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The Effects of 5-Aza-2´-deoxycytidine on Total Lipid and Fatty Acid Composition of *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae) and on Its Parasitized Host

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ABSTRACT

I investigated the changes in total lipid, total fatty acids, and fatty acid composition of endoparasitoid *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae) reared on *Achroia grisella* Fabr. (Lepidoptera: Pyralidae) that was exposed to various doses of 5-Aza-2´-deoxycytidine (5-Aza-dC), an epigenetically-effective agent. The combined effects of parasitism and 5-Aza-dC treatment on the total lipid and fatty acids of host larvae were also examined. 5-Aza-dC caused insignificant fluctuations in total lipid and fatty acid ratios of *A. galleriae* and parasitized host larvae (PHL). Total lipid and fatty acids reached considerably the highest values in PHL and the lowest ratios in parasitoid larvae for all of the control and experimental groups. All stages of *A. galleriae* and PHL contained the 14-24 carbon fatty acids. Palmitic acid was the most abundant fatty acid for PHL and parasitoid pupae in each testing group, while oleic acid for parasitoid larvae. 5-Aza-dC treatment caused significant changes in linolenic acid ratio for parasitoid pupa and in lignoceric acid ratio for PHL, while the percent of different fatty acid classes did not differ significantly for stages/PHL. However, eicosenoic acid could not be detected in both insect species at doses >0.1 mg/ml. There were significant differences among individual fatty acids and fatty acid classes (SFAs, UFAs, and PUFAs) for all stages/PHL. Besides, UFAs constituted the major proportion of fatty acids, whereas PUFAs were the smallest for all stages/PHL regardless of 5-Aza-dC treatment. 5-Aza-dC-induced changes for *A. galleriae-A. grisella* system seem to be considerable but the effect is reversible according to the result of fatty acid classes.

Key words: *Apanteles galleriae*, *Achroia grisella*, 5-Aza-dC, lipid, fatty acids.

INTRODUCTION

Living in a natural balance, organisms give similar reactions to similar conditions even though they have dissimilar genetic structures. For instance, frequent usage of chemicals such as medicals and pesticides has carcinogenic, teratogenic, and mutagenic effects on all living organisms. As being the first demethylating agents, 5-Aza-2´-deoxycytidine (5-Aza-dC) and 5-azacytidine (5-AzaC) also negatively affect DNA methylation by similar metabolic pathways (Osgood and Seward, 1989; Schauenstein *et al.*, 1991; Prakash and Kumar, 1997; Lantry *et al.*, 1999; Doerksen *et al.*, 2000; Sato *et al.*, 2003). Chemically synthesized initially in 1964 as a chemotherapeutic drug (Piskala and Sorm, 1964), 5-Aza-dC has a wide range of antimetabolic activities against cancer cells (Wijermans *et al.*, 2000; Christman, 2002;

Stresemann *et al.*, 2006; Gurion *et al.*, 2010). However, the influence of a chemical on an organism largely depends on the dose and the application method used. In this respect, it was shown that 5-Aza-dC and 5-AzaC could induce mutagenicity and cancer *in vivo* (Carr *et al.*, 1984; Jackson-Grusby *et al.*, 1997; Lantry *et al.*, 1999) and may be cytotoxic (Stresemann *et al.*, 2006). Hence, it becomes so important to determine the degree of toxic effects of these chemicals for all living organisms.

