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Original article (Orijinal araştırma)

Modeling of development and water consumption of mealworm, *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) larvae using nonlinear growth curves and polynomial functions

Büyüme eğrileri ve polinomial fonksiyonlar kullanılarak unkurdu, *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) larvalarının gelişim ve su tüketimlerinin modellenmesi

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Abstract

The water needs of *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) have to be met in either in-vitro culture or mass production. In this study, water needs of larvae were met directly by using a purpose-built water diffuser. The efficacy of larvae grown with the water diffuser (W) was tested against the control group (CONT). Various growth models were used to test their appropriateness to describe the experimental data. This research was conducted in the Population Genetic Laboratory of Animal Science Department of Isparta University of Applied Sciences in 2018. The highest larval weights were 138 mg in W and 144 mg in CONT treatments. The larvae in W entered the pupal period 2 weeks before the larvae in CONT. The growth of larvae in both groups was successively modeled with Gompertz and logistic growth curve models, and quadratic and cubic polynomial functions. The mean weekly water consumption of the W larvae was found to be between 58.4-129 mg. The water consumption of larvae can be described by polynomial functions. There were significant correlation coefficients for larval age, larval weight and water consumption. Consequently, using the diffuser instead of fresh vegetables or fruits is more suitable to meet the water requirement of the larvae.

Keywords: Growth curves, polynomial functions, *Tenebrio molitor*, water consumption

Öz

Tenebrio molitor L., 1758 (Coleoptera: Tenebrionidae)'un laboratuvar ortamında yetiştirilmesinde ve kitlesel üretimde su ihtiyaçlarının karşılanması gerekmektedir. Yapılan bu çalışmada larvaların su ihtiyaçları yeni bir yöntem kullanılarak doğrudan karşılanmıştır. Yöntemin (W) etkinliği oluşturulan kontrol grubuna (CONT) karşı test edilmiştir. Faklı büyüme modellerinin deneysel veri setini tanımlamadaki uygunluğu da test edilmiştir. Araştırma 2018 yılında, Isparta Uygulamalı Bilimler Üniversitesi Tarım Bilimleri ve Teknolojileri Fakültesi Zootekni Bölümü Popülasyon Genetiği Laboratuvarında yapılmıştır. Araştırmada W grubunda en yüksek larva ağırlığı 137.6 mg, CONT grubunda ise 144.2 mg bulunmuştur. Su uygulaması yapılan gruptaki larvalar, kontrol grubundaki larvalardan iki hafta önce pupa evresine girmişlerdir. Her iki grupta larvaların büyümesi, Gompertz, logistic büyüme eğrisi modelleri ve quadratik ve kubik polinomial fonksiyonları ile başarılı bir şekilde modellenmiştir. W grubu larvaların ortalama haftalık su tüketimleri 58.4 ile 128.8 mg aralığında bulunmuştur. Larvaların su tüketimleri polinomial fonksiyonlar kullanılarak modellenebilmektedir. Araştırmada larva yaşı, larva ağırlığı ve su tüketimi parametreleri arasında önemli korelasyonlar belirlenmiştir. Tüm bunlar dikkate alındığında *T. molitor* larvalarının su ihtiyacının ortama verilen taze sebze veya meyveler yerine, diffizor aracılığı ile karşılanmasının daha uygun bir yöntem olduğu sonucuna varılmıştır.

Anahtar sözcükler: Büyüme eğrileri, polinomial fonksiyonlar, *Tenebrio molitor*, su tüketimi

