Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

JAS

Use of Aquatic Plants (*Azolla Caroliniana* and *Lemna Spp*) as a Feed Source in Silkworm Culture

Süleyman Bekcan[a*], Hicran Yavuzcan[a], Hasan Hüseyin Atar[a]

[a]*Ankara University, Faculty of Agriculture, Department of Fisheries Engineering, Ankara, TÜRKIYE*

ARTICLE INFO
Research Article
Corresponding Author: Süleyman Bekcan, E-mail: bekcan@agri.ankara.edu.tr
Received: 15 October 2024 / Revised: 01 October 2024 / Accepted: 18 December 2024 / Online: 25 March 2025

Cite this article
Bekcan S, Yavuzcan H, Atar H H (2025). Use of Aquatic Plants (*Azolla Caroliniana* and *Lemna Spp*) as a Feed Source in Silkworm Culture. *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 31(2):532-537. DOI: 10.15832/ankutbd.1568035

ABSTRACT
This article focuses on the effects of the use of aquatic plants on the growth parameters of silkworm larvae in their artificial feeding. Due to the difficulty of obtaining mulberry leaves, different feeds and substances to be substituted for this food source were tested in various researches. In this study, the silkworm larvae were fed with the diet containing different proportion of azolla (*Azolla caroliniana)* meal and duckweed (*Lemna spp.)* meal found abundant and easily available compared to mulberry leaves in nature. The study aims at defining an appropriate combination of mulberry leaf meal, azolla meal and duckweed meal and the effects of these artificial feeds on the growth rate of silkworm larvae. Consequently, it was determined that 25%, 50%, 75% substitution of azolla meal and duckweed meal up to the 3rd instar (12th day) did not affect the growth and survival rates of silkworm larvae.

Keywords: Azolla meal, Duckweed meal, Mulberry leaves meal, Artificial feeding, Silkworm

1. Introduction

Aquatic plants have many potential benefit areas such as cheap food sources for human and animals and fertilizer due to their high nutritive value and short-term proliferation. The water fern azolla, in symbiosis with blue-green algae is remarkable with its easy cultivation, high productivity and nutrient-rich value (Singh & Subudhi 1978). The growth rate of different azolla species were researched in different environments and doubling time was observed as 3-7 days (Tran & Dao 1973; Talley et al. 1977). Azolla has been reported to contain 25-35% crude protein, 10-15% mineral, amino acids, 14.3% crude fibre (FAO 1989; Kathirvelan et al. 2015; Ara et al. 2015) and also vitamins (vitamin-A, vitamin B12, ß-Karoten), minerals, probiotics and biopolymers (Kamalasanana Pillai et al. 2005; Katayama et al. 2008; Kathirvelan et al. 2015). In addition, it has been shown that azolla in dried and fresh form is used as feed for fish (Gokcinar & Bekcan 2015), pork, chicken, duck, cattle and food source for human (FAO 1989; Indira et al. 2009; Leterme et al. 2010; Gauri-Mahadevappa et al. 2012; Kathirvelan et al. 2015; Rana et al. 2017).

Similar aquatic plant, duckweed includes up to 43% protein, 5% lipid, small amount of fibre and high amount of easily digestible dry matter (Leng et al. 1995; Saha et al. 1999). Duckweed as a natural protein source has a valuable amino acid profile similar to animal protein (Hillman & Culley 1978). It has potential using as a supplementary feed source in feeding of livestock, poultry and fish.

Another herbal product, mulberry leaf is the most important nutritional source in silkworm rearing. In last years, poor environmental conditions and the decrease of mulberry tree cultivation areas have negatively affected rearing of silkworm which is valuable product. These challenges in feeding silkworm lead to do further research on alternative nutrient sources. Due to the difficulty of finding mulberry leaves in the desired period in feeding silkworm, the researches have been recently concentrated on substituting easily available matters such as bovine milk, hen's eggs (Helaly 2018), royal jelly (Nguku et al. 2007) soybean, mushroom (Mahmoud 2013), soybean meal, corn meal (Pallavi et al. 2011) and Spirulina algae powder (Sahay et al. 2011) for mulberry leaves. Feeding materials have determinative effects on not only the developments of silkworm larvae but also the quality and productivity of cocoons in positive way. Besides the quality of cocoons and silk, these improvements reflect on by-products such as silkworm pupae (Ravikumar 1988; Seidavi et al. 2005). Indeed, silkworm pupae is also used as a valuable nutrient source for human (Yang et al. 2010) and animal such as fish (Radha & Geetha 2018) and poultry (Ijaiya & Eko 2009).

