



Investigation of reproductive efficiency, growth performance and survival of the southern medicinal leech, *Hirudo verbana* Carena, 1820 fed with mammalian and poultry blood



Murat Manav^a, Mustafa Ceylan^{b,*}, Hakan Murat Büyükçapar^a

^a Department of Fisheries, Agriculture Faculty, Kahramanmaraş Sütçü İmam University, Avsar Yerleskesi, 46100, Kahramanmaraş, Turkey

^b Medicinal Leech Research Laboratory, Fisheries Research Institute, 32500, Eğirdir, Isparta, Turkey

ARTICLE INFO

Keywords:

Aquaculture
Chicken
Cattle
Cocoon
Offspring

ABSTRACT

Medicinal leeches are one of the neglected organisms in animal reproduction studies. In this study, the effects of feeding two different types of blood, mammalian (cattle blood) and poultry (chicken blood), on the reproductive efficiency, growth performance and survival of the southern medicinal leech, *Hirudo verbana*, were investigated. The type of blood fed did not have a significant effect on the growth performance and survival of leeches, but the leeches fed chicken blood had a 2.5-fold greater performance compared to those fed cattle blood in terms of the gravidity process and reproductive efficiency. The final weight and survival for the cattle group was 10.79 ± 4.80 g and 87% and for the chicken group was 10.12 ± 5.26 g and 85%. Gravidity was initiated in the 7th month in both groups; however, while all gravidities in the leeches fed with chicken blood occurred in 1-month period, the gravidity of the leeches fed cattle blood occurred in a 3-month period. The cocoon efficiencies were 6.67 ± 1.50 cocoons/broodstock and 2.67 ± 3.37 cocoons/broodstock in the chicken and cattle groups, respectively. There were larger cocoons in the chicken compared to the cattle group. The offspring efficiencies were 12.59 ± 4.17 offspring/cocoon and 9.81 ± 3.67 offspring/cocoon in the chicken and cattle groups, respectively. The results of this study indicate poultry blood can be used in the management of broodstock with greater reproductive efficiency in medicinal leech aquaculture.

1. Introduction

The aquaculture sector has been growing rapidly in recent decades (Ponte et al., 2014; Nyman et al., 2017) and new species are being produced in the agricultural sector (Asche et al., 2009; Bostock et al., 2010). Leeches represent about 800 species in the world (Kvist et al., 2013) and have an important potential and are one of the neglected organisms in aquaculture studies especially with regard to leech reproduction (Ceylan et al., 2019). Leeches are macroinvertebrates and contribute positively to the digestion process of fish having the potential to be a source of live bait in aquaculture (Ceylan et al., 2017). There is a limited number of studies on the development of feeding protocols for leech species classified as "medicinal leeches" (Whitaker et al., 2004; Sket and Trontelj, 2008) which serve as a raw material in the pharmacy and cosmetic sectors being used in the treatment of some diseases in both traditional and modern medicine. There is, therefore, no recognized medicinal leech breeding protocol. In addition to the limited number of specialists studying leech reproduction/breeding, there is a need by commercial enterprises for greater information in production

* Corresponding author.

E-mail address: gm.ceylan@gmail.com (M. Ceylan).

<https://doi.org/10.1016/j.anireprosci.2019.05.004>

Received 27 March 2019; Received in revised form 1 May 2019; Accepted 8 May 2019

Available online 09 May 2019

0378-4320/ © 2019 Elsevier B.V. All rights reserved.

management of the marketing of medicinal leeches for about \$1,000/kg in the international market. Scientific studies to be conducted on feeding of medicinal leeches will significantly contribute to the development of leech breeding protocols and to the inclusion of leeches in commercial aquaculture sectors.

Medicinal leeches are fed with blood (Dickinson and Lent, 1984; Sawyer, 1986). Results of serological and molecular studies indicate the primary nutrients for medicinal leeches in the natural habitat consist of mammalian, reptile, bird and amphibian blood (Wilkin and Scofield, 1990; Keim, 1993; Tessler et al., 2018). Although the host preferences of medicinal leeches are known, the host effects on reproductive and growth performance are unknown. The determination of these effects will significantly contribute to the establishment of protocols for leech production in aquaculture. There is not direct feeding of the blood that is used for medicinal leech production (Davies and McLoughlin, 1996; Petrauskienė et al., 2009, 2011; Ceylan and Erbatır, 2012), with mammalian blood (cattle blood and pork blood) being the preferred food source, but there have been no previous studies using poultry blood.

The poultry industries which might have ample sources of blood for medicinal leeches, are expanding rapidly in some countries (Avcıoğlu and Türker, 2012; Mottet and Tempio, 2017). There can be discharge from poultry slaughterhouses of many wastes as well as blood and this can result in environmental pollution. Treatment units created to prevent environmental pollution in slaughterhouses require a large amount of energy (Aziz et al., 2018). The opportunity to utilize blood discharged from poultry slaughterhouses for leech production will help in addressing ecological sensitivities resulting from blood discharges, as well as contributing to development of an added value product for use in leech production.

In the present study, the effects of mammalian (cattle) and poultry (chicken) blood on the reproductive and growth performance were investigated for the first time in the medicinal leeches belonging to *Hirudo* genus. The medicinal leeches are widespread in the Palaearctic zoogeographical region and are important economically in the aquaculture sector. The growth of leeches, time to reach sexual maturity and reproductive efficiency of broodstock leeches were compared. The results of this study are expected to provide a basis for the attempts to develop a dietary ration for medicinal leeches that contributes useful scientific data for developing food sources for leeches and that further enhances the economic efficiencies of leech aquaculture enterprises.

2. Materials and method

2.1. Animals

The study was conducted on 120 offspring of the southern medicinal leeches (*Hirudo verbana* Carena, 1820) weighing 0.03 ± 0.01 g (min = 0.02 g, max = 0.05 g), which had recently erupted from the cocoon that had yet to be fed at the time of initiation of this study. The offspring were supplied by the Medicinal Leech Research Laboratory of Eğirdir Fisheries Research Institute, Turkey.

2.2. Experimental design

The experiment was conducted in Aquaculture Laboratory of Agriculture Faculty in Kahramanmaraş Sütçü İmam University, Turkey in which there was an environmental temperature of 24.56 ± 1.33 °C and a humidity of $46 \pm 7\%$. The leeches were divided into two separate groups that were fed with mammalian (cattle group) and poultry (chicken group) blood with four replicates and the animals were maintained in 5 L pet jars half filled with dechlorinated water. The leeches were evaluated daily and the water in the jars was changed twice a week. Dead leeches, if any, were noted and removed from the jars.

2.3. Study plan

There was initiation of the study in June 2017 and the study was conducted in two stages during a period of 9 months. In the first 7-month stage of the study, growth performance of the leeches from hatching to sexual maturity were investigated. In the second stage of the study, the reproductive performance of the gravid leeches was investigated for 2 months.