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Introduction

Mealworm, *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) is a good model organism due to its short life cycle and being easy to grow (Pölkki et al., 2012; Simon et al., 2013; Grau et al., 2017). In addition, *T. molitor* can be a good alternative to currently available protein sources for aquaculture (Ng et al., 2001; Gasco et al., 2016; Choi et al., 2018) and poultry production (Ramos-Elorduy et al., 2002; De Marco, 2015; Bovera et al., 2016) due to its high-quality lipid and high protein content (Finke, 2002; Ramos-Elorduy et al., 2002; Siemianowska et al., 2013; Hervé et al., 2014; Jin et al., 2016; Özsoy et al., 2017). *Tenebrio molitor* needs minimal physical space, has high conversion efficiency and can convert organic waste into usable animal feed. However, it has restricted use in poultry feed because of high production costs (Ramos-Elorduy et al., 2002). In the mass production of *T. molitor*, feed and water expenses constitute a significant part of the production costs. Wheat flour, bran, oatmeal, corn and corn flakes are used as the basic food in experimental *T. molitor* production. Similar to insect species such as *Ephestia kuehniella* Zeller, 1879 and *Sitodrepa panicea* L. 1758*, T. molitor* carbohydrate requirements are very high and carbohydrates in their diet need to be 80% or higher (Fraenkel et al., 1950). Well performance of *T. molitor* larvae requires meeting their feed and as well as water needs without causing any stress. The water needs of *T. molitor* in the laboratory and mass production can be met by variety of methods or mechanisms: i) absorbance of water through the cuticle in microclimate with high humidity (Fraenkel et al., 1950; Murray, 1968; Punzo & Rosen, 1984), ii) ingestion with feed of high moisture content (Aguilar-Miranda et al., 2002; Gholy & Alkoaik, 2009; Ravanzaadii et al., 2012; Siemiazauska et al., 2013), and iii) free-choice water supplement (Urs & Hopkins, 1973; Morales-Ramos et al., 2012). However, it has been reported that direct water supplement has advantages over other water intake mechanisms for speed of larval growth and early maturity with relatively higher weight, decreased mortality at any growth stage and reproductive success. Increasing humidity, supplying fresh fruits and/or vegetables and free-choice water given on cotton pads often stimulate growth of secondary organisms and cause physical degradation of the feed. Therefore, there is a need to overcome these deleterious effects of water supplement methods with a method not interfering with the feed while maintaining advantages of free-choice supplement.

Growth curve models and polynomial mathematical functions are used to express the change in the time dependent-weights of living organisms. Gompertz and logistic growth models are frequently used nonlinear regressions to describe the growth patterns of living organisms (Akbulut et al., 2004; Narinç et al., 2009; Aytekin & Zülkadir, 2013).

In this study, a new method was used to meet the water requirements of *T. molitor* larvae, which could be an alternative to the use of fresh vegetable pieces in the growth medium. The water needs of the larvae were met directly by using a purpose-built water diffuser. The water consumption or requirement of the larvae up to the pupal period was therefore determined at weekly intervals. In addition, the larvae grown with the water diffuser (W) and control larvae (CONT) were modeled using growth and water consumption growth curves (Gompertz and logistic) and polynomial functions (quadratic and cubic functions). In this way, the changes in water requirements of the larvae can be monitored by age.

Materials and Methods

Materials

A population of *T. molitor* was cultured at the Population Genetics Laboratory, Department of Animal Science, Faculty of Agricultural Sciences and Technologies, Isparta University of Applied Sciences in 2018. The gender identification was made at pupal period (Sokoloff, 1977), and then male and female pupae were transferred to separate growth boxes to develop to adults.

Methods

The mature insects were taken into the mating box that supplemented with nutrient for 48 h. Adult insects were removed from the nutrient medium at the end of the mating period and the eggs were kept in an incubation cabinet adjusted to 28°C and 60% RH in feed media for 7 d. The hatched larvae were kept together at 28°C for seven additional days and fed with 70% semolina, 20% wheat bran and 10% yeast mixture. Then, 100 randomly selected larvae from this population were placed in the 350 mL plastic boxes for each trial unit after precisely measuring their initial weight with a balance sensitive to 0.01 mg. The weekly weight gains were precisely measured till formation of pupae.

Four experimental groups were formed in the study. Two of these groups were control groups (CONT1 and CONT2, water requirement of the larvae was supplied fresh potatoes at 3-d intervals) and the other two were water-supplied groups (W1 and W2, water supplied directly with the water diffuser). The ration and water given to the groups are given in Table 1.