Investigating the effects of substances such as 5-Aza-dC on invertebrates as well as vertebrates will help us to have a better understanding the potential impact ways of toxic materials in all organisms as they descended from a common origin. However, there is little information about the extension of the toxicological effects of a cytosine analog, 5-Aza-dC especially on insects (Uçkan *et al.*, 2007; Amarasinghe *et al.*, 2014; Alvarado *et al.*, 2015). Besides, almost nothing is known about the effects of chemicals such as 5-Aza-dC on parasitoid species (Uçkan *et al.*, 2007; Pegoraro *et al.*, 2015). *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae) is a koinobiont, solitary, larval endoparasitoid of several lepidopterans, including the pyralid wax moths, *Galleria mellonella* L., *Achroia grisella* Fabr., *A. innotata* Walker, and *Vitula edmandsae* (Packard) (Shimamori, 1987; Watanabe, 1987; Whitfield *et al.*, 2001). Some data have been obtained previously on the changes of the lipid and the fatty acid ratio and composition during the development of the parasitoid, *A. galleriae* (Nurullahoğlu *et al.*, 2004; Uçkan *et al.*, 2009). The effects of 5-Aza-dC on the biological parameters of larval endoparasitoid *A. galleriae* have also been determined along the effect of this chemical on egg-adult development time of its host species, *A. grisella* (Uçkan *et al.*, 2007). 5-Aza-dC treatment increased time that is required to complete parasitoid immature development especially at 0.5 mg/ml dose. Adult longevity and size and the fecundity of parasitoid species were reduced by 5-Aza-dC exposure. The sex ratio of adults was in favor of males in 5-Aza-dC treatments. Moreover, exposure to 5-Aza-dC slightly increased the immature development of *A. grisella* and rarely caused some morphological disorders in the host such as reduced body size and curved-wings (Uçkan *et al.*, 2007). Therefore, I have developed our investigations on how this chemical affects lipid metabolism of the insects mentioned above in a host-parasitoid interaction. For the first time, the effects of 5-Aza-dC applied to host diet on total lipid, total fatty acids, and fatty acid composition were shown in *A. galleriae* and parasitized host larvae (PHL).

MATERIALS AND METHODS

Insects and bioassay

Laboratory colonies of the host, *A. grisella* and endoparasitoid, *A. galleriae* were established from adults that were collected from the honeycombs maintained from several beehives located in the vicinity of Rize, Turkey. Insect cultures and experimental groups were held in two different rooms at 25±1°C, 60 ± 5% RH, and a photoperiod of 12:12h (L:D). The details of the method for cultivating both host and parasitoid species were presented in the articles of Uçkan and Gülel (2000) and Uçkan

and Ergin (2003). Honey solution with 30% (wt:v) was used to feed adult parasitoids. Host colony was maintained with a diet described by Bronskill (1961) and modified by Sak *et al.* (2006).

Experimental procedure was designed according to Uçkan *et al.* (2007). Briefly, 5-Aza-dC (EEC no.219-089-4, Sigma-Aldrich, St. Louis, MO) solutions prepared in distilled water were used in all bioassays as water source of host diet**.** Early instars of *A. grisella* were exposed to four different doses (0.1, 0.5, 0.75, and 1.0 mg/ml) of 5-Aza-dC to evaluate the effects of the chemicals on lipid and fatty acid characteristics of stages/PHL (defining larvae and pupae of *A. galleriae* and PHL).

Lipid extraction

Lipid analyses were conducted with the stages of *A. galleriae* and parasitized 14- to 16-d-old *A. grisella* larvae (PHL) in which the third (last) instar parasitoids were collected. I selected random samples of 35 third instars and 25 pupae of *A. galleriae* and 35 PHL, which were obtained from 5-Aza-dC treated and chemical-free host individuals for analyzing. Total lipid and fatty acid extractions were repeated three times at different times for stages/PHL with the same number given above. Total wet weight of each group was calculated, and insects were kept in 3 ml chloroform-methanol (2:1 vol:vol) at -20 ^{o}C until extractions. The lipid fractions in stages/PHL were extracted and total lipid and fatty acid contents were determined using the method described by Folch *et al.* (1957). Total lipid and fatty acid contents as a percentage of wet weight were calculated. Thereafter, lipid extracts were brought to 3 ml with chloroform-methanol (2:1 vol:vol) and kept at -20^{o}C until fatty acid analysis.

Fatty acid analysis-gas chromatography

Fatty acid analysis was conducted at TUBITAK-BUTAL Research Centre (Bursa, Turkey) by using the direct methylation method. Briefly, solvents (chloroform-methanol) of lipid extracts were dried under N_2. The residue was dissolved with 1 ml methanol, mixed with 3 ml HCl, and kept in water bath for 60 min at 95^{o}C. Then 88% NaCl was added to the mixture and vortex-mixed. The esterified and methylated fatty acids were analyzed with a Perkin Elmer AutoSystem XL gas chromatograph equipped with a hydrogen flame ionization detector (FID). A fused silica capillary column (SP 2560; 100 m x 0.25 mm id, 0.20 µm film; Supelco, Supelco Park, PA) was used to separate the fatty acid samples. The FID parameters were optimized as follows: 260^{o}C for detector temperature, 35 ml/min flow rate for hydrogen, and 450 ml/min flow rate for air. The column oven condition was scheduled as 120°C for 5 min, 4°C increase/min up to 240°C, and 240°C for 25 min. The helium flow rate in column was 1 ml/min. Identification of fatty acid methyl esters was achieved by comparison of their retention times with those of standards attained from Supelco. The percentage of each peak area for each fatty acid was calculated for each of the tested group.

