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Orijinal araştırma (Original article)

Per os feeding with antisera specific to peritrophic matrix proteins stimulates larval growth in *Mamestra configurata* Walker (Lepidoptera: Noctuidae)

Peritrofik matriks proteinlerine spesifik antiserumların ağız yoluyla yedirilmesinin *Mamestra configurata* Walker (Lepidoptera: Noctuidae) larva gelişimine etkisi

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Summary

Peritrophic matrix (PM) is an acellular, porous sheath composed of chitin and proteins that lines the midgut epithelial cells. The PM serves as a barrier against food particles, pathogens and toxins. Several studies in dipteran systems revealed that antibodies specific to PM proteins retard insect development by binding to their target antigens in the PM and blocking PM pores. To test whether antisera specific to PM proteins also inhibit larval growth in a lepidopteran system, antisera specific to chitin deacetylase 1, insect intestinal mucin 2, insect intestinal mucin 4 and PM protein 1 were fed to 2nd instar *Mamestra configurata* Walker (Lepidoptera: Noctuidae) larvae, a major pest of Brassica plants in North America. Interestingly, all larvae feeding on antisera gained more weight than the larvae feeding on the diet containing non-immune sera or no antiserum. The anti-McPM1 and anti-McIIM4 antisera treatments showed the highest larval weight gains, followed by the anti-McCDA1 and anti-McIIM2 antisera treatments. The interaction of treatment with time was found significant by the 6th day and the interaction of concentration with time was found significant only by the 12th day. No difference was found between the larval weights from all treatments at concentrations of 1 or 4%.

Key words: Antiserum, feeding, peritrophic matrix, peritrophin, chitin deacetylase

Özet

Peritrofik matriks (PM) mide epitel hücreleri boyunca uzanan, kitin ve proteinden oluşan gözenekli ve hücresiz bir yapıdır. PM besin partikülleri, patojenler ve toksinlere karşı bir bariyer görevi görmektedir. Dipter sistemlerdeki çeşitli araştırmalar PM proteinlerine spesifik antibadilerin PM'deki hedef antijenlerine bağlanıp PM gözeneklerini tıkayarak böcek gelişimini geciktirdiğini göstermiştir. PM proteinlerine spesifik antibadilerin lepidopter bir sistemde de larva gelişimini engelleyip engellemediğinin belirlenmesi amacıyla, kitin deasetilaz 1, böcek barsak musini 2, böcek barsak musini 4 ve PM proteini 1 proteinlerine spesifik antiserumlar, Kuzey Amerika'daki Brassica bitkilerinin ana zararlısı durumunda olan *Mamestra configurata* Walker (Lepidoptera: Noctuidae)'nın 2. dönem larvalarına yedirilmiştir. İlginç olarak bu larvalar, antiserum içermeyen veya normal serum içeren besin üzerinde beslenen larvalara göre daha fazla ağırlık kazanmıştır. Anti-McPM1 and anti-McIIM4 antiserum denemeleri en yüksek larva ağırlık artışına neden olurken, anti-McCDA1 and anti-McIIM2 antiserum denemeleri daha az ağırlık artışına neden olmuştur. Antiserum-zaman ilişkisi 6. günden itibaren önemli bulunurken, konsantrasyon-zaman ilişkisi sadece 12. günde önemli bulunmuştur. Tüm antiserum uygulamalarının % 1 veya % 4'lük konsantrasyonlarındaki larva ağırlık artışları arasındaki fark önemli bulunmamıştır.

Anahtar sözcükler: Antiserum, beslenme, peritrofik matriks, peritrofin, kitin deasetilaz

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Introduction

Many insects contain an acellular, porous material lining the midgut epithelium and surrounding the midgut bolus called the peritrophic matrix (PM) (Hegedus et al., 2009). The PM compartmentalizes the midgut into two functional compartments (Terra et al., 1979); the endoperitrophic and ectoperitrophic spaces. The former is the midgut bolus including ingested food materials and digestive enzymes surrounded by the PM. The latter is the smaller region between the PM and the midgut epithelial cells which contains hydrolized food and other classes of digestive proteases (e.g. exopeptidases) involved in further digestion.