Table 1. Ration and water supplied to the experimental groups

Experimental groups	Rations	Water
W1- W2	70% semolina, 20% wheat bran, 10% yeast	Water diffuser
CONT1- CONT2	70% semolina, 20% wheat bran, 10% yeast	Fresh potatoes at 3-d interval

Water requirement of insects in W1 and W2 treatments was given to the larvae by a purpose-built water diffuser. It was assembled by combining a water container and a diffuser stick (Figure 1). Precisely measured water was consumed by the larvae through the diffuser. The larval weights in the experimental groups were measured at weekly intervals in groups of 10 using an analytical balance with 0.01 mg sensitivity (Radwag® AS 110.R2, Radom, Poland). Weighing results were recorded in milligrams. The insects in W groups consumed water ad libitum.

Figure 1. Water-diffuser design and water supplement to the larvae.

Data analysis and modeling

Larval ratio, pupal ratio, mortality ratio and individual water consumption were determined in this study using the following equations.

Larval ratio = number of alive larvae / number of individuals in the control treatment x 100,

Pupal ratio = number of alive pupae / number of individuals in the control treatment x 100,

Mortality ratio = number of dead individuals / number of individuals in the control treatment x 100, and

Water consumption of Individual = water consumption / number of alive larvae.

Gompertz and logistic growth models, and the mathematical models such as quadratic and cubic forms of polynomial functions used in the research and their parameters are given in Table 2.

The data set were subjected to one-way ANOVA procedure to separate treatment means. The statistical analysis such as ANOVA, growth curve, estimates of polynomial function parameters and correlations were performed in Minitab (Minitab, 2018) package program.

Table 2. Growth models and polynomial functions and their parameters

Growth models	
Gompertz growth model	Logistic growth model
$GGM(y) = exp(-exp(\theta_2 - \theta_3 t)$	$LGM(y) = \frac{\theta_1 + (\theta_2 - \theta_1)}{(1 + exp((t - \theta_3)/\theta_4))}$
$\theta_1; asymptote$	$\theta_1; asymptote$
$\theta_2; y - intercept$	$\theta_2; y - intercept$
$\theta_3; scale$	$\theta_3, \theta_4; scale$
$t; time$	$t; time$
Polynomial functions	
Quadratic function	Cubic function
$QF(y) = \theta_1 + \theta_2 t + \theta_3 t^2$	$CF(y) = \theta_1 + \theta_2 t + \theta_3 t^2 + \theta_4 t^3$
$\theta_1, \theta_2, \theta_3; scale$	$\theta_1, \theta_2, \theta_3, \theta_4; scale$
$t; time$	$t; time$

Results and Discussion

The weekly mean larval weight, pupae weight, larval ratio, pupal ratio, mortality ratio of the larvae is presented given in Table 3.

The highest larval weight was determined in the water-supplied group (W) 138 mg and in the control group (CONT) 144 mg. These maximums were obtained in week 12 in the W group and in week 11 in the CONT group. The larvae started to enter the pupal period starting from the week 9 in the CONT group and week 7 in the W group. The mean weight of larvae of the experimental groups started to differ from the week 2 onwards in favor of the water-supplied group. This superiority of water-supplied group continued up to week 9. Larval weights at weeks 9 and 10 of the two groups were not statistically different ($p > 0.05$). In the subsequent weeks, the larvae in the control group weighed more than the water-supplied group ($p < 0.01$). However, the highest larval weight for both treatments were obtained in week 9 and subsequent weeks. In both experimental groups, the differences in weight were negligible at weeks 1, 2 and 3, but they differed at weeks 4, 5, 6, 7 and 8. Weekly mean larval weights in the study were higher than those reported by Ramos-Erlorduy et al. (2002); lower than those of Kim et al. (2016a) and Ghaly & Alkoaik (2009) and similar to those of Özsoy et al. (2017) and Kim et al. (2016b). The differences between the values reported in the literature and this study is likely to be due to the nutrient value of the rations, genetic differences of the populations and experimental methods used.