Statistical analysis

Data for total lipid, total fatty acids, individual fatty acids, and fatty acid classes were subjected to one-way analysis of variance (ANOVA) to determine the main effects of

5-Aza-dC on each stage and PHL. The relationship between total lipid and stages/PHL, total fatty acids and stages/PHL, and the differences among the levels of individual fatty acids or fatty acid classes in all stages/PHL were also compared with one-way ANOVA for each control and 5-Aza-dC treated groups. Differences were separated by Tukey's honestly significant post hoc test (HSD) according to homogeneity of variances (SPSS Inc. 1999). An arcsine square-root transformation was performed on percentage values before analyses but untransformed means were presented. Results were considered statistically significant when $P<0.05$.

RESULTS

Total lipid and fatty acids

The ratio of total lipid and fatty acids as percentages of wet weight for different stages of *A. galleriae* and PHL are presented in Figs. 1 and 2. The lipid values of PHL slightly increased on exposure to 5-Aza-dC while the fatty acid ratios tended to decrease in general. The dose, 0.75 mg/ml had the highest lipid value about 19% in PHL. 5-Aza-dC treatment caused both increases and decreases at the percent lipid and fatty acid of stages when compared to the untreated group. The metabolite contents did not differ significantly between controls and 5-Aza-dC treated groups in PHL (lipid ratio: $F=1.385$; $df=4, 10$; $P>0.05$, fatty acid ratio: $F=2.214$; $df=4, 10$; $P>0.05$), parasitoid larvae (lipid ratio: $F=2.038$; $df=4, 10$; $P>0.05$, fatty acid ratio: $F=1.313$; $df = 4,10$; $P>0.05$), and parasitoid pupae (fatty acid ratio: $F=1.930$; $df=4,10$; $P>0.05$). However, there was a significant decrease only at 0.5 mg/ml according to 0.1 mg/ml in term of lipid values of parasitoid pupae ($F=4.100$; $df=4,10$; $P<0.05$).

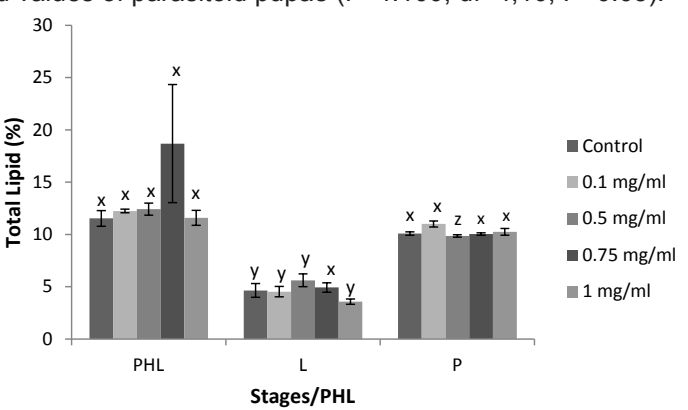

Fig. 1. 5-Aza-dC-related changes in total lipids of *A. galleriae* and *A. grisella*. PHL, parasitized host larva; L, parasitoid larva; P, parasitoid pupa. Stages/PHL (x-z) labeled with different letters are significantly different (Tukey's HSD test, $P<0.05$).

When stages and PHL were compared in terms of lipid and fatty acid ratios (Table 1), I found that both lipid and fatty acids of *A. galleriae* larvae were considerably lower than PHL (Figs. 1 and 2). However, the differencies were only insignificant for lipids at 0.75 mg/ml and for fatty acids at 0.1 and 1 mg/ml 5-Aza-dC doses. Besides, the percent

of lipid and fatty acids significantly increased from larval to pupal stage especially at 1 mg/ml dose. The increase rate was not statistically important for lipids at 0.75 mg/ml and for fatty acids at 0.1 mg/ml (Figs. 1 and 2). Another observation was slightly decreasing of lipid and fatty acid ratios in parasitoid pupa according to PHL, which was only significant at 0.5 mg/ml. The only exception of this tendency was significantly higher fatty acid value of parasitoid pupa according to PHL at 1 mg/ml (Fig. 2).

