Original Research Article

Comparative screening of elite bivoltine silkworm breeds under summerconditions of Kashmir

ABSTRACT

The success of the sericulture industry largely depends upon the quality of mulberry leaf and type of silkworm breed reared on these leaves. The rearing of season specific silkworm breeds can be instrumental in the successful rearing which will yield good cocoon crop and thereby leading to enhanced silk production. Data with respect to eleven economic parameters *viz.*, fecundity, hatching percentage, larvalweight, fifth instar larval duration, total larval duration, cocoon yield by number, cocoon yield by weight, single shell weight, single cocoon weigh, shell ratio and pupation rate was recorded, analysed statistically and subjected to multiple trait evaluation index. Significant differences were recorded in economic parameters but no specific silkworm breed performed best in all the economic parameters studied except SK-6 which performed best for most of the economic parameters, SK-1 **and** SK-28 showed best performance with respect to single cocoon weight and fecundity respectively. Out of nineteen silkworm breeds only nine breeds *viz.*, SK-6, SK-28, SK-30, KSO, SK-22, SK-24, SK-1, SK-13 and CSR-4 were found promising with average evaluation index of 66.7, 58.8, 57.2, 56.6, 55.5, 54.5, 53.1, 52.6 and 50.7 respectively. These breeds could be utilized in appropriate combination in evolution of new breeds/ hybrids for the region.

Keywords: breeds, hybrids, phenotypic, bivoltine, silkworm, cocoon

INTRODUCTION

Sericulture is both an art and science of raising silkworms for silk production. It is a farm based, labour intensive and commercially attractive economic activity falling under the cottage and small-scale sector. It provides income and employment to the rural people, especially the farmers with small land-holding and weaker sections of the society and is the major economic resource in countries such as China, India, Vietnam and Thailand. Sericulture in India is practiced predominantly in tropical environmental regions such as Karnataka, Tamil Nadu, Andhra Pradesh and West Bengal and to a limited extent in temperate environment of Jammu and Kashmir. Indian silk industry has registered a phenomenal growth over the years and presently is accounting for more than 18% of the global silk production and is the second largest producer of raw silk after China and the biggest consumer of raw silk. In J&K, though there is scope for taking two to three crops but only one crop is taken on large scale during spring, the production is below the production potential in both seasons especially in summer rearing. Attempts have been made to popularize the second silkworm rearing (Malik *et al.,* 2006)but the non-availability of diversified silkworm genetic resources came in way of achieving the goals. Sericulturally advanced countries like Japan and China have achieved a remarkable breakthrough in increasing the unit production of silk by evolving highly productive bivoltine silkworm breeds/hybrids suitable to their local conditions and agronomical practices. So, Silkworm breed is an influential factor in the development of sericulture and is evident that the production of good quality raw silk is based primarily on quality cocoons which in turn depend mainly on the superior silkworm breeds/hybrids. The selection of breeding resource material helps the breeder to successfully amalgamate the desired traits in the breed. An appropriate experimental design that includes selection methods employed over majority of economic traits contributing to the quality oriented quantitative productivity may lead to the success of any breeding programme. Breeding programme aimed at breed improvement has generally relied on the use of established breeds or elite lines.Therefore, it is imperative to evaluate diverse genotypes and to identify the distinct and different gene pools existing in a group of resource materials to be utilized in breeding programme by systematic evaluation. Since the genetic improvement of multiple traits being the objective of evolving the productive bivoltine hybrids, many silkworm breeders (Thaigrajan*et al.,* 1993; Kamilli, 1996; Rao *et al.,* 2000; Begum *et al.,* 2001; Malik *et al.,* 2006) followed specific methods to identify the suitable breeding material for developing summer specific silkworm hybrids. Jammu and Kashmir is the only traditional bivoltine belt in the countrybecause of salubrious climatic conditions for silkworm rearing and quality mulberry varieties can produce qualitysilk (Islam *et al.*, 2022a, 2022b; Islam, 2023; Islam *et al.*, 2023a; Islam *et al.*, 2024), but lack of season specific silkworm breeds/hybrids suited to agro-climatic conditions has been identified as one of the major constraints in boosting cocoon production (Trag*et al.*, 1992). Realizing this, some spring specific silkworm breeds were evolved by the Sericulture Institute of Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir by utilizing the genetic variability of existing and exotic germplasm resources (Kamili *et al.*, 2000). Season and region specific especially summer specific studies of silkworm *Bombyx mori* L. are of greater importance in identifying and understanding the adaptability of silkworm genotypes which are largely influenced by climatic factors (Vijayalakshmi *et al.*, 2014). Summer breeds are having significant importance in increasing cocoon production through rearing the hybrids in harsh summer. The advantages of rearing of summer silkworm breeds/hybrids are high pupation rate, adaptabilities to bad environmental conditions and inferior food quality during rearing. Stable cocooncrop under such conditions is difficult but summer specific breeds/hybrids have the potentiality for increasing the production under such unfavorable condition. Therefore, it is highly pertinent to**identify**robust breeds which can withstand the summer climatic conditions of Kashmir and accordingly the present research work was undertaken to evaluate the different silkworm breeds under Kashmir conditions for different economic parameters.