Table 3. Time-dependent variations of the measured parameters in the experimental groups

Time (week)	Group	LR[1] (%)	Weekly larval weight, LW[2] (mg)	PR[3] (%)	PW[4] (mg)	MR[5] (%)	Weekly LW means of groups (mg)[6]	
							W	CONT
1	W	100.0	0.39 ± 0.27 a	0.0	-	0.0	0.39 ± 0.27 f	0.39 ± 0.41 g
	CONT	100.0	0.39 ± 0.41 a	0.0	-	0.0		
2	W	99.0	1.26 ± 0.09 a	0.0	-	1.0	1.26 ± 0.09 f	1.00 ± 0.12 g
	CONT	97.0	1.00 ± 0.12 b	0.0	-	3.0		
3	W	97.0	5.53 ± 1.66 a	0.0	-	3.0	5.53 ± 1.66 f	2.90 ± 0.34 g
	CONT	95.5	2.90 ± 0.34 b	0.0	-	4.5		
4	W	97.0	22.1 ± 4.73 a	0.0	-	3.0	22.1 ± 4.73 e	8.92 ± 1.70 f
	CONT	94.5	8.92 ± 1.70 b	0.0	-	5.5		
5	W	97.0	50.5 ± 4.82 a	0.0	-	3.0	50.5 ± 4.82 d	26.8 ± 4.46 e
	CONT	94.5	26.8 ± 4.46 b	0.0	-	5.5		
6	W	97.0	87.1 ± 7.52 a	0.0	-	3.0	87.1 ± 7.52 c	62.7 ± 9.48 d
	CONT	92.5	62.7 ± 9.48 b	0.0	-	7.5		
7	W	94.0	116.2 ± 6.31 a	1.0	90.4	5.0	116.2 ± 6.31b	97.1 ± 8.07 c
	CONT	91.5	97.1 ± 8.07 b	0.0	-	8.5		
8	W	86.5	131.0 ± 8.46 a	7.5	101.7 ± 13.1	6.0	131.0 ± 8.46 a	122.3 ± 9.48 b
	CONT	89.5	122.3 ± 9.48 b	0.0	-	10.5		
9	W	60.0	134.8 ± 2.99 a	30.0	110.0 ± 14.8	10.0	134.8 ± 2.99 a	136.7[a] ± 9.25 a
	CONT	83.0	136.7 ± 9.25 a	5.5	107.7 ± 10.9	11.5		
10	W	53.5	136.8 ± 8.85 a	36.5	120.8 ± 15.2	10.0	136.8 ± 8.85 a	140.1 ± 9.05 a
	CONT	75.5	140.1 ± 9.05 a	12.0	112.6 ± 15.2	12.5		
11	W	44.0	133.4 ± 9.81 b	45.0	130.3 ± 30.9	11.0	133.4 ± 9.81 a	144.4 ± 8.05 a
	CONT	69.5	144.4 ± 8.05 a	18.0	121.0 ± 20.9	12.5		
12	W	39.5	137.6 ± 9.11 b	49.5	128.3 ± 13.0	11.0	137.6 ± 9.11 a	144.2 ± 5.07 a
	CONT	59.0	144.2 ± 5.07 a	28.0	125.7 ± 15.4	13.0		

[1] Larval ratio, [2] Results of variance analysis of weekly larval weight means between experimental groups, [3] Pupal ratio, [4] Pupal weight, [5] Mortality ratio, [6] Results of variance analysis of larval weight means within the experimental groups. The difference between the weights of larvae having the same letters is not important ($p < 0.05$).

The growth of larvae in W and CONT groups was modeled by using Gompertz and logistic growth models, and polynomial functions (quadratic and cubic) as given in Table 2. The model and function equations obtained from W group are given in Table 4 and the curves created using these models are given in Figure 2.

Table 4. General model of growth curve and polynomial functions of larvae in water-supplied group (W)

Model	Equations	R^2
Gompertz	$GM(y) = 139.4exp(-\exp(3.75 - 0.76t))$	90.0
Logistic	$LM(y) = \dfrac{136.8 + (-1.13 - 136.8)}{(1 + exp((t - 5.46)/0.88))}$	90.5
Quadratic	$QM(y) = -51.5 - 0.99t^2 + 28.4t$	93.0
Cubic	$CM(y) = 4.78 - 0.41t^3 + 7.03t^2 + 15.0t$	98.0

All of the equations obtained for the water-supplied group (Table 4) have a coefficient of determination of 90% and greater. In addition, R^2 values for each model (Table 4) were similar. In the case of water-supplied larvae, the development of larvae can be defined to a large extent by Gompertz, logistic growth model and polynomial functions. Also, the plots of the growth curves and polynomial functions (Figure 2) are largely consistent with observed values.