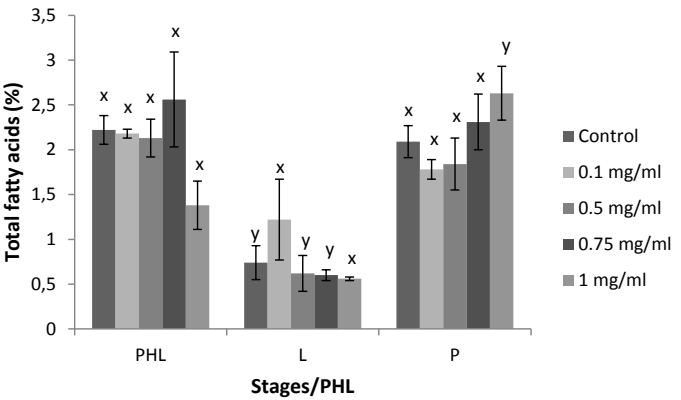

Fig. 2. 5-Aza-dC-related changes in total fatty acids of *A. galleriae* and *A. grisella*. PHL, parasitized host larva; L, parasitoid larva; P, parasitoid pupa. Stages/PHL (x-y) labeled with different letters are significantly different (Tukey's HSD test, P<0.05).

Table 1. ANNOVAs of the differences among stages/PHL for each testing group in terms of total lipid and fatty acid ratios.

	Statistics	Control	0.1 mg/ml	0.5 mg/ml	0.75 mg/ml	1 mg/ml
Lipid	F	38.877	144.677	49.930	4.520	82.551
	df	2, 6	2, 6	2, 6	2, 6	2, 6
	P	0.000	0.000	0.000	0.063	0.000
Fatty Acid	F	21.033	3.255	11.333	9.049	20.340
	df	2, 6	2, 6	2, 6	2, 6	2, 6
	P	0.002	0.110	0.009	0.015	0.002

Fatty acid composition

A total number of nine, sixteen, and twenty different fatty acids were identified in parasitoid larvae, pupae, and PHL, respectively. For stages/PHL, $C_{14:0}$, $C_{22:1}$, $C_{22:2}$, and $C_{24:1}$ fatty acids were evaluated statistically but were not shown in the tables since the ratios of these fatty acids were almost zero. Moreover, I could not detect $C_{14:1}$, $C_{15:0}$, $C_{15:1}$, $C_{17:1}$, $C_{18:3}$, $C_{20:2}$, $C_{20:3}$, $C_{20:4}$, $C_{21:0}$, $C_{22:6}$, and $C_{23:0}$ fatty acids for parasitoid larvae and $C_{14:1}$, $C_{15:1}$, $C_{20:3}$, and $C_{21:0}$ fatty acids for parasitoid pupae (Table 2-4). I found that all stages of *A. galleriae* and PHL contained the 14-24 carbon fatty acids.

Six fatty acids dominated in the composition in PHL and parasitoid pupae while five in parasitoid larvae. These fatty acids differed in order of plenitude in stages/PHL and were palmitic ($C_{16:0}$), palmitoleic ($C_{16:1}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$), eicosenoic ($C_{20:1}$), and arachidic ($C_{20:0}$) acids. Palmitic acid was considerably the most abundant fatty acid for PHL (except for 0.5 mg/ml) and parasitoid pupae in all control and experimental groups, but oleic acid was significantly the highest one for parasitoid larvae (Table 2-4).

