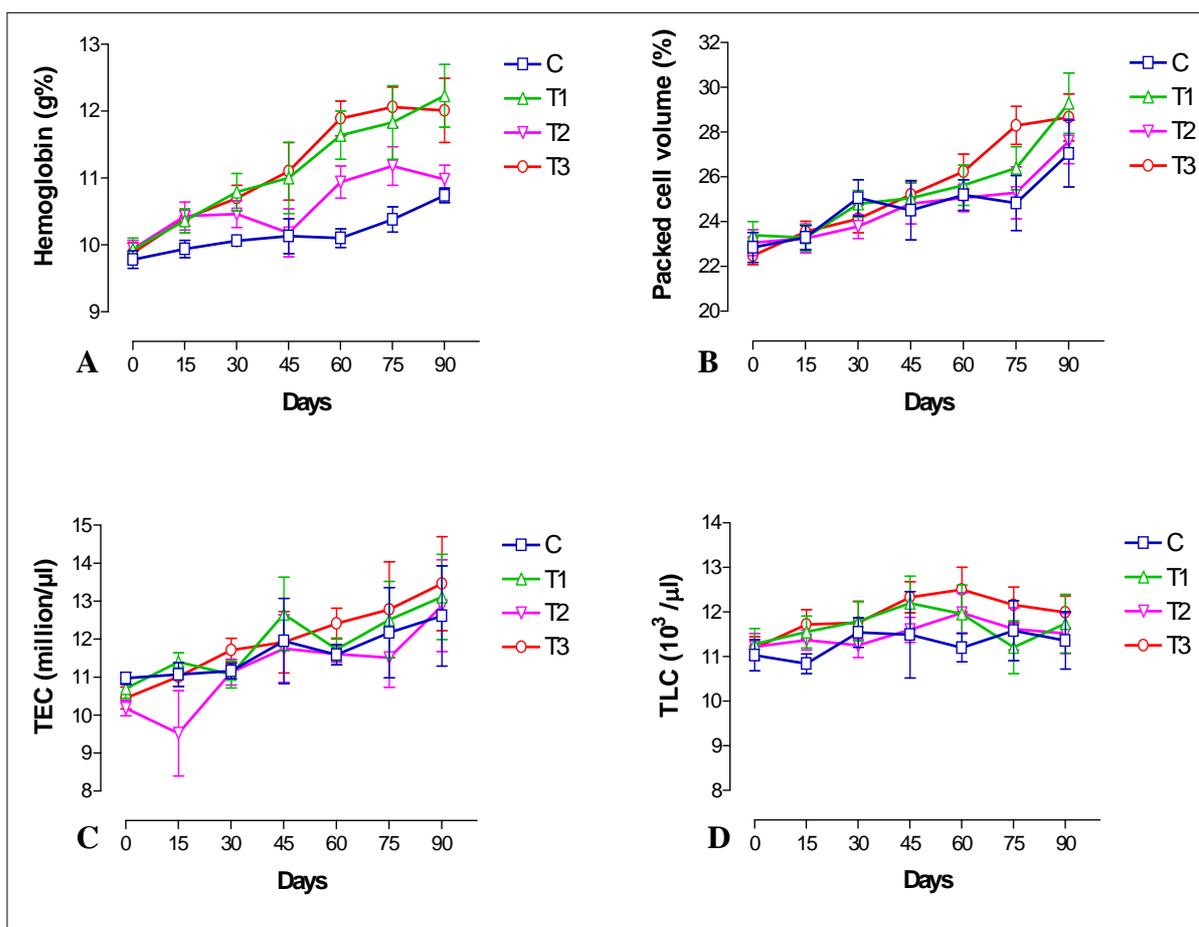


Fig 3: Effect of SM and SY supplementation on Hb (A), PCV (B), TEC (C) and TLC (D) in cold stressed Barbari goat.

2008) and TIG (Ghanem *et al.*, 2022) is accordant with the present findings.

From the present study SeY improves immune status in the experimental goats as Se plays an important role in the functioning of the immune system and prevents viral infections and increases immunity (Baum *et al.*, 2001) also slows the ageing process and inhibits the development of cancer (Ip and Dong, 2001). Se is essential for the efficient and effective functioning of the immune system in both humans and animals (Arthur *et al.*, 2003).

Hematological indices

The results of hematological indices are presented in the Fig 3. The mean values of Hb were found significantly ($P < 0.05$) greater in SM₆₀₀, SeY and SM₆₀₀+SeY groups as compare to CON group. There was found no statistical ($P < 0.05$) difference in the mean values of TEC and PCV among the groups. These values were found within the normal physiological range. The concentration of TLC increased in the supplemented group but observed no significant ($P < 0.05$) difference among the groups.

SM might improve the function of hematopoietic organs and their production of blood cells. In conjunction with our findings some experiments in laboratory animals have shown the therapeutic effect of milk thistle in prevention of hemolysis of erythrocytes (Zou *et al.*, 2001) and leucocytes (Locher *et al.*, 1998). SM supplementation increases TEC, PCV, Hb and TLC in nickel hematotoxicity and nephrotoxicity male albino wistar rats (Bouhalit *et al.*, 2017) and also found protective role of SM against ochratoxinosis in laying hen (Eid *et al.*, 2021). In the present study there was no effect of SeY supplementation on Hb, PCV and TEC whereas TIG concentration and lymphocyte (%) increases. Similar findings were also reported by Shi *et al.* (2018) in Taihang Black Goats supplemented with different level of SY.

CONCLUSION

The results of this study indicated that supplementation of SM and SY have important role in improving growth performance, feed intake and immune status along with improved health status of the animals during cold stress.

ACKNOWLEDGEMENT

The authors express their gratitude to Hon'ble Vice-Chancellor, SVPUAT, Meerut for providing research facilities for this study and Chaitanya Agro-Biotech. Pvt. Ltd., Malkapur, Buldana for providing selenium yeast for the experiment.

Conflict of interest

The authors declare that there is no conflict of interest for this manuscript.

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