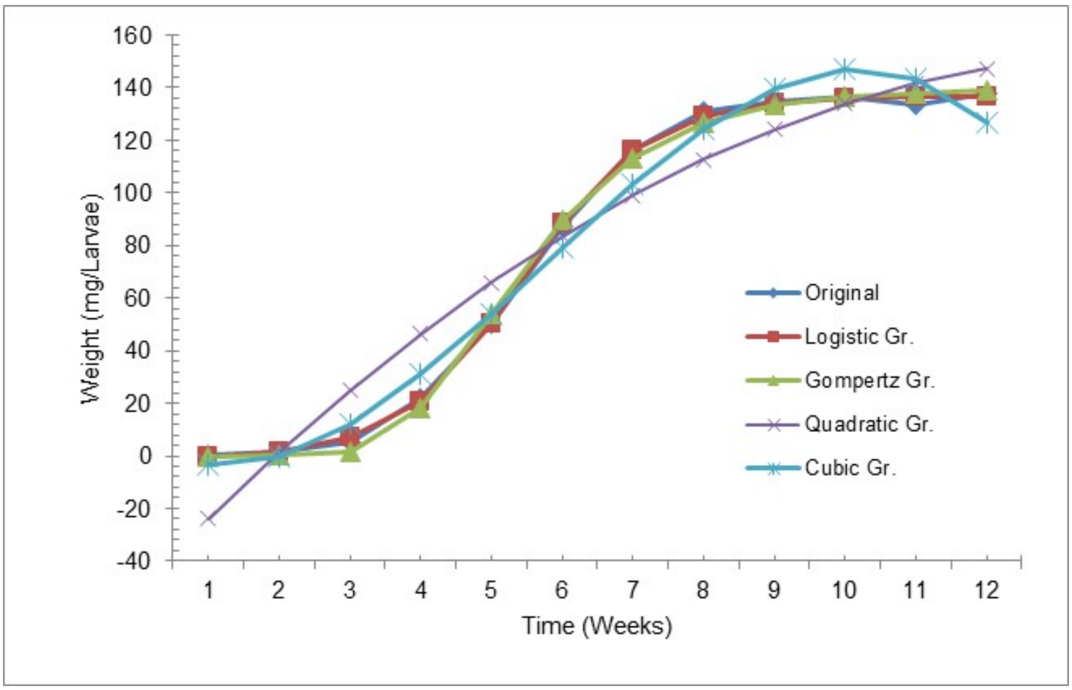

Figure 2. Growth curves of water applied larvae for different functions.

The equations of the growth curves and polynomial functions for the CONT group are given in Table 5. Accordingly, all of the R^2 values for the equations were high (92.5-99.0%). This indicated that the actual growth of *T. molitor* populations can be described to a large extent by the growth curve models and functions used.

The graphs of the general model equations and polynomial functions for the control group presented in Table 5 are shown in Figure 3. The observed values were close to the values predicted by growth curve and polynomial functions. Thus, any of these equations or functions can be successively used to describe the growth of the larval population. However, the quadratic model was less efficient for describing the growth in weeks 1, 3-5, 8, 9 and 12.

Table 5. General model of growth curve and polynomial functions of larvae in control group

Models	Equations	R^2
Gompertz	$GM(y) = 148.0exp(-\exp(4.05 - 0.70t)$	92.5
Logistic	$LM(y) = \dfrac{144.1 + (-1.16 - 144.1)}{(1 + exp((t - 6.29)/0.94))}$	92.6
Quadratic	$QM(y) = -40.0 - 0.17t^2 + 18.9t$	93.0
Cubic	$CM(y) = 27.5 - 0.49t^3 + 9.47t^2 + 33.2t$	99.0