There were significant fluctuations among the levels of individual fatty acids in all stages/PHL (Table 2-5). However, 5-Aza-dC treatment caused significant changes only in the ratio of linolenic acid ($C_{18:3}$) and lignoceric acid ($C_{24:0}$) for parasitoid pupa and PHL, respectively (Table 2, 4). Besides, eicosenoic acid slightly decreased at 0.1 mg/ml dose and could not be detected at higher doses of 5-Aza-dC in PHL. Similarly, 5-Aza-dC also caused a prominent reduction in the ratio of eicosenoic acid in larval and pupal stages of parasitoid species (Table 3, 4). Moreover, $C_{20:1}$ was the fifth most dominant fatty acid in controls of both PHL and parasitoid pupae. However, arachidic acid arose to the fifth abundant fatty acid level at all doses of 5-Aza-dC in place of eicosenoic acid in PHL and parasitoid pupae (Table 2, 4). Tables 2-4 also show the fatty acid pattern of stages/PHL. There were significant differences between fatty acid classes (SFAs, UFAs, and PUFAs) (except for PHL at 1.0 mg/ml) (Table 2-4, 6) but the percent of different fatty acid classes did not differ significantly with respect to 5-Aza-dC application in all stages/PHL (Table 2-4, 7). Furthermore, UFAs constituted the major proportion of fatty acids in all stages/PHL except for 1 mg/ml in PHL, whereas PUFAs were the smallest, regardless of 5-Aza-dC treatment.

CONCLUSIONS AND DISCUSSION

Parasitoid species alter the intermediary metabolism of their hosts and parasitization induces metabolic alterations in the fat body of hosts (Thompson, 1993; Salvador and Cônsoli, 2008). However, the regulation is species and host tissue-specific among parasitoid species (Thompson, 1993; Rivers and Denlinger, 1994; Bischof and Ortel, 1996) and do not cause a selective reduction or elevation of the lipid or fatty acid levels in the host body at any time (Thompson, 1982; Nurullahoğlu *et al.*, 2004; Uçkan *et al.*, 2009). In the *A. galleriae-A. grisella* (Nurullahoğlu *et al.*, 2004) and *A. galleriae-G. mellonella* (Uçkan *et al.*, 2009) systems parasitization did not cause a significant change in total lipid and fatty acids of host species. Hence, I decided to examine only the PHL for 5-Aza-dC-dependent effects on lipid and fatty acid ratios of host insect, *A. grisella* in the current paper. When all stages/PHL were evaluated collectively, the most noticeable changes (but insignificant) were an increase rate of 62% at 0.75 mg/ml for lipid values and a reduction ratio of 38% at 1 mg/ml for fatty acid values compared to controls in PHL. Besides, lipid and fatty acid ratios were sorted according to the abundance as PHL>parasitoid pupa>parasitoid larva in all groups except for 1 mg/ml dose being as parasitoid pupa>PHL>parasitoid larva. At this point, higher doses of 5-Aza-dC seem to have an adverse effect on PHL in terms of total lipid and fatty acid ratios. Significant decreasing of lignoceric acid ($C_{24:0}$) ratio at 0.75 and

1 mg/ml 5-Aza-dC doses according to control and 0.1 mg/ml groups and destruction of eicosenoic acid ($C_{20:1}$) at doses >0.1 mg/ml support this assumption for PHL.

Table 2. 5-Aza-dC-related changes in fatty acid composition (%) of parasitized host larva [a,b].