The PM has essential roles in insect physiology, such as protection of epithelial cells from physical damage by food particles and serving as a barrier against pathogens, parasites and toxins (Hegedus et al., 2009). The PM is permeable and allows digestive enzymes and nutrients to pass between the midgut compartments (Terra & Ferreira, 1994), as well as increases the efficiency of digestive processes by recycling enzymes through a countercurrent flow (Caldeira et al., 2007).

The PM is composed of chitin microfibrills and proteins (Hegedus et al., 2009). PM proteins have been the focus of many studies due to their essential roles in PM function. These proteins can be structural (peritrophins) or non-structural (enzymes) (Toprak et al., 2010c). Peritrophins have been proposed to contribute to PM stability by interlocking the chitin microfibrills via their chitin binding domains (e.g. non-mucin peritrophins), as well as to the protection of the PM against proteolysis via their mucin domains (e.g. insect intestinal mucins-IIMs) (Toprak et al., 2010c). PM-associated enzymes are primarily involved in digestion (e.g. serine proteases, insect intestinal lipases) and chitin-modification (e.g. chitin deacetylases-CDAs) (Toprak et al., 2010c).

Efforts have concentrated on the development of insect control strategies targeting the PM due to its essential roles in digestive physiology and its accessibility through *per os* feeding. One promising approach used antibodies (immune system-related proteins called immunoglobulins-IgG) specific to PM extracts or proteins (East et al., 1993; Casu et al., 1997; Tellam & Eisemann, 1998). These antibodies bind to their target antigens in the PM and block PM pores, this in turn inhibits nutrient uptake and/or digestive enzyme passage between the endo- and ectoperitrophic spaces (East & Eisemann, 1993; Casu et al., 1997). An anti-peritrophin-44 antibody ingested by *Lucilia cuprina* (Diptera: Calliphoridae) larvae inhibited the movement of small (6 nm) gold particles from the midgut lumen to the midgut epithelial cells (Casu et al., 1997). Furthermore, ingestion of antisera against peritrophins or PM extracts from *L. cuprina* (East et al., 1993; East & Eisemann, 1993; Casu et al., 1997) and *Chrysomya bezziana* (Diptera: Calliphoridae) (Sukarsih et al., 2000a; Natalia et al., 2007) inhibited the growth of the corresponding dipteran larvae.

Although targeting PM proteins by antibodies as an insect control strategy is promising, the studies are restricted to dipteran systems; and such hypothesis has not been tested in a lepidopteran system. To examine the effects of antisera on larval growth in a lepidopteran system, *Mamestra configurata* Walker (Lepidoptera: Noctuidae), a major pest of *Brassica napus* (canola) in North America, was used in the current study due to its well-defined PM structure (Shi et al., 2004; Toprak et al., 2008, 2010a, b, c). The effects of antisera specific to chitin deacetylase 1 (McCDA1) (Toprak et al., 2008), insect intestinal mucin 2 (McIIM2) (Toprak et al., 2010b), insect intestinal mucin 4 (McIIM4) (Toprak et al., 2010b) and peritrophic matrix protein 1 (McPM1) (Shi et al., 2004) at different concentrations (1 and 4%) on *M. configurata* larval growth are presented in the study.

Material and Methods

Insects and antisera

Mamestra configurata larvae were maintained at 21± 1 °C under a 16 h light/8 h dark photoperiod and fed *ad libitum* on artificial diet (Bucher & Bracken, 1976).

Four previously developed antisera anti-McCDA1 (Toprak et al., 2008), anti-McIIM2, anti-McIIM4 (Toprak et al., 2010b) and anti-McPM1 (Shi et al., 2004) and non-immune rabbit serum were used in this study. Anti-McCDA1 and anti-McIIM2 antisera were developed using recombinant proteins, while anti-McIIM4 and anti-McPM1 antisera were developed using derived peptides. Briefly, all antisera were developed using 100 mg of antigen in rabbits that were boosted twice. The antisera were shown to have high titres and to be highly specific to their antigens in western blot analyses (Shi et al., 2004; Toprak et al., 2008; 2010b). Protein concentrations were determined by Bradford assays (Bradford, 1976) to verify that the sera had equivalent amounts of total protein.