MATERIALS AND METHODS

Nineteen silkworm breeds of diverse geographical origin *viz.*, SK-1, SK-3, SK-6, SK-13, SK-22, SK-24, SK-28, SK-30, CSR-2, CSR-4,CSR-6, CSR-18, CSR-19, CSR-26, KSO, KH-Badami, M-106, DUN-6 and DUN-22 formed basis for the present study. The disease free layings of these selected breeds were obtained from the germplasm bank maintained at College of Temperate Sericulture, Mirgund, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K) and Central Sericulture Research and Training Institute (CSR&TI), Central Silk Board (Govt. of India), Pampore. The rearing was conducted at Silkworm Breeding and Genetics Section of College of Temperate Sericulture, Mirgund, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST Kashmir). The disease free layings of these breeds were incubated, brushed and reared using standard rearing method (**Krishnaswami, 1978**;Anonymous, 2003) during summer, July August, 2015. The experiment was laid out in a Completely Randomized Design (CRD) with three replications for each treatment comprised of 200 larvae (after third moult). The data pertaining to eleven metric traits *viz.,* fecundity, hatching percentage, average larval weight, fifth instar larval duration, total larval duration, cocoon yield/10,000 larvae by number, cocoon yield/10,000 larvae by weight, single cocoon weight, single shell weight, shell ratio and pupation rate were recorded and analyzed statistically and also subjected to multiple trait evaluation index and cluster analysis to come out with top performing breeds and different groups respectively. The Data on the economically important traits were collected, pooled and analyzed using R-software (3.2.1). The Evaluation Index (EI) was calculated as per the procedure outlined by Mano *et al.* (1993).

$$\text{Evaluation index} = \frac{A - B}{C} \times 10+50$$

Where, A= Value obtained for a particular trait of a particular breed/hybrid combination.

B= Mean values of a particular trait of all the breed/hybrid combinations.

C= Stand deviation of a particular trait of all the breed/hybrid combinations.

10= Stand Unit

50= Fixed Value

The E.I. value fixed for the selection of breed/hybrid is 50 or >50 for positive traits and 50 or <50 for negative traits. The breed/hybrid, which scored above the limit, is considered to possess greater economic value. The salient features of nineteen silkworm breeds of diverse geographical origin are given in Table-1. The following parameters were recorded during the research study:

Fecundity(no.):Average of eggs in three disease free layings.

Hatchingpercentage(%):Percentage of eggs hatched.

Averagelarvalweight(g):The weight of ten randomly picked mature larvae measured on the fifth day of the fish instar.

Fifthinstarlarvalduration(hours):Totaltimetakenfrom1st day of5thinstartodateof mounting.

Totallarvalduration(hours):Totaltimetakenfrom thedayofbrushingtomounting.

Cocoonyieldbynumber:

Thiswascalculatedbythefollowingformula:

$$= \frac{\text{No. of cocoons harvested}}{\text{No. of worms retained after } 3^{rd} \text{moult}} \times 10000$$

Cocoonyieldbyweight(kg)

Thiswascalculatedbythefollowingformula:

$$= \frac{\text{Wt.ofcocoonsharvested}}{\text{No. of worms retained after } 3^{rd}\text{moult}} \times 10000$$

Pupationrate(%):

Thiswascalculatedas:

$$= \frac{\text{Number of live pupae obtained from cocoons}}{\text{Total no. of cocoons harvested}} \times 100$$

Singlecocoonweight(g):

Average weight of twenty randomly selected male and female cocoons.