Fatty acids	Control	0.1 mg/ml	0.5 mg/ml	0.75 mg/ml	1 mg/ml
$C_{14:1}$	0.02 ± 0.02w a	---	0.13 ± 0.13w a	---	---
$C_{15:0}$	0.06 ± 0.03w a	0.04 ± 0.02w a	---	---	0.02 ± 0.02w a
$C_{15:1}$	---	0.02 ± 0.02w a	---	---	---
$C_{16:0}$	25.53 ± 0.51w b	25.02 ± 0.07w b	25.37 ± 0.55w b	27.17 ± 0.73w a	28.43 ± 1.44w b
$C_{16:1}$	21.99 ± 1.51w b	22.17 ± 0.59w b	21.31 ± 1.40w c	23.00 ± 1.17w b	15.54 ± 7.80w c
$C_{17:1}$	2.33 ± 1.23w a	2.73 ± 0.09w ac	---	---	---
$C_{18:0}$	2.34 ± 0.29w a	2.25 ± 0.18w ac	2.45 ± 0.13w a	2.28 ± 0.07w c	2.52 ± 0.40w a
$C_{18:1}$	14.09 ± 0.31w c	14.40 ± 0.21w d	15.55 ± 0.79w d	14.41 ± 0.63w d	16.54 ± 2.05w c
$C_{18:2}$	24.82 ± 1.89w b	24.69 ± 0.83w b	25.98 ± 1.00w b	24.76 ± 0.60w b	28.14 ± 3.53w b
$C_{18:3}$	0.88 ± 0.13w a	0.93 ± 0.18w ac	1.12 ± 0.28w a	0.88 ± 0.18w ce	0.60 ± 0.31w a
$C_{20:0}$	2.19 ± 2.08w a	2.15 ± 2.11w ac	6.49 ± 0.44w e	6.15 ± 0.45w f	7.12 ± 0.53w ac
$C_{20:1}$	4.04 ± 2.04w a	3.75 ± 1.88w c	---	---	---
$C_{20:3}$	---	---	---	---	0.21 ± 0.21w a
$C_{20:4}$	0.47 ± 0.03w a	0.61 ± 0.09w ac	0.76 ± 0.35w a	0.71 ± 0.38w ce	0.25 ± 0.13w a
$C_{21:0}$	---	---	---	0.03 ± 0.03w e	0.03 ± 0.02w a
$C_{22:0}$	0.44 ± 0.06w a	0.43 ± 0.01w ac	0.12 ± 0.12w a	0.28 ± 0.14w e	0.12 ± 0.12w a
$C_{22:6}$	---	---	0.16 ± 0.16w a	0.09 ± 0.09w e	0.08 ± 0.08w a
$C_{23:0}$	0.26 ± 0.14w a	0.22 ± 0.11w a	---	---	---
$C_{24:0}$	0.55 ± 0.08w a	0.58 ± 0.11w ac	0.23 ± 0.13wx a	0.08 ± 0.08x e	---
$C_{20:2}$	---	---	0.33 ± 0.17w a	0.16 ± 0.16w e	0.40 ± 0.21w a
SFAs[c]	31.36 ± 2.71w ab	30.69 ± 2.13w a	34.65 ± 0.54w ab	35.99 ± 0.70w a	38.24 ± 2.33w a
UFAs	42.47 ± 4.21w b	43.08 ± 2.68w b	37.00 ± 1.92w b	37.41 ± 0.77w a	32.08 ± 5.81w a
PUFAs	26.17 ± 1.98w a	26.23 ± 0.57w a	28.35 ± 1.57w a	26.60 ± 1.10w b	29.68 ± 3.48w a

[a]Data are means ± SE of three replicates using 35 parasitized host larva per replicate.

[b]Means in the same horizontal row and group (fatty acids or fatty acid classes) followed by the same letter (w-x) and means in the same vertical column and group followed by the same letter (a-f) are not significantly different from each other (Tukey HSD testi, P>0.05).

[c]SFAs, saturated fatty acids; UFAs, unsaturated fatty acids; and PUFAs, polyunsaturated fatty acids.

The lipid and fatty acid content of insects' show inter- and intraspecific variation (Downer, 1985) and defining the differences among insect species and different developmental stages contribute to the literature about insect physiology because of various metabolic functions (Ogg and Stanley-Samuelson, 1992; Ogg *et al.*, 1993; Çakmak *et al.*, 2007). Although PHL and parasitoid larvae had almost the same lipid

ratio, total fatty acids of parasitoid larvae were significantly higher than PHL according to our previous data (Nurullahoğlu *et al.*, 2004; Uçkan *et al.*, 2009). In contrast, I found in this study that both lipid and fatty acid values of *A. galleriae* larvae were considerably lower than PHL in control groups. The reason for this difference might be attributed to the use of different nutrients for host feeding, which was synthetic diet here versus natural blackened comb in our former studies (Nurullahoğlu *et al.*, 2004; Uçkan *et al.*, 2009). Considering all the results up to now, the significant difference in total fatty acids between the hosts and the parasitoid may also be related to variances among insect species. Another observation in this study was expressive increasing in lipid and fatty acid ratios during the pupal stage relative to the larval stage especially at 1 mg/ml dose.

Table 3. 5-Aza-dC-related changes in fatty acid composition (%) of *A. galleriae* larvae [a,b].