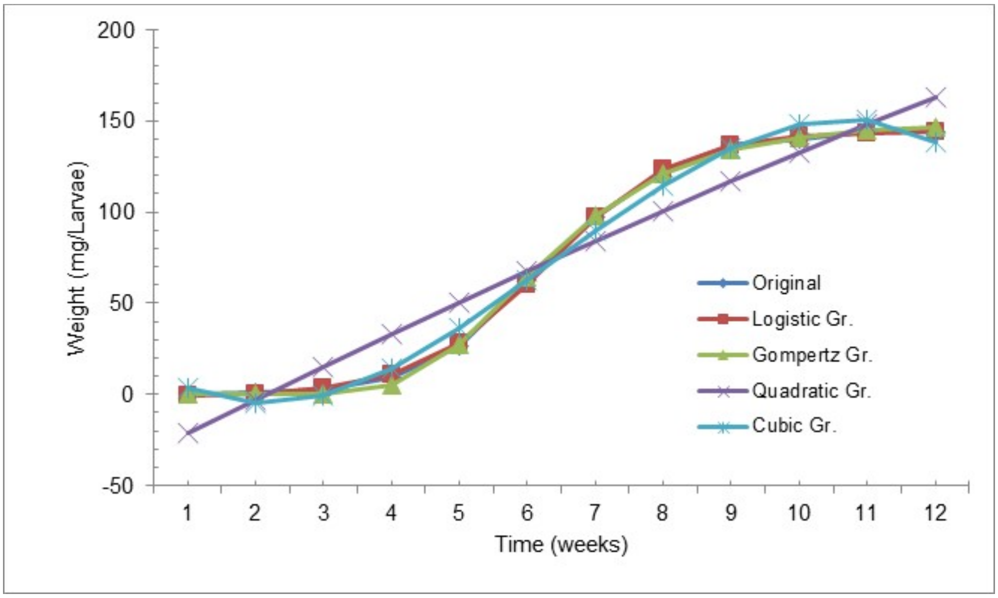

Figure 3. Growth curves of larvae in control treatment for different functions.

The individual weekly water consumption of the larvae found in the two groups are given in Table 6. Also, the differences between the weekly water consumption of larvae are presented in Table 6. There was no significant difference between the water consumption of the larvae from week 2 to 11^{th} week. However, the mean weight at week 12 (129 mg/larva) was clearly higher than in the preceding weeks. The fluctuation in the weekly mean water consumption of larvae may have due to various reasons. One reason could be the variation in the larval age. The 48-h mating period might have resulted in considerable variation in hatching time. Another variation source is the lack of synchrony in the molting of each individual. Given that larvae do not consume water during molting, this can result in significant differences in water consumption over the entire period of the experimental.

Water consumption estimations using quadratic and cubic polynomial functions were obtained from the time dependent individual water consumption data (Table 6). Water consumption estimation plots are given in Figure 4. As with the weight curves, quadratic and cubic polynomial functions had very high determination coefficients of 88.6 and 96.4%, respectively, in predicting actual water consumption of the *T. molitor*. This shows that quadratic and cubic polynomial functions can be used to predict the water consumption in in-vitro studies. The cubic function can reliably predict the water requirement of *T. molitor* until 7 weeks old, which was final growth stage before pupation. However, there were relatively higher errors in the preceding weeks due possibly to asynchrony in molting within the population.

However, the correlations between larvae age, water consumption and weights can give us different information. Correlation coefficient matrix for these parameters and water consumption is given in Table 7.

Table 6. The weekly individual water consumption of larvae in the water-supplied group

Group	Time (Week)										
	2	3	4	5	6	7	8	9	10	11	12
W1	64.9	64.8	77.5	75.0	59.1	64.0	56.1	64.5	76.2	94.9	134.2
W2	62.9	56.0	67.6	61.1	57.7	67.4	73.1	72.8	97.1	94.0	123.3
Average*	63.9 c	60.4 c	72.5 bc	68.1 bc	58.4 c	65.7 bc	64.6 bc	68.6 bc	86.6 bc	94.4 b	128.8 a

* Means followed by the same letter are not significantly different ($p < 0.05$).