Singleshellweight(g):

Average weight of twenty randomly selected male and female cocoon shells.

Shellratio(%):

Itwascalculatedbythefollowingformula:

$$= \frac{\text{Single shell weight}}{\text{Single cocoon weight}} \times 100$$

RESULTS AND DISCUSSION

Table 2 presents the evaluation index values of 19 breeds across 11 metric traits.The silkworm breeds performance recorded for fecundity in order of merit were SK-28 (535), SK-30 (529), SK-6 (524), KSO (515), SK-13 (510), SK-3 (503), DUN-6 (498), SK-22 (495), CSR-2 (478), DUN-22 (475), CSR-4 (472), CSR-6 (471), SK-1 (468), CSR-26 (462), KH-Badami (459), SK-24 (457), CSR 18 (429), CSR-19 (425), and M-106 (420). Significant differences were observed in fecundity with respect to **breeds** being highest in SK-28 and lowest in M-106 (Table-2).The silkworm breeds performance recorded for hatchingpercentage in order of merit were SK-6 (94.00), SK-28 (90.24), SK-24 (88.35), SK-22 (87.33), KSO (86.83), SK-13 (86.64), SK-30 (82.33), CSR-4 (81.43), CSR-2 (81.36), DUN-6 (80.66), CSR-26 (79.26), KH-Badami (78.81), DUN-22 (77.31), SK-1 (77.00), CSR-6 (76.63), M-106 (76.58), SK-3 (76.00), CSR-19 (72.93) and CSR-18 (72.00). Significant differences were observed in hatching percentage being highest in SK-6 and lowest in CSR-18 (Table-2).The silkworm breeds performance recorded for average larval weight in order of merit were SK-6 (42.70), SK-30 (41.16), SK-24 (41.13), SK-28

(40.60), SK-13 (40.36), KSO (40.12), CSR-4 (39.76), SK-22 (39.57), CSR-2 (39.20), DUN-6 (39), DUN-22 (38.77), SK-1 (38.60), KH-Badami (38.20), CSR-26 (37.20), M-106 (37.10), SK-3 (36.80), CSR-19 (36.76), CSR-18 (35.33) and CSR-6 (35.32) (Table-2). Significant differences were observed in fifth instar larval duration with respect to treatments/breeds being highest in SK- 6 and lowest in CSR-6.The silkworm breeds performance recorded for fifth instar larval duration (hrs) in order of merit were SK-6 (192), CSR-4 (192), CSR-26 (194), CSR-6 (195), KSO (196), CSR-2 (196), DUN-6 (196), SK-24 (198), KH-Badami (198), SK-1 (200), SK-28 (202), SK-30 (202), M-106 (202), SK-22 (204), SK-13 (204), DUN-22 (204), SK-3 (204), CSR-18 (210) and CSR-19 (218). Significant differences were observed in fifth instar larval duration being lowest in SK- 6 and highest in CSR-19 (Table-2).The silkworm breeds performance recorded for total larval duration (hrs) in order of merit were SK-6 (672), CSR-26 (674), CSR-6 (675), KSO (676), SK-28 (676), SK-1 (676), DUN-6 (676), SK-22 (678), SK-13 (678), KH-Badami (678), SK-3 (678), SK-24 (678.66), M-106 (682), DUN-22 (682), SK-30 (685.33), CSR-18 (686), CSR-2 (686), CSR-4 (686) and CSR-19 (698). **Larval duration of fifth instar larvae, and the total larval duration, were similar, lowest in SK-6 and longest in CSR-19**(Table-2). The silkworm breeds performance recorded for cocoon yield by number in order of merit were SK-6 (8914), SK-30 (8780), SK-28 (8687), SK-24 (8557), KSO (8398), SK-13 (8193), DUN-6 (8112), SK-22 (8039), KH-Badami (7921), CSR-6 (7866), CSR-2 (7829), CSR-4 (7778), SK-1 (7736), DUN-22 (7467), CSR-26 (7330), CSR-18 (7283), SK-3 (7097), CSR-19 (6999) and M-106 (6769). Cocoon yield by number showed significant difference among the treatments being highest in SK- 6 and lowest in M-106 (Table-2).The silkworm breeds performance recorded for Cocoon yield by weight in order of merit were SK-6 (15.75), SK-30 (14.94), KH-Badami (14.85), SK-24 (14.68), SK-28 (14.43), KSO (14.19), CSR-2 (13.93), DUN-6 (13.83), CSR-6 (13.76), M-106 (13.65), CSR-4 (13.35), DUN-22 (13.11), SK-22 (13.02), CSR 26 (12.87), SK-13 (12.71), SK-1 (11.80), SK-3 (11.76), CSR-18 (11.24) and CSR-19 (10.67). Cocoon yield by weight again showed significant differences among the treatments being highest in SK- 6 and lowest in CSR-19 (Table-2).The silkworm breeds performance recorded for pupation rate (%) in order of merit were SK-6 (92.30), SK-28 (91.30), SK-30 (87.30), SK-22 (86.30), KSO