2.4. Feed (blood) source and leech feeding

Feeding of the leeches was performed using the methods described in previous studies (Zhang et al., 2008; Ceylan and Erbatır, 2012). Leeches were fed on a monthly basis until there was sexual maturity. Fresh cattle blood was obtained from the Slaughterhouse of Kahramanmaraş Municipality, and chicken blood was obtained from a private chicken Slaughterhouse in the Onikisubat Municipality (Kahramanmaraş, Turkey). Both slaughterhouses are licensed by Ministry of Agriculture of Turkey. The hemogram and serum biochemical analyses of the blood samples were performed by the Vetform Veterinary Clinic Laboratory (Etiler/Istanbul, Turkey).

The blood obtained from the slaughterhouses was treated with 6 ml/L of heparin to prevent coagulation. There was 6 ml of heparin placed in empty 1 L pet jars and the jars were subsequently filled with fresh blood while exsanguination of animals was occurring at the slaughterhouse. After blood collection, the jars were gently shaken for 1 min to mix the blood and heparin. The blood was quickly transported to laboratory in a portable refrigerator at 4 °C within 1 h and the feeding regimen was initiated using these blood samples. Considering the mean body temperature of cattle and chickens, the temperature of cattle blood was maintained at 38.5 °C (Kendall and Webster, 2009) and the temperature of chicken blood was maintained at 41.5 °C (Dawson and Whittow, 2000) in a circulating water bath before feeding occurred. Blood was inserted into 15-cm of a cow intestine that was placed in a warm water bath for 10 min during which tissue softening occurred. The leeches were fed *ad libitum* with the other leeches in the treatment group.

The individual weight (g) of all leeches was determined before and after each feeding.

2.5. Leech reproduction

To compare the reproductive efficiency of the leeches fed with different blood types, 15 gravid leeches weighing 13.49 ± 2.47 g in the group fed chicken blood and 15 gravid leeches weighing 13.35 ± 2.95 g in the group fed cattle blood with variables related to sexual maturation being evaluated. Gravidity was evaluated by local swelling and there was assessment of the discoloration indicators in the clitellum region of the leeches (Wilkin, 1989; Elliott, 2008; Ceylan et al., 2015). The initial weight (g) of the gravid leeches was determined and leeches were individually placed in 2 L pet jars while being covered with half-moist peat (humidity level: 70%–75%), and the reproductive performance was assessed for 2 months. The jars were assessed three times a week for determining the time of cocoon development. The weight of the cocoons was determined using a 0.01 g precision scale, and the length, inner length, diameter and inner diameter of the cocoons were determined using a 0.01 mm precision digital calliper. The cocoons were individually incubated in 350 mL pet jars while being embed in half moist peat (humidity: 70%–75%) for 30 days at an environmental temperature of 25 °C. At the end of the incubation period, the cocoons were manually opened, the offspring number was determined, and the individual weight of the offspring was determined using a 0.1 mg precision scale (Davies and McLoughlin, 1996; Utevskaia and Atramentova, 2002; Petrauskienė et al., 2011; Ceylan et al., 2017, 2019).

2.6. Statistical analyses

The normality of the data (leech body weight, cocoon weight, cocoon length, cocoon diameter and number of offspring) was analyzed using the Kolmogorov-Smirnov test and the homogeneity of the variances was analyzed using the Levene's test. Depending on the determinations from the preliminary tests, transformation (logarithmic, inverse, square root, etc.) occurred for the data of the groups with non-normal distribution and non-homogeneous variances. The Independent Samples *t*-test was used to compare data with normal distribution and with homogeneous group variances on the basis of feeding groups, while the Mann-Whitney U test was used for data that were non-normally distributed or with non-homogeneous group variances. One-way analysis of variance was used for the analysis of data with normal distribution and with homogeneous group variances according to months and the Duncan's multiple comparison test was used for assessment of treatment differences, while the Kruskal-Wallis test was used for the analysis of data with non-normal distribution and with non-homogeneous group variances and the Dunn's multiple comparison test was used to determine treatment differences. To determine the correlation between the values of variables, the Pearson's correlation coefficient or Spearman's rank correlation coefficient was used depending on the pre-test results, while the presence and strength of correlation between the variables were analyzed with regression analysis. The mortality rates were analyzed using the Chi-square test. With the analyses, the significance level was considered as $\alpha = 0.05$. The values were given as mean \pm standard deviation. The data were analyzed on the IBM SPSS Statistics version 25.0 software package for Windows (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Analyses of cattle and chicken bloods

For values resulting from the hemogram analysis, white blood cell (WBC), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values were greater in leeches fed the chicken blood, while erythrocyte (RBC), hemoglobin (HGB), hematocrit (HCT) and mean corpuscular hemoglobin concentration (MCHC) were greater in leeches fed cattle blood. Serum analysis, glucose, cholesterol, triglyceride and calcium values were greater in leeches fed chicken blood, while phosphate, albumin and total protein

Table 1

Hemogram and serum biochemical analysis results with the feeding of cattle and chicken blood to the southern medicinal leech, *Hirudo verbana*; Mean values are based on results of two analyses performed at different times for cattle and chicken blood types.

Variables	Unit	Cattle blood (n = 2)	Chicken blood (n = 2)
White blood cells (WBC)	$\times 10^9/L$	9.2 ± 1.6	22.9 ± 16.5
Erythrocyte count (RBC)	$\times 10^{12}/L$	8.6 ± 0.1	5.7 ± 0.2
Hemoglobin (HGB)	g/dl	15.2 ± 3.0	11.3 ± 0.4
Hematocrit (HCT)	%	38.9 ± 0.7	34.0 ± 1.4
Mean corpuscular volume (MCV)	fL	45.3 ± 1.4	60.2 ± 0.2
Mean corpuscular hemoglobin (MCH)	pg	17.6 ± 3.3	20.0 ± 0.0
MCH concentration (MCHC)	g/dl	39.0 ± 8.6	33.2 ± 0.1
Blood glucose (GLU)	mg/dl	164.0 ± 14.8	259.7 ± 9.3
Phosphate	mg/dl	2.5 ± 2.0	0.4 ± 0.3
Cholesterol	mg/dl	84.4 ± 2.7	99.3 ± 18.8
Triglyceride (TG)	mg/dl	199.9 ± 3.1	226.0 ± 7.1
Albumin (Alb)	g/dl	5.1 ± 0.8	3.1 ± 0.2
Calcium (Ca)	mg/dl	7.3 ± 0.6	9.0 ± 0.5
Total protein (TP)	g/dl	6.6 ± 0.7	4.0 ± 0.2

amount were greater in leeches fed cattle blood. The data obtained with hemogram and serum biochemical analyses for leeches fed chicken and cattle blood are included in Table 1.

3.2. Growth performance

The initial mean weight of leeches was 0.03 ± 0.01 g, and after the last feeding the leeches in the cattle group had a mean weight of 12.91 ± 6.05 g (final weight: 10.79 ± 4.80 g), while the leeches chicken group had a mean weight of 13.59 ± 8.47 g (final weight: 10.12 ± 5.26 g) at the time of the last feeding. Throughout the experiment the heaviest leech weighed 38.49 g in those fed chicken blood and weighed 27.61 g in those fed cattle blood.