Biossays

Each antiserum, as well as the non-immune serum, was mixed homogeniously at 1 and 4% into the artificial diet (Bucher & Bracken, 1976) in a Waring blender when the temperature was below 40 °C. The diets were poured into insect rearing cups, cooled at room temperature for 2 h to solidify and kept at 4 °C until used.

In the bioassays, 10 2nd instar *M. configurata* larvae were used for each concentration and for each antiserum. Newly molted larvae were starved for 4 h and then allowed to feed on the artificial diets containing the antisera. In parallel, diet without serum and diet containing non-immune serum served as controls for each concentration. The diet cups were changed every two days. Total weight of the 10 larvae from each cup was recorded every 2 days over a period of 12 days, which roughly corresponds to the end of *M. configurata* larval stage under these experimental conditions. All bioassays were conducted in an insect rearing cabinet set to 21±1 °C and replicated three times.

Stastical analyses

One way-ANOVA was used to compare the weight of larvae fed antisera and non-immune serum to those fed diet alone for each concentration (1 ve 4%) at each time point. A repeated measures ANOVA was used to examine the effects of treatment and concentration over time. In this analysis, two between-subject factors: (I) treatment (anti-McCDA1, anti-McIIM2, anti-McIIM4, anti-McPM1 antisera and non-immune serum), and (II) concentration (1 ve 4%) were evaluated together with the within-subject factor: time (0, 2, 4, 6, 8, 10 and 12 days). The alpha level was set at 0.05. The larval weights for each treatment were summarized as means±standard error (SE). ANOVA was performed using SPSS software (version 18, SPSS, Inc, Chicago, IL, USA) and means were compared using the MSTAT package for Duncan's Multiple Range Test.

Results and Discussion

The initial statistical analysis using one way ANOVA revealed that larval weights when feeding on diets containing anti-McIIM2, anti-McIIM4 and anti-McPM1 were significantly higher compared to the non-immune serum or diet only treatments through the 2nd to 12th day at a concentration of 1%. Similarly, the anti-McIIM2, anti-McIIM4 and anti-McPM1 treatments showed significantly higher larval weights than other treatments by the 8th day at a serum concentration of 4% ($p<0.05$) (Table 1). However, anti-McCDA1 treatment was also found to cause a significant increase in larval weight by 12th day at the concentration of 4%. This analysis indicated that treatments of antisera specific to PM proteins could be

considered as “a real treatment” and revealed a significant stimulative effect on larval growth. Furthermore, no significant difference was found between the regular diet and non-immune sera treatments for both concentrations at each time point, indicating both could be considered as a negative control (Table 1). Since the primary goal of this research was to understand the effects of antisera specific to PM proteins at two concentrations on larval development, the non-immune serum which was also applied at the same concentrations, was considered to be the control for the second statistical analysis. Therefore, the second analysis using repeated measures ANOVA focused on the comparison of antisera specific to PM proteins amongst each other, as well as with the non-immune serum (negative control) at the same concentrations.

Repeated measures ANOVA indicated the absence of a triple interaction between treatment, concentration and time ($p>0.05$). However, dual interactions such as the interaction of treatment with time and the interaction of concentration with time were found ($p<0.05$). The presence of such interactions suggests that treatment-time interactions have occurred at both concentrations, and concentration-time interactions occurred for all treatments. No interaction of treatment with concentration was found ($p>0.05$), suggesting treatment-concentration interaction did not occur at any of the time points.