The aim of this research was to define an appropriate combination of the artificial feeds used in the trial and to determine their effects on the growth rate of silkworm larvae. For this reason, silkworm larvae were fed with the meals of mulberry leaf, azolla, duckweed and their different combinations. These mentioned aquatic plants are found abundant and easily available compare to mulberry leaf in nature. Thus, it was possible to conduct the research without considering the period of silkworm rearing dependent on mulberry leaves growing season either the plants growing season.

2. Material and Methods

A wooden frame of the size of 2 m x 2m x 0.2 m was covered by a tarpaulin. By preparing a mixture made of 20 kg flower soil, 3 kg cow dung and 40 g super phosphate fertilizer, this slurry was poured on the tarpaulin. Then water was poured into the mixture until total height reached to 20 cm. Azolla was inoculated in the environment. Growing azolla was harvested, dried in the shade and turned into meal. The same processes were also made for obtaining duckweed. The mulberry leaves collected in the season were dried in the shade and turned into meal. All feeds prepared in this way, except vitamins autoclaved at 121 °C for 15 minutes under 15 lbs pressure. The substitution rates of mulberry leaf and azolla meal in the artificial diet were determined to range from 0 to 20, 40, 60, and 80 g, corresponding to 0%, 25%, 50%, 75%, and 100%. The same replacement rates were also applied to mulberry leaf and duckweed meal. Nutrient elements were prepared as paste in the ratio of 1 g of dry matter and 2.6 g of pure water, then stored in glass jars in refrigerator at -18 °C (Tables 1 and 2). The pastes were brought to room temperature before given to the larvae and they renewed every 12 hours. The trial was conducted in a rearing cupboard providing the control of lightening, temperature and humidity. Temperature values were checked three times per day (0.1 sensitivity). Four replicates of 10 larvae per plastic box were established for each treatment. Hatching larvae were randomly selected, transferred into experiment medium and started to feed with artificial diet at the same time, namely 12 hours later (Bhattacharyya et al. 2017). Experiment period was edited as 47 days.

Considering that the larvae could be damaged, weighing was started from the third instar, namely 12th day (0.001 sensitivity). The vapor machine connected to the timer was used for humidity adjustment (Table 3). Light/darkness ratio was set 16hr: 8hr with a timer (Khairmode et al. 2019).

Data obtained were analyzed by analysis of variance (ANOVA) and means were grouped by Duncan's test ($P<0.05$), All percentage and ratio data were transformed to arcsin values prior to analysis (Zar 1984).

Table 1- Composition of the artificial diet ingredients quantities/100 g dry weight (prepared with azolla)

Ingredients	***K***	***A1***	***A2***	***A3***	***A4***
Mulberry leaf meal (g)	80	60	40	20	-
Azolla meal (g)	-	20	40	60	80
Citric Acid (g)	4	4	4	4	4
Ascorbic Acid (g)	2	2	2	2	2
Vitamin Mixture (g)[1]	3	3	3	3	3
Sorbic Acid (mg)	200	200	200	200	200
Propionic Acid (mg)	690	690	690	690	690
Chloramphenicol (mg)	10	10	10	10	10
Agar (g)	7.1	7.1	7.1	7.1	7.1
Sucrose (g)	3.0	3.0	3	3	3

[1]Supradyn: Vitamin A 3333 I.U., vitamin B1 20 mg, vitamin B 25 mg, calcium D-pantothenate 11.6 mg, vitamin B6 10 mg, vitamin B12 5 mcg, vitamin C 150 mg, vitamin D3 500 I.U., vitamin E 10 I.U., D-biotin (vitamin H) 250 mcg, Nicotinamide 50 mg, Folic acid 1 mg; Iron 10 mg, Phosphorus 23.8 mg, Calcium 51.3 mg, Magnesium 21.2 mg, Manganese 0.5 mg, copper 1 mg, Zinc 0.5 mg, Molybdenum 0.1 mg