Fatty acids	Control	0.1 mg/ml	0.5 mg/ml	0.75 mg/ml	1 mg/ml
$C_{14:1}$	---	---	---	---	---
$C_{15:0}$	---	---	---	---	---
$C_{15:1}$	---	---	---	---	---
$C_{16:0}$	12.95 ± 6.50w ab	16.26 ± 2.42w a	11.65 ± 4.51w a	20.67 ± 0.85w a	17.99 ± 0.43w a
$C_{16:1}$	15.77 ± 0.84w a	11.02 ± 2.75w ab	7.73 ± 4.96w a	19.79 ± 2.04w a	16.60 ± 1.10w a
$C_{17:1}$	---	---	---	---	---
$C_{18:0}$	5.92 ± 1.15w abc	5.38 ± 1.02w bcd	2.62 ± 1.84w a	5.31 ± 0.60w b	5.47 ± 0.58w b
$C_{18:1}$	59.12 ± 5.67w d	54.28 ± 6.11w e	72.29 ± 10.95wb	46.94 ± 0.56w c	53.58 ± 2.95w c
$C_{18:2}$	4.77 ± 1.79w bc	9.26 ± 1.08w abc	4.45 ± 3.69w a	6.48 ± 2.50w b	6.04 ± 2.76w b
$C_{18:3}$	---	---	---	---	---
$C_{20:0}$	0.20 ± 0.20w c	0.19 ± 0.19w d	0.54 ± 0.54w a	---	0.31 ± 0.31w d
$C_{20:1}$	---	0.65 ± 0.65w cd	---	---	---
$C_{20:3}$	---	---	---	---	---
$C_{20:4}$	---	---	---	---	---
$C_{21:0}$	---	---	---	---	---
$C_{22:0}$	0.84 ± 0.84w c	0.18 ± 0.18w d	---	0.16 ± 0.16w d	---
$C_{22:6}$	---	---	---	---	---
$C_{23:0}$	---	---	---	---	---
$C_{24:0}$	0.43 ± 0.43w c	2.79 ± 2.79w bcd	0.71 ± 0.71w a	0.65 ± 0.65w d	---
$C_{20:2}$	---	---	---	---	---
SFAs[c]	20.34 ± 4.34w a	24.80 ± 5.52w a	15.53 ± 6.07w a	26.79 ± 1.03w a	23.78 ± 0.39w a
UFAs	74.89 ± 6.13w b	65.95 ± 4.52w b	80.03 ± 7.10w b	66.73 ± 2.36w b	70.18 ± 2.37w b
PUFAs	4.77 ± 1.79w a	9.26 ± 1.08w a	4.45 ± 3.69w a	6.48 ± 2.50w c	6.04 ± 2.76w c

[a]Data are means ± SE of three replicates using 35 third instars per replicate.

[b]Means in the same horizontal row and group (fatty acids or fatty acid classes) followed by the same letter (w) and means in the same vertical column and group followed by the same letter (a-e) are not significantly different from each other (Tukey HSD testi, P>0.05).

[c]SFAs, saturated fatty acids; UFAs, unsaturated fatty acids; and PUFAs, polyunsaturated fatty acids.

Table 4. 5-Aza-dC-related changes in fatty acid composition (%) of *A. galleriae* pupae [a,b].