The weight increases of the leeches during the treatment period before and after feeding were significant during all months of the treatment period ($P < 0.05$). There was the greatest weight increase at the second feeding with $504 \pm 34\%$ in the leeches fed cattle blood, while this occurred at the third feeding with $536 \pm 68\%$ in the chicken group. As the leeches aged, the rate of weight increases before and after feeding gradually decreased. The least rate of weight increase occurred at the last feeding with the increase being $70 \pm 21\%$ in the leeches fed chicken blood, while this occurred at the second-to-last feeding with the rate being $91 \pm 56\%$ in the leeches fed cattle blood.

As an indicator of food digestion performance, the weight loss rates between the feeding periods in both groups were significant ($P < 0.05$). The greatest weight loss occurred between the second and third feeding periods with the loss being $46 \pm 3\%$ in the group fed cattle blood, while this occurred between the third and fourth feeding periods in the group fed chicken blood with the weight loss being $41 \pm 5\%$. The digestion process of the blood consumed was prolonged with growth, and the least rate of weight loss occurred in the last three times feeding occurred. The least weight loss between the feeding periods occurred in the last month both in the leeches fed cattle or chicken blood with the weight loss being $17 \pm 4\%$ and $25 \pm 8\%$, respectively.

The values for monthly changes of the body weight, proportional increase and decrease in body weight in the two feeding groups are included in Table 2 and the growth curves are depicted in Fig. 1.

3.3. Gravidity rate

Gravidity of the leeches in both feeding groups started in the 7th month. At the end of 9th month, two leeches in both groups were not gravid, while all other leeches were gravid. All leeches ($n = 49$) that were alive and found to be gravid in the group fed chicken blood became gravid in the 7th month. Unlike the group fed chicken blood, gravidity increased gradually in the leeches in the group fed cattle blood with the number of gravid leeches from the 7th to 9th months being 34, 45 and 50, respectively.

Table 2

Data for monthly changes in body weight, rate of increase and decrease in body weight between the feeding periods in the southern medicinal leech, *Hirudo verbana* fed cattle or chicken blood; Body weight was different between months for the two groups ($P < 0.05$); Growth of the leeches was not affected by blood type (cattle or chicken) fed based on the body weight values at the end of the experiment ($P > 0.05$); Rate of both increase and decrease in body weight was different between months ($P < 0.05$).

Months	Feeding period	Body weight (g) [*]		Rate of increase in body weight (%) [#]		Rate of decrease in body weight (%) [¥]	
		Cattle group	Chicken group	Cattle group	Chicken group	Cattle group	Chicken group
0	Before feeding (Initial of trial)	0.03 ± 0.01^g	0.03 ± 0.01^g	125 ± 42^{Bd}	250 ± 64^{Aab}		
1	After feeding	0.06 ± 0.03^{Bf}	0.10 ± 0.05^{Af}			18 ± 5^{Bde}	33 ± 2^{Aabc}
	Before feeding	0.05 ± 0.03^{Bfg}	0.07 ± 0.03^{Ag}	504 ± 34^{Aa}	188 ± 118^{Bab}		
2	After feeding	0.33 ± 0.17^{Aef}	0.21 ± 0.18^{Bef}			40 ± 8^{ab}	32 ± 9^{abc}
	Before feeding	0.20 ± 0.11^{Aef}	0.14 ± 0.11^{Bef}	362 ± 72^{Bb}	536 ± 68^{Aa}		
3	After feeding	0.91 ± 0.58^{ed}	0.86 ± 0.66^{ed}			46 ± 3^{Aa}	29 ± 2^{bbc}
	Before feeding	0.49 ± 0.30^{de}	0.61 ± 0.47^{de}	329 ± 40^b	260 ± 113^{ab}		
4	After feeding	2.09 ± 1.06^{cd}	1.97 ± 1.26^{cd}			36 ± 2^{bc}	41 ± 5^a
	Before feeding	1.34 ± 0.65^{cd}	1.17 ± 0.71^{cd}	225 ± 11^{Bc}	300 ± 41^{Aab}		
5	After feeding	4.37 ± 2.15^{bc}	4.61 ± 2.66^{bc}			36 ± 1^{bc}	38 ± 2^{Aab}
	Before feeding	2.82 ± 1.40^{bc}	2.87 ± 1.55^{bc}	121 ± 20^d	156 ± 25^{ab}		
6	After feeding	6.16 ± 3.12^{ab}	7.41 ± 4.65^{ab}			20 ± 9^{de}	27 ± 4^c
	Before feeding	4.96 ± 2.80^{ab}	5.29 ± 2.84^{ab}	91 ± 56^d	116 ± 34^b		
7	After feeding	9.02 ± 4.89^{ab}	11.16 ± 6.28^a			27 ± 9^{cd}	27 ± 10^c
	Before feeding	6.48 ± 3.25^{Bab}	8.03 ± 3.67^{Aa}	99 ± 15^d	70 ± 21^b		
8	After feeding	12.91 ± 6.05^a	13.59 ± 8.47^a			17 ± 4^e	25 ± 8^c
	Before feeding (End of trial)	10.79 ± 4.80^a	10.12 ± 5.26^a				

[#]: Proportional weight increases before and after feeding, [¥]: Proportional weight decrease in the 1-month period between feeding activities. [#],[¥]: For the relevant variables, the difference between the means is indicated with uppercase letters in the same line and with lowercase letters in the same column is different ($P < 0.05$); *Lettering with lowercase letters in the same column does not include considerations for the entire column, and are independent evaluations on the basis of feeding periods (“Before feeding” and “After feeding”).

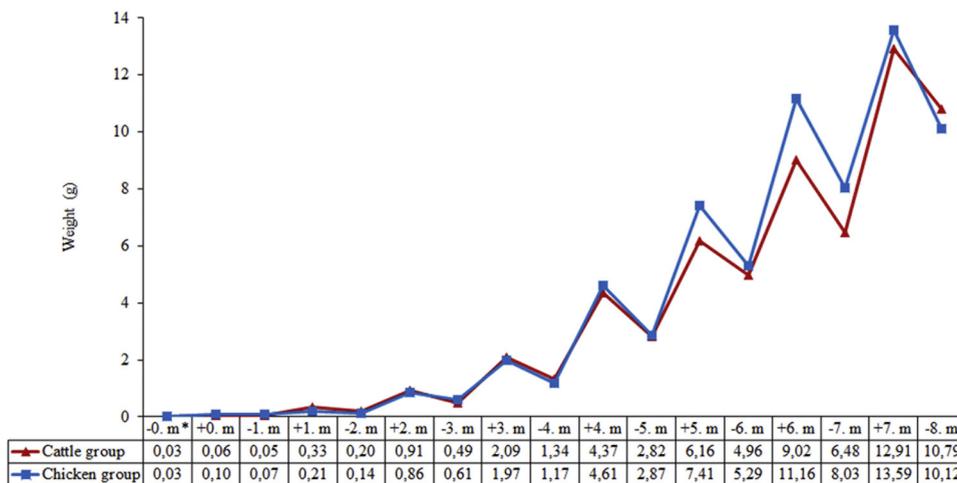


Fig. 1. Growth curve of the southern medicinal leech, *Hirudo verbana*, fed cattle or chicken blood; Growth model of the leeches indicated there was a marked increase in growth of both rate of both groups as result of the feedings; According to the body weight values at the end of the experiment the growth of the leeches was not affected by feeding either cattle or chicken blood ($P > 0.05$); *Initiation of the study; The "-" sign in front of the months refers to the pre-feeding period and "+" sign refers to the post-feeding period.