[2] The pastes were prepared in ratio of 1 g of dry matter and 2.6 g of pure water

Table 2- Composition of the artificial diet ingredients quantities/100 g dry weight (prepared with duckweet)

Ingredients	*K*	*L1*	*L2*	*L3*	*L4*
Mulberry leaf meal (g)	80	60	40	20	-
Duckweet meal (g)	-	20	40	60	80
Citric Acid (g)	4	4	4	4	4
Ascorbic Acid (g)	2	2	2	2	2
Vitamin Mixture (g)[1]	3	3	3	3	3
Sorbic Acid (mg)	200	200	200	200	200
Propionic Acid (mg)	690	690	690	690	690
Chloramphenicol (mg)	10	10	10	10	1
Agar (g)	7.1	7.1	7.1	7.1	7.1
Sucrose (g)	3	3	3	3	3

[1] Supradyn: Vitamin A 3333 I.U., vitamin B1 20 mg, vitamin B 25 mg, calcium D-pantothenate 11.6 mg, vitamin B6 10 mg, vitamin B12 5 mcg, vitamin C 150 mg, vitamin D3 500 I.U., vitamin E 10 I.U., D-biotin (vitamin H) 250 mcg, Nicotinamide 50 mg, Folic acid 1 mg; Iron 10 mg, Phosphorus 23.8 mg, Calcium 51.3 mg, Magnesium 21.2 mg, Manganese 0.5 mg, copper 1 mg, Zinc 0.5 mg, Molybdenum 0.1 mg

[2] The pastes were prepared in ratio of 1 g of dry matter and 2.6 g of pure water

Table 3- The room temperature and humidity during the experimental period

Treatments	*Temperature*	*Humidity*
1st instar	25.06±0.203	82.1±2.98
2nd instar	25.39±0.171	81.4±2.96
3rd instar 12th day	25.56±0.146	79.5±5.40
4th instar 18th day	25.51±0.113	81.36±2.53
5th instar 28th day	25.24±0.094	77.13±2.25
32nd day	25.44±0.108	78.53±1.73
47th day	25.19±0.160	77.00±2.02

3. Results

In the research, hatching larvae were put into feeding medium in 12 hours and feeding process started. All larvae in groups A4 and L4 died before third instar. By considering that the larvae would be sensitive, they were not weighed until third instar (12th day). At the third instar, considering all groups, there was no significant difference in weight gain from each other ($P>0.05$). At the fourth instar, while there was significant difference statistically in weight gain between group K and other groups ($P<0.05$), there was not found significant difference between the groups A1, A2, A3, L1 ($P>0.05$). Nevertheless, all larvae in groups L2 and L3 died. At the fifth instar, on 28th day and 32nd day, while there was a large difference in weight gain between group K and other groups A1, A2 and L1 ($P<0.05$), all larvae in groups A3, L2 and L3 died. Most of the larvae in group K started to cocoon on 34th day (Figure 1).

The groups of A1 and L1 continued to feed until 37th and 43rd day. The mean weights of the individuals in A1 and L1 reached 0.663 g. and 0.378 g. on 43rd day in respectively. Nevertheless, these individuals died after a few days (Table 4).

By the third instar (12th day), it is noteworthy that no deaths occurred across all groups, and the weight gains, particularly in group K (control) and other groups, did not show statistically significant differences (Table 5).

The weights of cocoons and mortality rates of silkworm larvae in control group (K) are demonstrated in (Table 6).