Treatment and time interactions revealed that the larval weights by the 6^{th} day (shown by “D”) are significantly higher than the weights by the 2^{nd} day (shown by “E”) for each antiserum treatment ($p<0.05$) (Table 2). This trend continued until end of the experiment; thus, the larval weights significantly increased by the 8^{th} (shown by “C”), 10^{th} (shown by “B”) and 12^{th} (shown by “A”) days ($p<0.05$). A similar trend occured for the non-immune serum also by the 6^{th} day (shown by “CD”) and continued until end of the experiment ($p<0.05$). When the antisera treatments were compared to the non-immune sera treatment, the larval weight differences were significant by the 8^{th} day (shown by small letters) and this trend continued until end of the experiment. By end of the experiment, all antisera caused more larval weight gain than the non-immune serum treatment ($p<0.05$) (Table 2). This suggests that the larval weight gain is not due to the use of antisera as a protein source because the non-immune serum had a similar amount of protein. Therefore, antisera specific to PM proteins stimulated the growth of *M. configurata* larvae. By contrast, in various dipteran systems antisera developed against crude PM extracts (East et al., 1993; East & Eisemann, 1993; Eisemann & Binnington, 1994; Sukarsih et al., 2000a) or PM proteins (Casu et al., 1997; Tellam & Eisemann, 1998; Tellam et al., 2001; Natalia et al., 2007) inhibited insect development. Furthermore, insect mortality was reported in some cases (East & Eisemann, 1993; Sukarsih et al., 2000a); however, all larvae succesfully pupated in the current study. To our knowledge, the only study reporting an increase in insect weight was conducted by Sukarsih et al. (2000b) where *C. bezziana* larvae feeding on sheep vaccinated with recombinant peritrophins, Cb15, Cb42 or Cb48, showed marginally greater weight gain and survival. Furthermore, *in vitro* feeding of *C. bezziana* larvae with anti-Cb15 or anti-Cb42 antiserum also produced small increases in larval weight. Therefore, immune related larval response may differ not only between different insect orders, but also between the species of the same order, although the antisera targeted structurally and functionally similar proteins, peritrophins in this case.

When the antisera were compared to one another, the anti-McPM1 and anti-McIIM4 antisera treatments (shown by “a”) showed the highest larval weight gains, which were followed by the anti-McCDA1 and anti-McIIM2 antisera treatments (shown by “b”) (Table 2). It is not clear why the anti-McPM1 and anti-McIIM4 antisera produced higher larval weights than the others. However, their target antigens, McPM1 and McIIM4, are major peritrophins and predominantly found in the PM (Shi et al., 2004; Toprak et al., 2010b), suggesting these antisera interact more intimately with the PM. By contrast, McCDA1 is loosely associated with the PM and acts more as a secreted enzyme (Toprak et al., 2008), therefore, anti-McCDA1 antiserum is likely to interact only transiently with the PM.

Table 1. Total larval weight[I] (g) of the *Mamestra configurata* larvae fed various anti-PM protein antisera (Anti-McCDA1, Anti-McIIM2, Anti-McIIM4, Anti-McPM1), non-immune serum (at concentrations of 1 and 4%) or diet alone at each time point (0, 2, 4, 6, 8, 10, 12 days)