3.4. Reproductive performance

The final weight of the gravid leeches fed cattle blood with a mean weight of 13.35 ± 2.95 g had a reduction in weight to 7.68 ± 2.62 g which is a decrease of $40 \pm 22\%$ in mean weight. The mean weight of the gravid leeches of the group fed chicken blood was 13.49 ± 2.47 g with a reduction in weight to 5.01 ± 1.55 g which was a $63 \pm 8\%$ decrease in weight. The difference between the two groups in terms of the proportional weight loss and final weights of the gravid leeches was significant ($P < 0.05$). The data for frequency distribution of proportional weight loss of the broodstock leeches during the reproductive period are depicted in Fig. 2.

Cocoons were obtained from 8 of 15 gravid leeches that were fed cattle blood and from all 15 gravid leeches that were fed chicken blood. Although there was a positive correlation between the initial weight of the gravid leeches in both groups and the number of cocoons produced (chicken $r = 0.486$, cattle $r = 0.453$), this correlation was not significant ($P > 0.05$). The frequency of cocoon production as evaluated as the first cocoon produced for each broodstock leech was 7.03 ± 4.00 days in leeches fed cattle blood and 6.46 ± 2.74 days in those fed chicken blood. Although the broodstock leeches in the group fed chicken blood produced cocoons more frequently, the difference between the two groups in terms of frequency of cocoon production was not significant ($P > 0.05$).

A total of 40 cocoons were obtained from the leeches fed cattle blood and 100 cocoons were obtained from those fed chicken

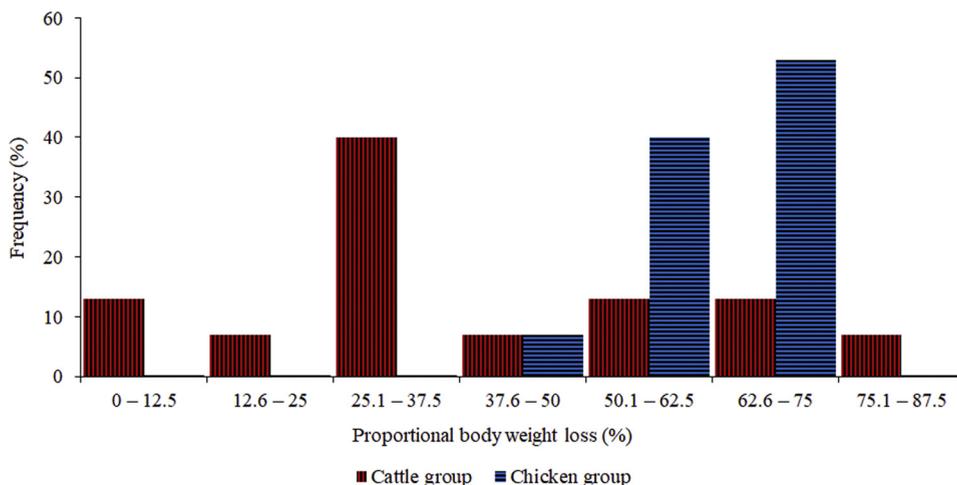


Fig. 2. Frequency distribution of the proportional body weight loss of the broodstock leeches fed cattle or chicken blood during the reproductive period; Rate of loss in body weight between the feeding periods in both groups was significant ($P < 0.05$); The most frequent range in loss of body weight was 62.6%–75% with leeches fed chicken blood having a greater productive performance, while the range was 25.1%–37.5% in leeches fed cattle blood.

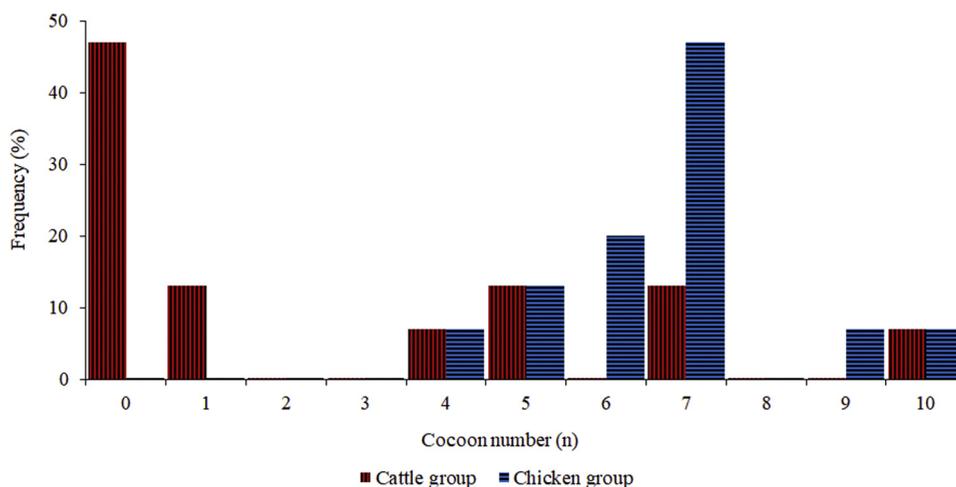


Fig. 3. Frequency distribution of the number of cocoons obtained from the broodstock leeches fed cattle or chicken blood; Cocoons were deposited from 8 of 15 broodstock leeches by leeches fed cattle blood, while there was cocoon depositions from all 15 broodstock leeches fed chicken blood; Mean deposited cocoon number per broodstock was 2.67 ± 3.37 in leeches fed cattle blood and 6.67 ± 1.50 in leeches fed chicken blood; Difference between leeches fed cattle or chicken blood for cocoon efficiency was significant ($P < 0.05$); The most frequent class in deposited cocoon number was seven with leeches fed chicken blood having the greater productive performance, while this value was 0 in leeches fed cattle blood.

blood. The cocoon production efficiency was 2.67 ± 3.37 cocoons/broodstock in leeches fed cattle blood and 6.67 ± 1.50 cocoons/broodstock in those fed chicken blood. There was a difference between the two groups in terms of cocoon production efficiency ($P < 0.05$). Offspring erupted from 36 (90%) of 40 cocoons obtained from leeches fed cattle blood and 74 (74%) of 100 cocoons from the leeches fed chicken blood, and there were no detectable offspring development in the other cocoons. The data for frequency distribution of the number of cocoons from the broodstock leeches are depicted in Fig. 3.