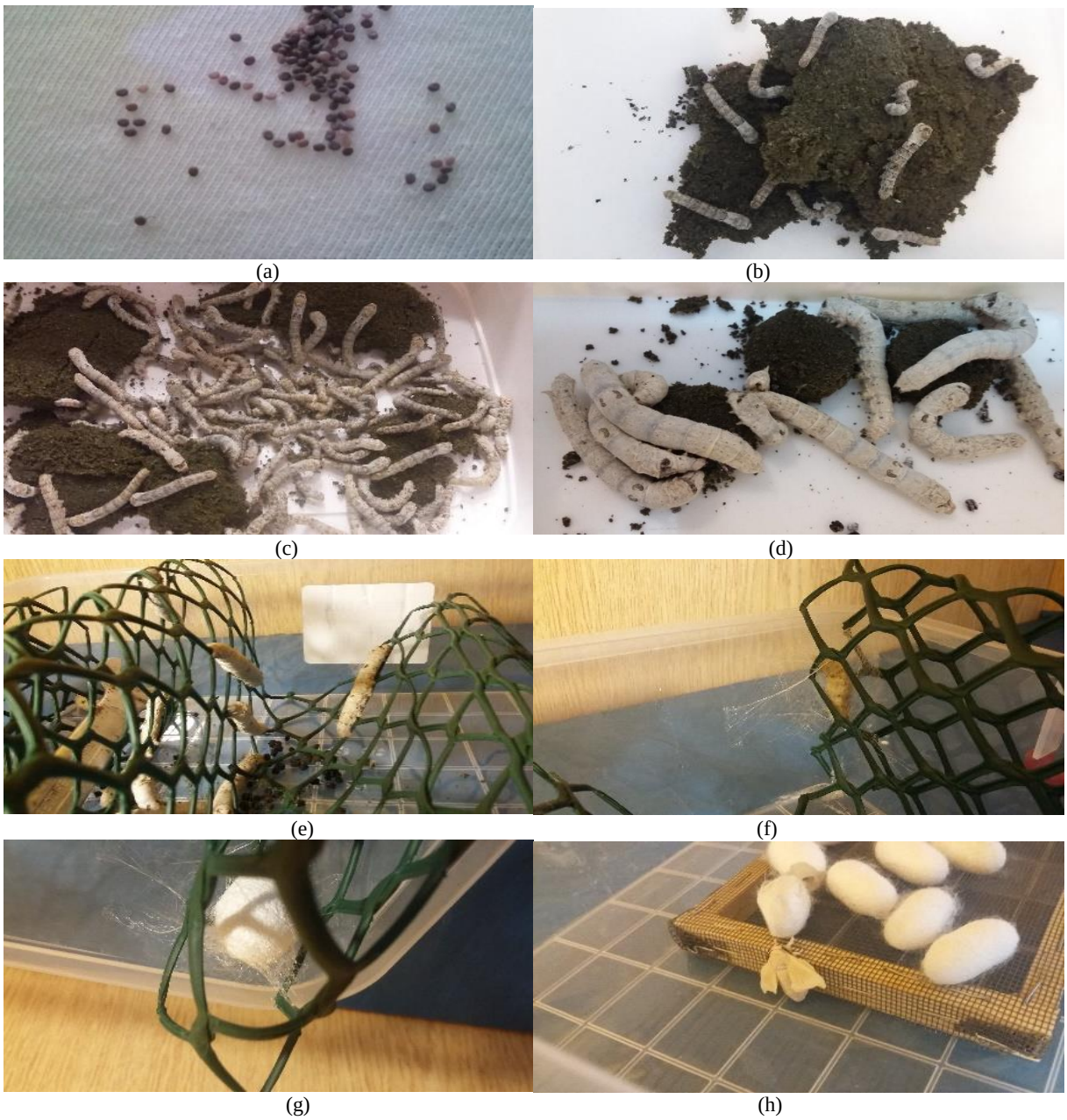

Figure 1- Silkworm larvae and silk moth periods during trial (a. silkworm eggs used in the experiment b. c. d. larvae aged between 12 and 28 days e. larvae on the 32nd day f. g. cocoon weaving stage, h. emergence from the cocoon)