(85.70), SK-13 (85.00), CSR-4 (83.00), DUN-22 (82.70), SK-24 (82.00), SK-1 (81.00), DUN-6 (80.30), CSR-2 (80.00), KH-Badami (79.00), CSR-6 (77.30), CSR-19 (76.00), CSR-18 (74.30), SK-3 (74.00), CSR-26 (71.00) and M-106 (71.00). Significant differences were observed in pupation rate being highest in SK- 6 and lowest in M-106 (Table-2).The silkworm breeds performance recorded for single cocoon weight in order of merit were SK-1 (1.87), SK-6 (1.86), CSR-6 (1.77), KSO (1.74), SK-24 (1.71), CSR-18 (1.71), SK-22 (1.71), CSR-26 (1.69), CSR-4 (1.67), SK-28 (1.66), SK-30 (1.65), M-106 (1.65), SK-13 (1.63), CSR-19 (1.63), SK-3 (1.60), KH-Badami (1.59), DUN-6 (1.54), CSR-2 (1.47) and DUN-22 (1.38). Single cocoon weight showed significant differences among the treatments/breeds being highest in SK- 6 and lowest in DUN-22 (Table-2).ThesilkwormbreedsperformancerecordedforsingleshellweightinorderofmeritwereSK-6(0.40),SK-1(0.39),SK-22(0.34),CSR-6(0.34),KSO (0.32),CSR-26(0.32),SK-28(0.31),SK-24(0.31),SK-30(0.31),SK-3(0.30), CSR-4(0.29),SK-13(0.28),DUN-6(0.27),CSR-19(0.27),CSR-18(0.25),M-106(0.25),KH-Badami(0.24),DUN-22(0.20)andCSR-2(0.19).Significant differenceswerealsoobservedinsingleshellweightbeinghighestinSK-6andlowestinCSR-2(Table-2).The silkworm breeds performance recorded for shell ratio in order of meritwereSK-6(21.50),SK-1(20.85),SK-22(19.58),CSR-6(19.20),CSR-26 (18.93),SK-30(18.78),SK-3(18.75),SK-28(18.67),KSO(18.39),SK-24 (18.12),DUN-6(17.53),CSR-4(17.36),SK-13(17.17),CSR-19(16.56),M-106 (15.15),KH-Badami(15.09),CSR-18(14.61),DUN-22(14.49)andCSR-2 (12.92).Shellratioalsoshowedsignificantdifferencesamongthetreatments/breedsbeinghighest inSK-6andlowestinCSR-2(Table-2).Evaluation of genetic resources promotes effective and higher utilization of the germplasm, particularly in breeding and crop improvement programme. As the goal of breeding changes rapidly, the evaluation needs to be adaptive. Evaluation of genetic resources is the most important aspect of germplasm management, which determines the use of genotypes in various breeding programmes of race improvement. The germplasm stocks can be utilized for direct utilization as local breeds or as a parent material, whereas the international need focuses towards germplasm systems that emphasize the use and employment of materials rather than mere acquisition and storage. Improvement of silk productivity depends on the magnitude of genetic variability and the extent to which the associated traits are heritable in silkworm. Among the nineteen breeds studied SK-6 showed best performance for nine parameters *viz.,* hatching percentage, average larval weight, larval duration (hrs), cocoon yield by number, cocoon yield by weight, single cocoon weight, single shell weight, shell ratio and pupation rate whereas SK-28 and SK-1 performed best for fecundity and single cocoon weight respectively. The present study clearly establishes the superiority of these breeds over the rest, which is due to the superior genetic constitution of these breeds and their buffering ability towards summer environmental conditions. These breeds have immense potential as breeding material as they displayed variability for various economic traits, higher the variability more scope for selection for segregants. The present study corroborates the observations of Harada (1961) for cocoon shell weight followed by cocoon weight and survival rate, Subbha Rao and Sahai (1989) for cocoon yield followed by cocoon weight. Cocoon yield being a complex trait is contributed by more than 21 components (Thaigrajan*et al.,* 1993). Hence identification of robust breed/hybrid calls for consideration of cumulative effects of all the component traits on silk yield whether acting in a positive or negative direction. Judging the superiority of the silkworm breed on the basis of its performance in individual traits cannot lead to fruitful results, a common index method was found very much essential (Bhargava *et al.,* 1994). The evaluation index (E.I) method developed by Mano *et al.* (1993) was found to be very useful in selecting potential parents/hybrids in silkworm breeding programme. Many**researchers**(Kumaresan *et al.,* 2000; Rao *et al.,* 2006; Moorthy *et al.,* 2007; Rayar, 2007) used this evaluation index method for selecting potential hybrid. The selection of the breeds/hybrids ultimately depends on the excellence and performance of breeds/hybrids in many individual traits. In present study the multiple trait evaluation index method was employed giving equal weightage to economic traits *viz.,* fecundity, hatching%, average larval weight, fifth instar larval duration, total larval duration, cocoon yield by number, cocoon yield by weight, single shell weight, single cocoon weigh, shell ratio and pupation rate, only nine breeds scored evaluation index above 50. Based on the evaluation index values obtained for nine positive characters (higher the values, better the economic viability i.e. fecundity, hatching %, average larval weight, cocoon yield by number, cocoon yield by weight, single cocoon weight, single shell weight, shell ratio and pupation rate) and two negative characters (lesser the values, better the economic viability i.e. 5th age larval duration and total larval duration), top nine ranking breeds *viz.*, SK-6, SK-28, SK-30, KSO, SK-22, SK-24, SK-1, SK-13 and CSR4 were identified based on the higher average index values (>50) obtained for positive characters and lower index value (<50) obtained for negative characters. Significant differences were recorded in all the eleven economic parameters, however no specific silkworm breed performed best in all the eleven economic parameters except SK-6 which performed best for most of the economic parameters and SK-1 and SK-28 showed best performance with respect to single cocoon weight and fecundity respectively.