The size of cocoons obtained from the gravid leeches fed chicken blood were larger than those fed cattle blood. The cocoon weight, length, inner length, diameter and inner diameter values were, respectively, 1.23 ± 0.28 g, 23.23 ± 2.87 mm, 14.57 ± 1.59 mm, 18.37 ± 2.78 mm and 10.32 ± 1.62 mm in the leeches fed cattle blood, while values for these variables were, respectively, 1.30 ± 0.27 g, 24.35 ± 2.68 mm, 15.24 ± 2.40 mm, 18.54 ± 2.37 mm and 10.82 ± 1.37 mm in the leeches fed chicken blood. There was a difference between the two groups in terms of cocoon length ($P < 0.05$); however, the difference in values between groups for the other cocoon variables was not significant ($P > 0.05$).

In both groups, there were correlations between broodstock leech size (weight) and cocoon size ($P < 0.05$). When evaluated generally, the correlation coefficients were greater in leeches fed chicken blood compared to those fed cattle blood.

There were a total of 353 offspring produced by the leeches fed cattle blood, while there was a total of 932 offspring produced by those fed chicken blood. The offspring efficiency was 12.59 ± 4.17 offspring/cocoon in leeches fed chicken blood, while this efficiency was 9.81 ± 3.67 offspring/cocoon in those fed cattle blood, and there was a difference between the two groups in terms of offspring efficiency ($P < 0.05$). The mean offspring weight was 38.5 ± 17.4 mg in the leeches fed cattle blood, while this value was 36.3 ± 13.7 mg in the leeches fed chicken blood, and there was not a significant difference between the two groups in terms of offspring weight ($P > 0.05$).

The values for frequency distribution of the offspring from the cocoons in the leeches fed cattle or chicken blood are depicted in Fig. 4. The data for weight-frequency distribution of the offspring are depicted in Fig. 5. The data for reproductive performance of the leeches in the two groups are provided in Table 3. The data from correlation and regression analyses of broodstock leech weight and cocoon size are presented in Table 4.

3.5. Survival rate

A total of 17 leeches, eight leeches fed cattle blood and nine fed chicken blood, died during the period the study was being conducted. None of the gravid leeches died during the period leeches' were reproductively functional. Accordingly, the survival rate was 87% for the leeches fed cattle blood and 85% for those fed chicken blood. The mortality rates were evenly distributed ($\chi^2 = 0.059$, $P > 0.05$). During the last 4-months of the 9 month study there were about 59% ($n = 13$) of deaths that occurred during the entire study period. The depiction of data for monthly survival rates in leeches fed cattle or chicken blood is in Fig. 6.

4. Discussion

In the present study, the type, cattle or chicken, blood fed to the southern medicinal leeches, *H. verbana*, affected the developmental breeding pattern. The group fed chicken blood had a markedly greater gravidity and reproductive performance, although

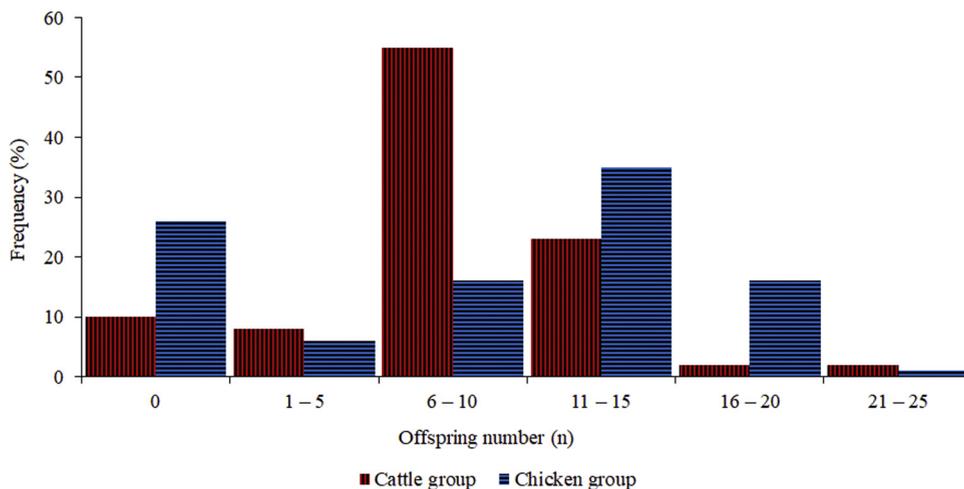


Fig. 4. Frequency distribution of the number of offspring leeches from cocoons of leeches fed cattle or chicken blood; A total of 353 offspring were produced by leeches fed cattle blood, while 932 offspring were produced by leeches fed chicken blood; The mean number of offspring in the cocoons was 12.59 ± 4.17 in leeches fed chicken and 9.81 ± 3.67 in those fed cattle blood; Difference between leeches fed cattle or chicken blood was significant in terms of offspring number ($P < 0.05$); The most frequent values in offspring number per cocoon was 11–15 in leeches fed chicken blood and was 6–10 in those fed cattle blood.

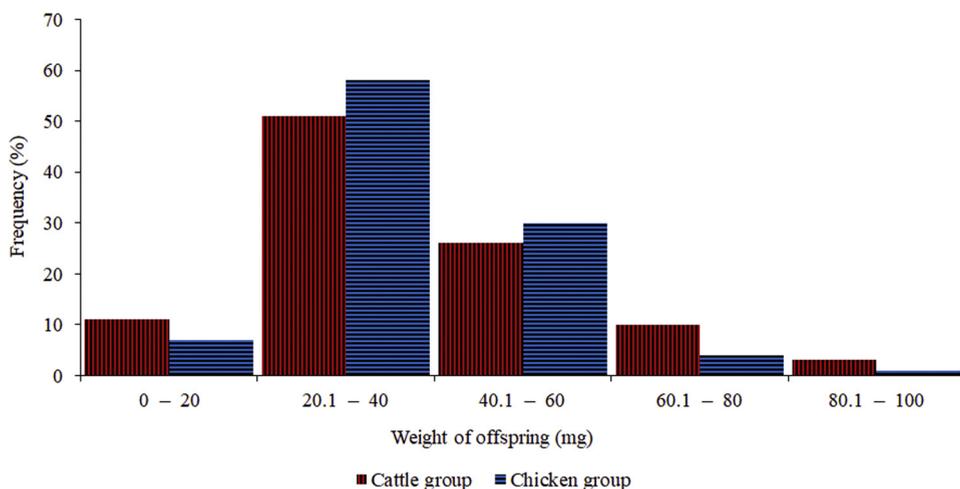


Fig. 5. Weight-frequency distribution of the offspring leeches from cocoons of broodstock leeches fed either cattle or chicken blood; Mean offspring weight was 38.5 ± 17.4 mg in leeches fed cattle blood, while this value was 36.3 ± 13.7 mg in leeches fed chicken blood; Difference between the leeches fed cattle or chicken blood in terms of offspring weight was not significant ($P > 0.05$); The most frequent class in offspring weight was 20.1–40 mg for both groups.

there was no effect on growth performance and survival rates.

There was a marked growth pattern in both groups. The leeches consumed a large volume of blood at each feeding on a monthly basis, but the digestion of the blood that was consumed occurred at a slower rate. These patterns of blood consumption and digestion resulted in the marked increase in growth rate of *H. verbana*, similar to that previously reported (Sawyer et al., 1981; Wilkin and Scofield, 1991; Pfeiffer et al., 2005) in a closely related species, *Hirudo medicinalis* and the giant glossiphoniid leech, *Haementeria ghilianii*.