Table 4- Means weight of larvae during the experimental period

	Weight of larvae (mg)					
Treat.	***3rd instar 12th day***	***4th instar 18th day***	***5th instar 28th day***	***32nd day***	***37th day***	***43rd day***
K	17.43±0.836[a]	131.73±7.08[a]	876.37±57.6[a]	1598.4±103[a]	-	-
A1	19.95±0.623[a]	29.63±1.73[b]	116.20±8.97[b]	185.3±15.8[b]	411.87±43.6	662.77±47.6
A2	19.10±0.812[a]	15.71±1.33[b]	45.50±1.50[b]	55.5±3.50[c]	-	-
A3	17.25±0.735[a]	11.56±0.584[b]	-	-	-	-
A4	-	-	-	-	-	-
L1	15.80±0.626[a]	21.14±2.24[b]	60.63±6.75[b]	133.3±23.6[c]	187.33±24.6	378.50±76.2
L2	13.20±0.750[a]	-	-	-	-	-
L3	12.35±0.990[a]	-	-	-	-	-
L4	-	-	-	-	-	-
F	13.45	153.37	71.30	68.87	9.96	10.67
P	0.0001	0.0001	0.0001	0.0001	0.003	0.002

Means with different superscripts (a, b and c) are significantly different (Duncan's multiple range test (P<0.05)). The F and P values were obtained from the analysis of variance (ANOVA)

Table 5- The mortality rates during the experimental period

Treatments	*Mortality %*					
	3rd instar 12th day	*4th instar 18th day*	*5th instar 28th day*	*32nd day*	*37th day*	*43rd day*
K	0.0	0.0	0.0	0.0	-	-
A1	0.0	12.087±3.66	29.888±1.92	29.888±1.92	29.888±1.92	36.002±3.96
A2	0.0	33.055±2.59	77.913±3.66	77.913±3.66	100	-
A3	0.0	47.884±1.67	100	-	-	-
A4	100	-	-	-	-	-
L1	0.0	32.898±3.66	50.832±2.41	56.945±2.59	56.945±2.59	56.945±2.59
L2	0.0	100	-	-	-	-
L3	0.0	100	-	-	-	-
L4	100	-	-	-	-	-
F		23.88	75.93	73.05	70.57	19.60
P		0.0001	0.0001	0.0001	0.0001	0.004

All percentage and ratio data were transformed to arcsin values prior to analysis. In respect of replicates, values 0and 100 were calculated as 1 and 99. The F and P values were obtained from the analysis of variance (ANOVA)

Table 6- The weights of cocoons and mortality rates during the experimental period*

Treatments	*Weight of cocoon (mg)*	*Min*	*Max*	*Mortality %*
K	971.1±17.0	821.0	1084.0	39.17±2.41
A1	-	-	-	100
A2	-	-	-	100
A3	-	-	-	100
A4	-	-	-	100
L1	-	-	-	100
L2	-	-	-	100
L3	-	-	-	100
L4	-	-	-	100

*: Cocoons were weighed on the 47th day

4. Conclusions

In the research, the substitution rates of mulberry leaf and Azolla meal in the artificial diet ranged from 0 to 20, 40, 60, and 80 g, corresponding to 0%, 25%, 50%, 75%, and 100% of the mulberry leaf meal for the four groups (A1, A2, A3, A4). The same substitution rates were also applied to mulberry leaf and duckweed meal for the four groups (L1, L2, L3, L4). Then it was tried to examine the reactions of silkworm larvae to these artificial feeds. All larvae in group A4 fed with 100% azolla meal and group L4 fed with 100% duckweed died before third instar. Up to third instar in all groups except A4 and L4, the fact that the mortality rates are 0% and weight gains are not significantly difference show that there will be a potential of the substitution of aquatic plants for mulberry leaf in early stages of rearing silkworm larvae. After third instar in all groups except control group, the decreases in survival rates and in mean weight gains and after fifth instar not completing the cocooning process point out that even 25% substitution of azolla meal and duckweed meal for mulberry leaf meal is not appropriate. Therefore, it can be suggested doing detailed research on lower substitution than 25%.