Day	Concentration	Treatment[II]	P-Value[III]	Mean±SE Mean
0	1%	Only Diet	0.728	0,0188±0,0003
		Non-Immune serum		0,0195±0,0014
		Anti-McCDA1		0,0200±0,0008
		Anti-McIIM2		0,0204±0,0022
		Anti-McIIM4		0,0196±0,0015
		Anti-McPM1		0,0217±0,0006
	4%	Only Diet	0.954	0,0188±0,0003
		Non-Immune serum		0,0188±0,0018
		Anti-McCDA1		0,0198±0,0005
		Anti-McIIM2		0,0195±0,0004
		Anti-McIIM4		0,0195±0,0011
		Anti-McPM1		0,0199±0,0012
2	1%	Only Diet	0.006**	0,0238±0,0007(B)
		Non-Immune serum		0,0239±0,0005(B)
		Anti-McCDA1		0,0249±0,0013(B)
		Anti-McIIM2		0,0301±0,0012(A)
		Anti-McIIM4		0,0273±0,0012(A)
		Anti-McPM1		0,0275±0,0011(A)
	4%	Only Diet	0.876	0,0237±0,0007
		Non-Immune serum		0,0249±0,0014
		Anti-McCDA1		0,0251±0,0003
		Anti-McIIM2		0,0247±0,0011
		Anti-McIIM4		0,0253±0,0007
		Anti-McPM1		0,0251±0,0012
4	1%	Only Diet	0.007**	0,0439±0,0034(C)
		Non-Immune serum		0,0452±0,0011(C)
		Anti-McCDA1		0,0542±0,0050(C)
		Anti-McIIM2		0,0767±0,0098(A)
		Anti-McIIM4		0,0643±0,0063(B)
		Anti-McPM1		0,0656±0,0017(B)
	4%	Only Diet	0.235	0,0439±0,0034
		Non-Immune serum		0,0557±0,0083
		Anti-McCDA1		0,0561±0,0019
		Anti-McIIM2		0,0616±0,0030
		Anti-McIIM4		0,0568±0,0026
		Anti-McPM1		0,0570±0,0058
6	1%	Only Diet	0.000**	0,0845±0,0042(CD)
		Non-Immune serum		0,0786±0,0017(D)
		Anti-McCDA1		0,0967±0,0067(BC)
		Anti-McIIM2		0,1220±0,0046(A)
		Anti-McIIM4		0,1054±0,0081(AB)
		Anti-McPM1		0,1127±0,0030(AB)
	4%	Only Diet	0.131	0,0845±0,0042
		Non-Immune serum		0,0926±0,0133
		Anti-McCDA1		0,0954±0,0030
		Anti-McIIM2		0,1078±0,0055
		Anti-McIIM4		0,1064±0,0012
		Anti-McPM1		0,1103±0,0077
8	1%	Only Diet	0.002**	0,1372±0,0029(C)
		Non-Immune serum		0,1208±0,0073(C)
		Anti-McCDA1		0,1605±0,0210(BC)
		Anti-McIIM2		0,2536±0,0303(A)
		Anti-McIIM4		0,2073±0,0262(AB)
		Anti-McPM1		0,2237±0,0115(AB)
	4%	Only Diet	0.029*	0,1372±0,0029(C)
		Non-Immune serum		0,1534±0,0302(BC)
		Anti-McCDA1		0,1765±0,0032(ABC)
		Anti-McIIM2		0,2195±0,0182(A)
		Anti-McIIM4		0,2041±0,0054(AB)
		Anti-McPM1		0,2102±0,0223(A)

Table 1. (continued)

Day	Concentration	Treatment[II]	P-Value[III]	Mean±SE Mean
10	1%	Only Diet	0.000**	0,2674±0,0064(CD)
		Non-Immune serum		0,2293±0,0103(D)
		Anti-McCDA1		0,3408±0,0493(BC)
		Anti-McIIM2		0,4653±0,0241(A)
		Anti-McIIM4		0,4046±0,0317(AB)
		Anti-McPM1		0,4351±0,0117(A)
	4%	Only Diet	0.012*	0,2674±0,0064(B)
		Non-Immune serum		0,3061±0,0634(B)
		Anti-McCDA1		0,3436±0,0099(AB)
		Anti-McIIM2		0,4313±0,0272(A)
		Anti-McIIM4		0,4195±0,0099(A)
		Anti-McPM1		0,4322±0,0365(A)
12	1%	Only Diet	0.049*	0,4503±0,0092(BC)
		Non-Immune serum		0,3933±0,0280(C)
		Anti-McCDA1		0,5778±0,0790(ABC)
		Anti-McIIM2		0,5427±0,0938(ABC)
		Anti-McIIM4		0,6911±0,0691(A)
		Anti-McPM1		0,6341±0,0583(AB)
	4%	Only Diet	0.000**	0,4503±0,0092(C)
		Non-Immune serum		0,4514±0,0640(C)
		Anti-McCDA1		0,6204±0,0055(B)
		Anti-McIIM2		0,6314±0,0323(B)
		Anti-McIIM4		0,7544±0,0218(A)
		Anti-McPM1		0,7596±0,0337(A)