In the present study, the highest rate of blood consumption compared to their own body weight occurred at the 2nd and 3rd feedings with about a five-fold increase in weight (Table 2). This value was less than the value of 8.9 reported by Dickinson and Lent (1984) for the related taxon, *H. medicinalis* species. It is thought that the species difference, as well as the relative amount of starvation and individual differences may affect these outcomes from blood feeding. Considering that medicinal leeches can survive for as long as 6 months (Kikuchi et al., 2009), and in some cases for even as long as 23 months (Ceylan, 2016) without feeding, it is predicted that the large blood volume that can be consumed is an important adapted survival trait of *Hirudo* leeches. The amount of blood consumed compared to body weight reached a peak in the 2nd to 3rd month and there was a gradual decrease subsequent to that time. This indicates the proportional amount of blood based on amount consumed relative to body weight by leeches decreases

Table 3

Reproductive performance of the southern medicinal leeches, *Hirudo verbana* fed chicken blood or cattle blood; Differences of final weight of broodstock leeches, cocoon number per broodstock, length of cocoons and offspring number per cocoon between groups fed the two different types of blood were significant ($P < 0.05$).

Variables		Chicken group	Cattle group
Broodstock	Gravid leeches used in production study (n)	15	15
	Initial weight (g)	13.49 ± 2.47 (9.36 – 16.11)	13.35 ± 2.95 (9.70 – 18.20)
	Final weight (g)	5.01 ± 1.55 ^b (2.99 – 8.16)	7.68 ± 2.62 ^a (3.59 – 11.89)
	Proportional weight loss (%)	63 ± 8 ^a (49 – 75)	40 ± 22 ^b (8 – 78)
Cocoon	Cocoon deposition frequency (day)	6.46 ± 2.74 (3 – 15)	7.03 ± 4.00 (3 – 20)
	Total obtained cocoon (n)	100	40
	Cocoons obtained offspring (n)	74 (974)	36 (%90)
	Cocoon number per broodstock (n)	6.67 ± 1.50 ^a (4 – 10)	2.67 ± 3.37 ^b (0 – 10)
	Cocoon weight (g)	1.30 ± 0.27 (0.67 – 1.98)	1.23 ± 0.28 (0.65 – 1.79)
	Cocoon length (mm)	24.35 ± 2.68 ^a (18.48 – 29.99)	23.23 ± 2.87 ^b (16.04 – 28.89)
	Cocoon inner length (mm)	15.24 ± 2.40 (11.33 – 24.03)	14.57 ± 1.59 (11.67 – 16.87)
	Cocoon diameter (mm)	18.54 ± 2.37 (13.56 – 25.02)	18.37 ± 2.78 (13.9 – 23.16)
Offspring	Cocoon inner diameter (mm)	10.82 ± 1.37 (7.24 – 13.81)	10.32 ± 1.62 (6.18 – 13.21)
	Total offspring (n)	932	353
	Offspring number per cocoon (n)	12.59 ± 4.17 ^a (3 – 21)	9.81 ± 3.67 ^b (4 – 23)
	Weight of offspring (g)	36.3 ± 13.7 (1.6 – 109.5)	38.5 ± 17.4 (4.2 – 98.9)

^aValues between parentheses indicate the minimum and maximum values for the relevant variable. The difference between the means is indicated with lowercase letters in the same line is different ($P < 0.05$).

with age. Because of the greater mass-specific metabolism required by small leeches there is need for more energy in the long period of satiation compared to larger leeches; therefore, the relative amount of blood consumed decreases as the leech develops (Lent et al., 1988). The decrease in relative amount of weight loss with age, which is an indicator of the digestion process between the feeding periods, is an important indicator in terms of the nutritional physiology of leeches and leech aquaculture. The rates of weight increase after feeding, which is an indicator of the amount of blood consumed is dependent on age of the leeches, and the relative rates of weight loss between the feeding periods, which is an indicator of the digestion rate. This information is useful and practical for determining the amount of blood required to feed leeches in commercial aquaculture enterprises.

Because of the need for osmotic and volume regulation after feeding on blood, there is a marked urine discharge in leeches in the initial 8 h after blood consumption (Zerbst-Boroffka, 1973). The medicinal leech, *H. verbana* can vomit some of the blood consumed even 66 days after feeding (Ceylan and Erbatur, 2012). In the present study, frequent blood vomiting of some leeches after each feeding, especially in the first week, resulted in the color of water in the jars being pinkish/reddish. The fact that deaths usually occurred in the jars with blood discharge indicates that the blood in the stomach of satiated leeches is discharged after death. Furthermore, the deterioration in the hygiene quality of the water in containers where there was blood vomiting by the leeches gives rise to the thought that these conditions might have caused the death of other leeches in the container. The fact that most of the deaths (about 59%) occurred in the last 4 months of the study indicates the negative effect of waste discharge on water quality and the amount of this discharge increased with stocking density of the leeches. It, therefore, appears as though the effects of the stocking density of juvenile leeches on the growth performance and survival rates should be investigated. Such a study would provide useful results for leech aquaculture enterprise development.

Deaths resulting from cannibalism as previously reported by Kutschera and Roth (2005) and Ceylan et al. (2019) in medicinal leeches did not occur in the present study. It is predicted that regular feeding of the leeches on a monthly basis was effective in avoiding this problem.

Although the leeches fed with chicken blood had a greater reproductive efficiency than the leeches fed cattle blood, it was noted that the rate of cocoon development with offspring emergence was less. The rate of cocoon development from which offspring were obtained was 90% in the leeches fed cattle blood, while this rate was 74% in the group fed chicken blood. It is thought that the results reported by Petrauskienė et al. (2011) contribute to the interpretation of the results in the present study in this regard. In the previous study with the *H. medicinalis* species, there was a lesser reproductive performance compared to what occurred with the *H. verbana* species in terms of number of cocoons and offspring. The weight of offspring emerging from cocoons and the survival rate of leeches during the growth phase were greater in the *H. medicinalis* species that have a lesser reproductive performance. For this reason, it is predicted that the lesser rate of cocoon opening in leeches fed with chicken blood might have been a negative consequence as a result of the relatively greater reproductive outcome of this species. A study involving the rates of cocoon opening and analysis of the biotic and abiotic factors affecting cocoon opening would be beneficial for both testing of this hypothesis and in development of an economic leech aquaculture sector. Considering that more offspring were obtained than there was larger cocoon development (Ceylan et al., 2015), it would be important to ascertain whether there is larger cocoon development in leeches fed chicken blood. This information would be valuable for planning in development of commercial medicinal leech production enterprises.