On the other hand, feeding with this kind of diets makes it possible to cultivate silkworm larvae all year round independent of the period of harvesting mulberry leaves, provided that appropriate conditions are met. Besides, the durability of substitute materials provides a great advantage compared to mulberry leaf. On the 32nd day, statistically significant difference between groups A1 and L1 means that azolla meal is more advantageous than duckweed meal. However, since the structure of the diets will affect the quality of cocoon and nutritional value of pupae, further researches are necessary. Additionally, it is also suggested further researches to determine the reasons for shortening life span of larvae as the amount of azolla and duckweed meal increases in diet and to detect whether or not completing the cocooning process by feeding larvae only mulberry leaf meal after 12th day.

Acknowledgements

The authors would like to express their gratitude to Kozabirlik Cooperative for assistance for the supply of silkworm eggs.

References

Ara S, Adil S, Banday M T & Khan M A (2015). Feeding Potential of Aquatic Fern-Azolla in Broiler Chicken Ration. *J. Poult. Sci. Technol* 3(1): 15-19. Corpus ID: 55082440.

Bhattacharyya P, Jha S, Ghosh A & Mandal P (2017). Effect of artificial diet components with antioxidant activity on mulberry leaf dependent silkworm rearing system. *Harvest* 2: 31-48

FAO (1989). Azolla and its multiple uses with emphasis on Africa, 52 pp

Gauri-Mahadevappa D, Sanganal-Jagadeesh S, Gopinath C R & Kalibavi C M (2012). Importance of azolla as a sustainable feed for livestock and poultry - A review. *Agricultural Reviews* 33: 93-103. www.arccjournals.com / indianjournals.com. Corpus ID: 82198573.

Gokcinar N C & Bekcan S (2015). The Effects of Partially Replacing Fishmeal with azolla (*Azolla* Sp.) on Growth Parameters of Shabbout Fish (*Tor grypus* H. 1843). *Journal of Applied Biological Sciences* 9(1): 43-46, 2015 ISSN: 1307-1130, E-ISSN: 2146-0108, www.nobel.gen.tr

Helaly W M M Y (2018). Evaluation of two food additives on *Bombyx mori* L. Characters. *Journal of Entomology and Zoology Studied*. 6(2): 3119-3123. E-ISSN: 2320-7078 P-ISSN: 2349-6800.

Hillman W S & Culley D D (1978). The uses of duckweed. American Scientist, 66:442-451. This content downloaded from 193.255.222.254 on Thu, 22 Feb 2024 10:31:15 + 00:00 All use subject to https://about.jstor.org/terms

Ijaiya A T & Eko E O (2009). Effect of replacing dietary fishmeal with silkworm (*Anapheefracta*) caterpillar meal on growth digestibility and economics production of starter broiler chicken. *Pakistan J. Nutr*., 8: 845-849. https://doi.org/10.3923/pjn.2009.845.849

Indira D, Rao K S, Suresh J, Naidu K V & Ravi A (2009). Azolla (*Azolla pinnata*) as feed supplement in buffalo calves on growth performance. *Indian Journal of Animal Nutrition* 26: 345-348. Corpus ID: 85714327

Kamalasanana Pillai P, Premalatha S & Rajamony S (2005). Azolla: a sustainable feed for livestock. Leisa Magazine, 21 (3) September, pp. 26-27. The full article was published in LEISA India, Volume 4 number 1, March 2002. It is available on the website www.leisa.info.

Katayama N, Masamichi Y, Yoshiro K, Chung C L, Watanabe I & Hidenori W (2008). Space Ag. task force-azolla as a component of the space diet during habitation on Mars, *Acta Astronautica*. 63: 1093-1099. doi:10.1016/j.actaastro.2008.01.023

Kathirvelan C, Banupriya S & Purushothaman M R (2015). Azolla- an alternate and sustainable feed for livestock. *Int. J. Sci. Environ. Technol*., 4(4): 1153-1157. ISSN 2278-3687 (O) 2277-663X (P). Published Aug 2, 2015 www.ijset.net

Khairmode P V, Shewale V S, Lawand S T & Tare V S (2019). Photoperiod induced alterations in growth pattern of silkwormScanner. International Conference on Advences in Pure and Applied Sciences ICAPAS. https://www.researchgate.net/publication/333901012.