[I] Means followed by different letters in the same line are significantly different at the 0.05 level using Duncan's test

[II] Antisera or non-immune serum were incorporated into artificial diet at 1% or 4% supplied in insect rearing cups. Ten 2nd instar larvae were fed on artificial diet containing sera in each cup and the bioassays were repeated three times

[III] P values denoted by * and ** indicate significant difference between the treatments and control (diet alone) at the 0.05 and 0.01 levels, respectively.

Table 2. Average total larval weight[I] (g) of the *Mamestra configurata* larvae fed various anti-PM protein antisera or a non-immune rabbit serum

	Mean±SE Mean				
Time (day)	Non-immune serum[II]	Anti-McCDA1[II]	Anti-McIIM2[II]	Anti-McIIM4[II]	Anti-McPM1[II]
0	0,0192±0,0010 (a) (E)	0,0199±0,0004 (a) (E)	0,0199±0,0010 (a) (E)	0,0196±0,0008 (a) (E)	0,0208±0,0007 (a) (E)
2	0,0244±0,0007 (a) (E)	0,0250±0,0006 (a) (E)	0,0274±0,0014 (a) (E)	0,0263±0,0007 (a) (E)	0,0263±0,0009 (a) (E)
4	0,0504±0,0044 (a) (DE)	0,0551±0,0024 (a) (DE)	0,0692±0,0057 (a) (DE)	0,0606±0,0035 (a) (DE)	0,0613±0,0033 (a) (DE)
6	0,0856±0,0068 (a) (CD)	0,0960±0,0033 (a) (D)	0,1149±0,0045 (a) (D)	0,1059±0,0036 (a) (D)	0,1115±0,0037 (a) (D)
8	0,1371±0,0157 (c) (C)	0,1685±0,0099 (bc) (C)	0,2366±0,0175 (a) (C)	0,2057±0,0120 (ab) (C)	0,2169±0,0116 (ab) (C)
10	0,2677±0,0335 (c) (B)	0,3422±0,0225 (b) (B)	0,4483±0,0179 (a) (B)	0,4120±0,0152 (a) (B)	0,4337±0,0171 (a) (B)
12	0,4224±0,0338 (c) (A)	0,5991±0,0367 (b) (A)	0,5870±0,0486 (b) (A)	0,7228±0,0354 (a) (A)	0,6968±0,0412 (a) (A)

[I] Means followed by different capital letters in the same column or small letters in the same line are significantly different at the 0.05 level using Duncan's test

[II] Antisera or non-immune serum were incorporated into artificial diet at 1% and 4% supplied in insect rearing cups. Ten 2nd instar larvae were fed on artificial diet containing antiserum in each cup and the bioassays were repeated three times.

The interaction of concentration with time was found to be significant, but only at one time point (by the 12th day) (Table 3). Indeed, no interaction of treatment with concentration was found as mentioned above, suggesting that there is no significant difference between the larval weights from all treatments at concentrations of 1 or 4%. Likewise, there was no correlation between larval weight and antibody titre in *C. bezziana* (Sukarsih et al., 2000a). However, higher antibody concentrations led to reduced larval weight in *L. cuprina* (Casu et al., 1997), suggesting the effect of antiserum titer could differ even in species of the same order.