Medicinal leeches have a wide range of hosts such as fish, birds, amphibians and mammals (Elliott and Tullett, 1984; Wilkin and Scofield, 1990; Elliott and Dobson, 2015). The amount of energy content in the blood sourced from hosts varies; mammalian blood has a greater energy content than amphibian blood (Merilä and Sterner, 2002). The greater energy content in the host blood positively affects the reproductive performance of medicinal leeches. For example, compared to frog blood, both the sexual maturation

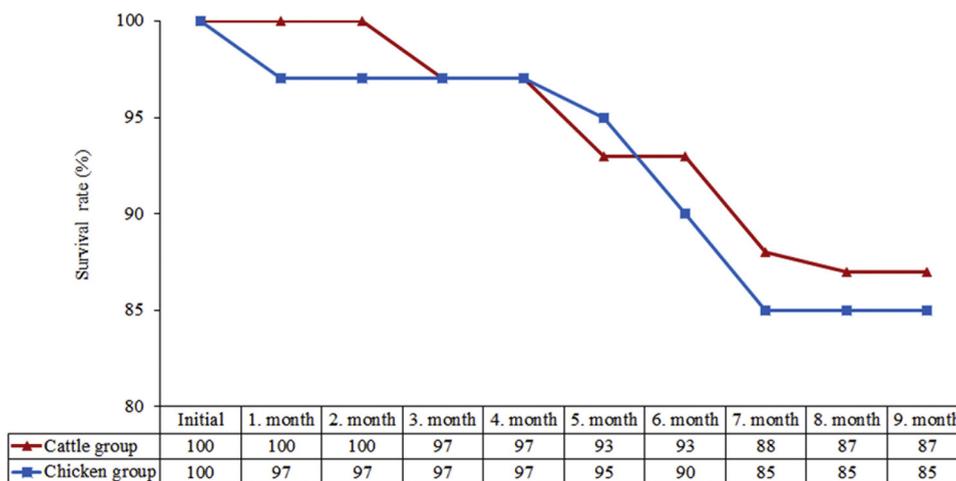


Fig. 6. Monthly change of the survival rates in leeches fed cattle or chicken blood; A total of 17 leeches, eight of leeches fed cattle blood and nine of leeches fed chicken blood, died during the study; Survival rates were 87% and 85% in leeches fed cattle or chicken blood, respectively; Survival rates were evenly distributed between the groups ($\chi^2 = 0.059$, $P > 0.05$).

time shortens, and the fecundity rate increases in medicinal leech populations primarily fed mammalian blood (Davies and McLoughlin, 1996). In the present study, it is predicted that there was approximately 50% more glucose in chicken blood compared to cattle blood and this factor may contribute to the greater reproductive performance of the leeches fed chicken blood. This hypothesis should be supported by further detailed studies focusing on this specific topic. While all gravidities in the leeches fed with chicken blood occurred in one month, the gravidity of the leeches fed cattle blood occurred during a 3-month period. In addition, the amount of energy in chicken blood is thought to be a determinative factor for the greater developmental capacity in terms of both cocoon and juvenile development in leeches fed chicken blood.

5. Conclusions

The results of the present study revealed that mammalian and poultry blood had no significant effect on the growth patterns of the southern medicinal leech, *H. verbana*, but had a significant effect on the capacity for sexual maturity and reproductive performance. The results of this study which indicate the feeding of chicken blood could be incorporated into management practices of broodstock leech production in commercial aquaculture enterprises in which the leeches have a high capacity for reproductive performance, thus, providing a value-added product. There appears to be need for scientific studies that focus on the modifications in blood components fed to leeches, especially protein and glucose, based on the results of the present study. These findings provide valuable information for development of feed rations that will form a basis for optimum growth and reproductive performance. This type of information is important if there is to be further development of the aquaculture sector for medicinal leech production, which have been safely used in the treatment of some diseases in traditional medicine as well as many branches of modern medicine.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgment

This study was supported by Kahramanmaraş Sütçü Imam University, Coordination Unit of Scientific Research Projects with the project number of 2017/1-2 YLS. We would like to thank the Eğirdir Fisheries Research Institute from which offspring of the medicinal leeches were supplied.

References

- Asche, F., Roll, K.H., Trollvik, T., 2009. New aquaculture species—the whitefish market. *Aquacult. Econ. Manag.* 13, 76–93. <https://doi.org/10.1080/13657300902881641>.
- Avcioglu, A.O., Türker, U., 2012. Status and potential of biogas energy from animal wastes in Turkey. *Renew. Sust. Energ. Rev.* 16, 1557–1561.
- Aziz, H., Puat, N., Alazaiza, M., Hung, Y.T., 2018. Poultry slaughterhouse wastewater treatment using submerged fibers in an attached growth sequential batch reactor. *Int. J. Env. Res. Pub. He.* 15, 1734. <https://doi.org/10.3390/ijerph15081734>.
- Bostock, J., McAndrew, B., Richards, R., Jauncey, K., Telfer, T., Lorenzen, K., Little, D., Ross, L., Handisyde, N., Gatward, I., Corner, R., 2010. Aquaculture: global status and trends. *Philos. Trans. Biol. Sci.* 365, 2897–2912. <https://doi.org/10.1098/rstb.2010.0170>.
- Ceylan, M., 2016. Determination of Ecology, Population Size and Catch Efficiency of Medicinal Leech (*Hirudo verbana* Carena, 1820) Populations in Wetlands Around Lake Eğirdir. PhD Thesis. Süleyman Demirel University.