Leng R A, Stambolie J H & Bell R (1995). Duckweed – a potential high-protein feed resource for domestic animals and fish. Livestock Research for Rural Development 7(1): http://www.lrrd.org/lrrd7/1/3.htm.

Leterme P, Londoño A M, Muñoz J E, Súarez J, Bedoya C A, Souffrant W B & Buldgen A (2010). Nutritional value of aquatic ferns (*Azolla filiculoides Lam.* and *Salvinia molesta* Mitchell) in pigs. *Animal Feed Science and Technology*, 149(1-2): 135-148. doi:10.1016/j.anifeedsci.2008.04.013

Mahmoud M M (2013). Effect of Various Kinds of Dietary Proteins in Semi – Artificial Diets on the Mulberry Silkworm Bombyx mori L. *Egypt. Acad. J. Biolog. Sci*., 6(1): 21-26. ISSN: 1687–8809 DOI:10.21608/eajbsa.2013.13815

Nguku E K, Muli E M & Raina S K (2007). Larvae, cocoon and post-cocoon characteristics of Bombyx mori L. (lepidoptera:bombycidae) fed on mulberry leaves fortified with Kenyan royal jelly. In J. Appl. Sci. Environ. Manage. 11(4): 85-89. JASEM ISSN 1119-8362 Full-text Available Online at www.bioline.org.br/ja.

Pallavi K, Muthuswami M, Bhaskar R N & Naveen V (2011). Role of Food Additives on young age Silkworm (Bombyx mori L.) Rearing. *Int. J. Pure Appl. Sci. Technol.* 7(2): 132-140. ISSN 2229 - 6107 Corpus ID: 18768828

Radha S & Geetha B (2018). Replacement of Fish Meal with Supplementatıon of Silkworm (Bombyx Morı) Pupae Meal On Growth In Common Carp. *Journal of Emerging Technologies and Innovative Research (JETIR*) 5(6): 284-288, www.jetir.org (ISSN-2349-5162)

Rana D, Katoch S, Mane B G, Rani D & Sankhyan V (2017). Biological Evaluation of Azolla in Ration of Commercial Chicken Broiler *Journal of Animal Research* 7(3): 601-607. DOI: 10.5958/2277-940X.2017.00091.2

Ravikumar C (1988). Western ghat as a bivoltine region prospects, challenges and strategies for its development. Indian Silk, 26 (9):39-54

Saha J K, Rahmatullah S M & Mazid M A (1999). Optimization of stocking density of duckweed, *Wolffia arhiza* (Linn.) and *Lemna* sp. *Bangldesh J. Fisheries Res.* 7: 161-168

Sahay A, Satpathy S & Sharan S K (2011). Field trial experiment of artificial diet on tasar silkworm, Antheraea myltta D., Nature Proceedings: hdl:10101/npre. 2011.6701.1: https://core.ac.uk/download/pdf/290525.pdf

Seidavi A R, Bizhannia A R, Sourati R & Mavvajpour M (2005). The nutritional effects of different mulberry varieties on biological characters in silkworm. *Asia Pac J Clin Nutr*.pp 14. Qingdao University*ISSN:* 0964-7058

Singh P K & Subudhi B P R (1978). Utilization of azolla in poultry feed. Indian Farming. 27:37-39

Talley S N, Talley B J & Rains D W (1977). Nitrogen fixation by azolla in rice fields. In Alexander Hollaender, ed. Genetic Engineering for Nitrogen Fixation. Plenum Press, New York and London, pp. 259-281. DOI 10.1007/978-1-4684-0880-5_17

Tran Q T & Dao T T (1973). Azolla a green compost. Vietnamese studies, Agric, Problem 38:119-127

Yang Y, Tang L, Tong L, Liu Y, Liu H & Li X (2010). Initial ground experiments of silkworm cultures living on different feedstock for provision of high quality animal protein for human in space [J]. *Advances in Space Research* 46(6): 707-711. https://doi.org/10.1016/j.asr.2010.04.007.

Zar J H (1984). Biostatistical analysis. Prentice Hall Englewood Cliffs, NJ, 718 pp. ISBN: 978-0-13-100lW6·5