Table 1: Silkworm breeds and their salient features

Treatment	Silkworm genotype	Larval marking	Cocoon shape	Origin	Parentage
T1	SK-1	Marked	Constricted	TSRI, SKUAST-K, Mirgund, Kashmir	Shunerei×Shogetsi
T2	SK-3	Marked	Dumb Bell	-do-	Shunerei×Shogetsi
T3	SK-6	Plain	Oval	-do-	Shogetso×Hoshu
T4	SK-13	Marked	Constricted	-do-	Foyo×Tokai
T5	SK-22	Plain	Oval	-do-	Jam104×Jam103
T6	SK-24	Plain	Oval	-do-	Kinsko×Showa
T7	SK-28	Marked	Constricted	-do-	Evolved Under Broad Based Germplasm Complex, Comprising 10 Breeds With Marked Larvae (Kamili *et. al.*, 2000)
T8	SK-30	Plain	Oval	-do-	Evolved Under Broad Based Germplasm Complex, Comprising 10 Breeds With Marked Larvae (Kamili *et. al.*, 2000)
T9	KH-Badami	Marked	Constricted	-do-	
T10	M-106	Marked	Constricted	-do-	
T11	KSO	Plain	Oval	KSSRDI, Karnataka	
T12	CSR-2	Plain	Oval	CSRTI, Mysore	Shunrei×Shogetsu
T13	CSR-4	Plain	Dumb Bell	-do-	(BN18×BCS25)×NB4D2
T14	CSR-6	Marked	Oval	-do-	Shunrei×Shogetsu
T15	CSR-18	Mixed	Dumb Bell	-do-	B201×BCS12
T16	CSR-19	Mixed	Dumb Bell	-do-	B201×BCS12
T17	CSR-26	Marked	Constricted	-do-	C135×N134
T18	DUN-6	Plain	Oval	Dehradun, Uttarakhand	CC1 x NN6D