- Ceylan, M., Erbatur, İ., 2012. A study on nutrition of medicinal leech (*Hirudo verbana* Carena, 1820): cannibalism? *Ege J. Fish. Aqua. Sci.* 29, 167–170.
- Ceylan, M., Çetinkaya, O., Küçükçara, R., Akçimen, U., 2015. Reproduction efficiency of the medicinal leech *Hirudo verbana* Carena, 1820. *Turk. J. Fish. Aquat. Sc.* 15, 411–418. https://doi.org/10.4194/1303-2712-v15_2_27.
- Ceylan, M., Küçükçara, R., Akçimen, U., Yener, O., 2017. Reproduction efficiency of the horse leech, *Haemopsis sanguisuga* (Linnaeus, 1758). *Int. J. Inver. Rep. Dev.* 61, 182–188. <https://doi.org/10.1080/07924259.2017.1318096>.
- Ceylan, M., Küçükçara, R., Akçimen, U., 2019. Effects of broodstock density on reproduction efficiency and survival of southern medicinal leech, *Hirudo verbana* Carena, 1820. *Aquaculture*. 498, 279–284. <https://doi.org/10.1016/j.aquaculture.2018.08.016>.
- Davies, R., McLoughlin, N., 1996. The effects of feeding regime on the growth and reproduction of the medicinal leech *Hirudo medicinalis*. *Freshw Biol.* 36, 563–568. <https://doi.org/10.1046/j.1365-2427.1996.00121.x>.
- Dawson, W.R., Whittow, G.C., 2000. Regulation of body temperature. In: Whittow, G.C. (Ed.), *Sturkie's Avian Physiology*. Academic Press, San Diego, CA, pp. 343–379.
- Dickinson, M.H., Lent, C.M., 1984. Feeding behavior of the medicinal leech, *Hirudo medicinalis* L. *J. Comp. Physiol. A* 154, 449–455. <https://doi.org/10.1007/BF00610160>.
- Elliott, J.M., 2008. Population size, weight distribution and food in a persistent population of the rare medicinal leech, *Hirudo medicinalis*. *Freshw Biol.* 53, 1502–1512. <https://doi.org/10.1111/j.1365-2427.2008.01978.x>.
- Elliott, J.M., Dobson, M., 2015. *Freshwater leeches of Britain and Ireland: keys to the Hirudinea and a review of their ecology*. Freshwater Biological Association. Scientific Publication, London No. 69.
- Elliott, J.M., Tullett, P.A., 1984. The status of the medicinal leech *Hirudo medicinalis* in Europe and especially in the British Isles. *Biol. Conserv.* 29, 15–26. [https://doi.org/10.1016/0006-3207\(84\)90011-9](https://doi.org/10.1016/0006-3207(84)90011-9).
- Keim, A., 1993. Studies on the host specificity of the medicinal blood leech *Hirudo medicinalis* L. *Parasitol. Res.* 79, 251–255. <https://doi.org/10.1007/BF00931901>.
- Kendall, P.E., Webster, J.R., 2009. Season and physiological status affects the circadian body temperature rhythm of dairy cows. *Livest. Sci.* 125, 155–160. <https://doi.org/10.1016/j.livsci.2009.04.004>.
- Kikuchi, Y., Bomar, L., Graf, J., 2009. Stratified bacterial community in the bladder of the medicinal leech, *Hirudo verbana*. *Environ. Microbiol.* 11, 2758–2770. <https://doi.org/10.1111/j.1462-2920.2009.02004.x>.
- Kutschera, U., Roth, M., 2005. Cannibalism in a population of the medicinal leech (*Hirudo medicinalis* L.). *Biology Bull.* 32, 626–628. <https://doi.org/10.1007/s10525-005-0154-7>.
- Kvist, S., Min, G.S., Siddall, M.E., 2013. Diversity and selective pressures of anticoagulants in three medicinal leeches (Hirudinida: Hirudinidae, Macrobdellidae). *Ecol. Evol.* 3, 918–933. <https://doi.org/10.1002/ece3.480>.
- Lent, C.M., Fliegner, K.H., Freedman, E., Dickinson, M.H., 1988. Ingestive behaviour and physiology of the medicinal leech. *J. Exp. Biol.* 137, 513–527.
- Merilä, J., Sterner, M., 2002. Medicinal leeches (*Hirudo medicinalis*) attacking and killing adult amphibians. *Ann. Zool. Fenn.* 39, 343–346.
- Mottet, A., Tempio, G., 2017. Global poultry production: current state and future outlook and challenges. *World. Poultry Sci. J.* 73, 245–256. <https://doi.org/10.1017/S0043933917000071>.
- Nyman, A., Huyben, D., Lundh, T., Dicksved, J., 2017. Effects of microbe-and mussel-based diets on the gut microbiota in Arctic charr (*Salvelinus alpinus*). *Aquacult. Rep.* 5, 34–40. <https://doi.org/10.1016/j.aqrep.2016.12.003>.
- Petrauskienė, L., Utevska, O., Utevsky, S., 2009. Can different species of medicinal leeches (*Hirudo* spp.) interbreed? *Invertebr. Biol.* 128, 324–331. <https://doi.org/10.1111/j.1744-7410.2009.00180.x>.
- Petrauskienė, L., Utevska, O., Utevsky, S., 2011. Reproductive biology and ecological strategies of three species of medicinal leeches (genus *Hirudo*). *J. Nat. Hist.* 45, 737–747. <https://doi.org/10.1080/00222933.2010.535918>.
- Pfeiffer, I., Brenig, B., Kutschera, U., 2005. Molecular phylogeny of selected predaceous leeches with reference to the evolution of body size and terrestrialism. *Theory Biosci.* 124, 55–64. <https://doi.org/10.1016/j.thbio.2005.05.002>.
- Ponte, S., Kelling, I., Jespersen, K.S., Kruijssen, F., 2014. The blue revolution in Asia: upgrading and governance in aquaculture value chains. *World Dev.* 64, 52–64. <https://doi.org/10.1016/j.worlddev.2014.05.022>.
- Sawyer, R.T., 1986. *Leech Biology and Behavior, vol. I, II, III Oxford University Press, New York*.
- Sawyer, R.T., Lepont, F., Stuart, D.K., Kramer, A.P., 1981. Growth and reproduction of the giant Glossiphoniid leech *Haementeria ghilianii*. *Biol. Bull.* 160, 322–331. <https://doi.org/10.2307/1540892>.
- Sket, B., Trontelj, P., 2008. Global diversity of leeches (Hirudinea) in freshwater. *Hydrobiologia*. 595, 129–137. <https://doi.org/10.1007/s10750-007-9010-8>.
- Tessler, M., Weiskopf, S.R., Berniker, L., Hersch, R., Mccarthy, K.P., Yu, D.W., Siddall, M.E., 2018. Bloodlines: mammals, leeches, and conservation in southern Asia. *Syst. Biodivers.* 16, 488–496. <https://doi.org/10.1080/14772000.2018.1433729>.
- Utevska, O.M., Atramentova, L.A., 2002. Heritability of reproductive traits in the medicinal leech *Hirudo medicinalis* L. *Russ. J. Genet.* 38, 44–49. <https://doi.org/10.1023/A:1013711710927>.
- Whitaker, I.S., Rao, J., Izadi, D., Butler, P.E., 2004. Historical article: *Hirudo medicinalis*: ancient origins of, and trends in the use of medicinal leeches throughout history. *Brit. J. Oral Max. Surg.* 42, 133–137. [https://doi.org/10.1016/S0266-4356\(03\)00242-0](https://doi.org/10.1016/S0266-4356(03)00242-0).
- Wilkin, P.J., 1989. The medicinal leech, *Hirudo medicinalis* (L.) (Hirudinea: Gnathobdellae), at Dungeness. *Kent. Bot. J. Linn. Soc.* 101, 45–57. <https://doi.org/10.1111/j.1095-8339.1989.tb00135.x>.
- Wilkin, P.J., Scofield, A.M., 1990. The use of a serological technique to examine host selection in a natural population of the medicinal leech, *Hirudo medicinalis*. *Freshw Biol.* 23, 165–169. <https://doi.org/10.1111/j.1365-2427.1990.tb00261.x>.
- Wilkin, P.J., Scofield, A.M., 1991. Growth of the medicinal leech, *Hirudo medicinalis*, under natural and laboratory conditions. *Freshw Biol.* 25, 547–553. <https://doi.org/10.1111/j.1365-2427.1991.tb01398.x>.
- Zerbst-Boroffka, I., 1973. Osmotic and volume regulation in *Hirudo medicinalis* following food intake. *J. Comp. Physiol.* 84, 185–204. <https://doi.org/10.1007/BF00697606>.
- Zhang, B., Lin, Q., Lin, J., Chu, X., Lu, J., 2008. Effects of broodstock density and diet on reproduction and juvenile culture of the leech, *Hirudinaria manillensis* Lesson, 1842. *Aquaculture*. 276, 198–204. <https://doi.org/10.1016/j.aquaculture.2008.02.003>.