Table 7. Pearson's correlation coefficients between larval age, weight and individual water consumption

Parameters	Larval age (Weeks)	Larval weight (mg)
Larval age	1	
Larval weight	0.72**	1
Water consumption	0.93**	0.45*

* and ** indicate significance at $p < 0.05$ and ** at $p < 0.01$, respectively.

The correlation coefficients clearly revealed that there were linear relationships among larvae age, larvae weight and water consumption. There was a similar type of relation between larvae weight and water consumption.

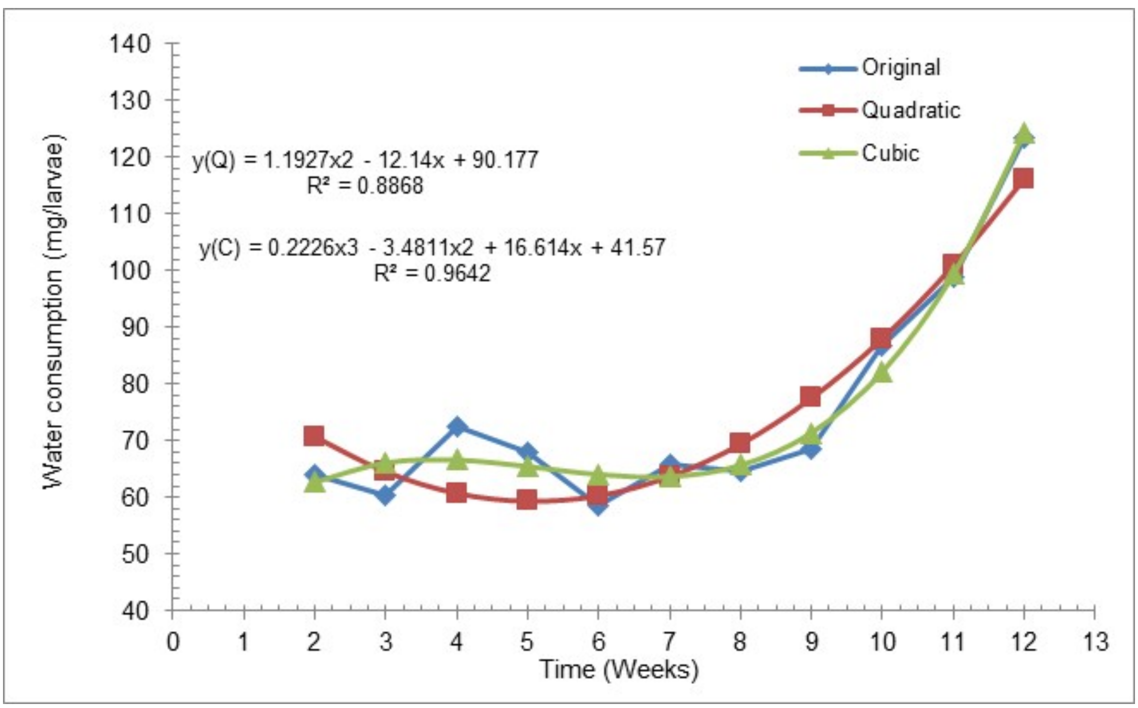

Figure 4. Weekly water consumption plots of quadratic and cubic polynomial functions.

Conclusions

Gompertz and logistic growth curves, and quadratic and cubic polynomial functions can be successfully used to describe the growth performance of mealworms in both experimental groups. The water requirements of *T. molitor* ranged from 54.4 to 94.4 mg per week with a weekly mean of 70.3 mg. Water consumptions is likely to be linearly related to larvae weight and age.

Using a water diffuser to meet the water needs of *T. molitor* larvae, which is a model organism and can be an alternative protein source in animal feed, can advance pupal development by up to 2 weeks in mealworm cultivation. This means an earlier larval harvest or shorter cultivation period, which would have economic benefits for commercial production. Direct supplementation of water requirement of mealworm larvae by means of a water diffuser can result in a shorter intergenerational period than the control group. This is an important feature for such model organisms. The usage of water diffuser can reduce labor costs as well as enable supplementation of mealworm diets with a variety of water-soluble nutrients, such as amino acids and carbohydrates.

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