Table 3. Average total larval weight[I] (g) of the *Mamestra configurata* larvae fed two different concentrations of antisera specific to PM proteins or non-immune serum

Time	Mean±SE Mean	
(day)	Concentration	
	1%	4%
0	0,0202±0,0006 (a)	0,0195±0,0004 (a)
	(F)	(F)
2	0,0267±0,0007 (a)	0,0250±0,0004 (a)
	(EF)	(EF)
4	0,0612±0,0036 (a)	0,0575±0,0019 (a)
	(E)	(E)
6	0,1031±0,0044 (a)	0,1025±0,0034 (a)
	(D)	(D)
8	0,1932±0,0149 (a)	0,1927±0,0097 (a)
	(C)	(C)
10	0,3750±0,0249 (a)	0,3865±0,0193 (a)
	(B)	(B)
12	0,5678±0,0376 (b)	0,6434±0,0331 (a)
	(A)	(A)

[I] Means followed by different capital letters in the same column, and small letters in the same line, are significantly different at the 0.05 level using Duncan's test.

Inhibiton of the larval growth in dipteran systems has been shown to be based on the blockage of PM pores (East & Eisemann, 1993; Casu et al., 1997). For instance, the anti-peritrophin-44 antibody ingested by *L. cuprina* larvae inhibited the free movement of small 6 nm gold particles from the midgut lumen to the midgut epithelial cells (Casu et al., 1997). Such blockage prevents the passage of nutrients and digestive enzymes between midgut epithelial cells and the midgut bolus, and as a consequence retards larval development. The absence of an immune serum related inhibitory effect and the stimulation of larval growth by antisera in *M. configurata* is an unresolved question; however, several factors could have affected the outcome of these experiments. One possibility may be related to the PM structure and formation in lepidopteran larvae. The lepidopteran larval PM is a Type I PM, formed by the entire midgut and composed of multiple layers reaching a thickness of 5-20 mm (Ryerse et al., 1992; Lehane, 1997). The larval PM in the dipterans, is a Type II PM formed by a special organ called the cardia which resides at the foregut-midgut boundary. It usually lacks multiple layers and typically does not exceed 5 mm in thickness (Lehane, 1997). Therefore, it may be difficult to affect small molecule transfer in the thicker PM in lepidopteran larvae, and binding of the antisera to their antigens may not be sufficient to inhibit the larval development. Indeed, several studies on mosquitoes, which also have a Type I PM, reported no inhibitory effect of midgut-specific antibody on larval longevity (Ramasamy et al., 1992; Suneja et al., 2003), also suggesting the type of PM could be the main determinant in the inhibition of insect development by antisera.

The size of the PM pores may be another important determinant in the response of larve to immune sera. If the pores are large enough to allow the antibodies to pass through the midgut, blockage would never occur. In accordance with this, the PM pore size of most dipteran larvae is around 10 nm (Lehane, 1997), while it is 36-800 nm in various lepidopterans (Adang & Spence, 1983; Barbehenn & Martin, 1995). IgG molecules have a diameter of about 10 nm (Valentine & Green, 1967), therefore, the possibility of PM pore blockage by the antiserum is lower in lepidopterans than dipterans.

One other possibility is the degradation of the antisera in the midgut, the site of digestive proteolysis. Antibodies have a relatively long half-life in *L. cuprina* larvae, whose growth is retarded by antiserum feeding (Eisemann et al., 1993). In contrast, ingested IgG was fully digested and lost its antigen-binding activity within 4 h in the gut of the buffalo fly, *Haemotobia irritans* (Diptera: Muscidae), which lacks an immune related larval response (Allingham et al., 1998). Although *M. configurata* larvae had continuous access to artificial diet containing the antisera throughout 12 days in this study, it is well-known that *M. configurata* larval midgut is rich in digestive proteases (Hegedus et al., 2003) and these proteases are active throughout the larval development and even elevated during feeding stages (Toprak et al., 2010b). Therefore, the antisera used in this study could have lost its activity due to proteolysis.

In conclusion, *M. configurata* larvae did not reveal any developmental retardation during feeding on antisera specific to PM proteins, instead they gained more weight than the regular diet or non-immune sera treatments, which is in contrast to the reports from dipteran larval systems. The immune related-larval retardation appears to occur as a combination of multiple insect and methodology related parameters. These may be the type of PM structure, the mechanism for PM formation, as well as antiserum stability and titer. Each should be carefully examined to develop a successful insect control strategy using antisera.

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