T19	DUN-22	Marked	Constricted	Dehradun, Uttarakhand	(KS x NB4D2) x (AT x NB4D2

Table2:Evaluationindexvaluesofdifferenteco-geographicsilkwormbreeds

Treatment	Average larvalweight	5^{th}instar larvalduration	Totallarvalduration	Cocoon yield bynumber	Cocoon yield byweight	Singlecocoonweight	Singleshellweight	Shellratio	Pupation rate	Averageei Values	Rank	Fecundity	Hatching
SK1	48.9	49.4	43.3	51.5	38.1	67.9	68.4	63.9	49.8	53.1	7	46.4	42.8
SK3	39.9	55.6	46.6	47.7	37.8	44.9	51.7	54.9	38.4	45.9	14	56.7	41.2
SK6	69.2	36.9	36.8	58.4	67.6	67.0	70.2	66.4	68.3	66.7	1	62.7	70.9
SK13	57.6	55.6	46.6	54.1	44.9	47.5	48.0	48.2	56.4	52.6	8	58.5	58.8
SK22	53.6	55.6	46.6	53.2	47.2	54.3	59.1	59.7	58.5	55.5	5	54.3	59.9
SK24	61.4	46.3	47.7	51.3	59.6	53.4	53.6	54.4	51.5	54.5	6	43.2	61.5
SK28	58.8	52.5	43.3	57.0	57.7	50.0	53.6	54.8	66.7	58.8	2	65.8	64.7
SK30	61.6	52.5	58.6	57.6	61.5	49.2	53.6	55.2	60.1	57.2	3	64.2	51.6
M-106	41.4	52.5	53.0	45.8	51.9	49.2	42.5	39.2	33.6	42.0	17	32.5	42.1
KH-Badami	46.9	46.3	47.0	52.5	60.8	44.1	40.6	39.0	46.6	46.7	13	43.9	45.9
CSR2	51.9	43.2	59.7	52.0	54.0	33.9	31.3	29.3	48.2	44.2	15	49.2	50.0
CSR4	54.6	36.9	59.7	51.7	49.7	50.9	49.9	49.0	53.1	50.7	9	47.7	50.1
CSR6	32.7	41.6	41.7	52.2	52.7	59.4	48.0	55.6	43.9	48.2	11	47.3	42.2
CSR18	32.7	64.9	59.7	48.8	33.9	54.3	42.4	37.1	39.0	39.7	19	35.0	34.6
CSR19	39.7	77.5	79.0	47.2	29.7	47.5	46.2	45.4	42.8	40.9	18	34.1	36.1
CSR26	41.9	40.1	40.0	49.1	46.1	52.6	55.5	55.7	33.6	47.2	12	44.6	46.5
KSO	56.4	43.2	43.0	55.3	55.9	56.9	55.5	54.6	57.4	56.6	4	60.1	59.0
DUN6	50.8	43.2	43.0	53.7	53.2	39.9	46.2	49.6	48.8	49.5	10	55.1	48.9
DUN22	49.7	55.7	53.0	49.9	47.9	26.3	33.2	36.5	52.5	43.1	16	48.6	43.3

CONCLUSION

The superior silkworm breeds attain significance as it is crucial for the production of bumper silk production apart from the nutritious mulberry varieties. Significantdifferenceswererecordedinalltheeleveneconomic parameters,howevernospecificsilkwormbreedperformedbestinall the eleven economic parameters except SK-6 which performed best formost of the economic parameters andSK-1andSK-28showedbestperformancewithrespecttosinglecocoonweightandfecundityrespectively. Now based on the criteria of evaluationindex SK-6, SK-1andSK-28 excelled in most of the parameters and can be recommended for the silkworm rearing and development of new hybrids for the farmers for enhancing the raw silk production.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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