Fatty acids	Control	0.1 mg/ml	0.5 mg/ml	0.75 mg/ml	1 mg/ml
$C_{14:1}$	---	---	---	---	---
$C_{15:0}$	---	---	---	0.04 ± 0.04w a	0.01 ± 0.01w a
$C_{15:1}$	---	---	---	---	---
$C_{16:0}$	25.42 ± 0.73w a	26.33 ± 0.30w a	26.75 ± 0.37w a	26.32 ± 0.52w b	27.22 ± 0.27w b
$C_{16:1}$	22.30 ± 1.00w ab	21.77 ± 0.65w b	20.78 ± 1.38w b	22.52 ± 1.09w c	20.39 ± 0.43w c
$C_{17:1}$	2.47 ± 1.25w c	2.14 ± 1.08w cd	---	---	---
$C_{18:0}$	2.79 ± 0.29w c	2.46 ± 0.14w cd	3.08 ± 0.34w c	3.31 ± 0.22w d	3.19 ± 0.15w d
$C_{18:1}$	21.12 ± 0.33w b	21.30 ± 0.29w b	21.97 ± 0.50w b	21.93 ± 0.48w c	22.16 ± 0.12w e
$C_{18:2}$	20.37 ± 1.26w b	21.40 ± 1.00w b	21.22 ± 0.40w b	19.35 ± 1.42w e	21.50 ± 0.56w e
$C_{18:3}$	0.03 ± 0.03wx c	0.02 ± 0.02w c	0.15 ± 0.01y d	0.12 ± 0.01xy a	0.11 ± 0.00wxy a
$C_{20:0}$	1.67 ± 1.64w c	3.11 ± 1.56w d	5.50 ± 0.44w e	5.96 ± 0.91w f	5.16 ± 0.43w f
$C_{20:1}$	3.55 ± 1.81w c	1.33 ± 1.33w cd	---	---	---
$C_{20:3}$	---	---	---	---	---
$C_{20:4}$	0.02 ± 0.02w c	0.09 ± 0.06w c	0.22 ± 0.09w d	0.14 ± 0.10w a	0.05 ±0.01w a
$C_{21:0}$	---	---	---	---	---
$C_{22:0}$	0.21 ± 0.02w c	0.05 ± 0.05w c	0.16 ± 0.08w d	0.22 ± 0.02w a	0.06 ± 0.06w a
$C_{22:6}$	---	---	---	0.08 ± 0.08w a	---
$C_{23:0}$	0.05 ± 0.02w c	---	---	---	---
$C_{24:0}$	---	---	0.06 ± 0.06w d	---	---
$C_{20:2}$	---	---	0.10 ± 0.10w d	---	0.15 ± 0.08w a
SFAs[c]	30.14 ± 2.43w a	31.95 ±1.74w a	35.56 ± 0.87w a	35.85 ± 0.78w a	35.64 ± 0.23w a
UFAs	49.44 ± 3.65w b	46.53 ± 1.47w b	42.75 ± 0.90w b	44.45 ± 0.61w b	42.55 ± 0.56w b
PUFAs	20.42 ± 1.30w a	21.52 ± 1.02w c	21.69 ± 0.32w c	19.69 ± 1.38w c	21.81 ± 0.53w c

[a]Data are means ± SE of three replicates using 25 pupae per replicate.

[b]Means in the same horizontal row and group (fatty acids or fatty acid classes) followed by the same letter (w-y) and means in the same vertical column and group followed by the same letter (a-f) are not significantly different from each other (Tukey HSD testi, P>0.05).

[c]SFAs, saturated fatty acids; UFAs, unsaturated fatty acids; and PUFAs, polyunsaturated fatty acids.

Referring to Figs. 1 and 2, the reason of this accumulation for lipid and fatty acid levels are seen to be different. Observable reduction in the percent of lipids at 1 mg/ml dose for larvae compared to the control, while almost unchanged values for pupae indicate that 5-Aza-dC affects the lipid levels of larvae much more than pupae at this dose. Unlike lipids, total fatty acid values of pupae increased visibly at 1 mg/ml dose according to the control. However, larvae had almost constant levels of fatty acids for the same dose compared to pupae. In this case, it is plausible to assume that pupae were more sensible than larvae.

Significant increasing of linolenic acid ($C_{18:3}$) ratio at 0.5 mg/ml dose with respect to control and 0.1 mg/ml groups and destruction of eicosenoic acid ($C_{20:1}$) at doses

>0.1 mg/ml support this assumption for parasitoid pupa. It is showed in a number of studies that the changes in lipid and fatty acid milieu are closely correlated with the physiological needs of insects and are influenced by environmental factors such as pesticides and other chemicals (Stanley-Samuelson *et al.*, 1988; Ogg and Stanley-Samuelson, 1992; Sak *et al.*, 2006).

Table 5. ANNOVAs of the differences among individual fatty acids for each testing group in *A. galleria* and parasitized host larva (PHL).

Stages/PHL	Statistics	Control	0.1 mg/ml	0.5 mg/ml	0.75 mg/ml	1 mg/ml
Parasitoid Larvae	F	46.040	51.793	28.744	227.274	179.365
	df	23, 48	23, 48	23, 48	23, 48	23, 48
	P	0.000	0.000	0.000	0.000	0.000
Parasitoid Pupae	F	156.255	250.982	641.544	381.268	2256.853
	df	23, 48	23, 48	23, 48	23, 48	23, 48
	P	0.000	0.000	0.000	0.000	0.000
PHL	F	100.140	175.941	393.183	575.438	22.849
	df	23, 48	23, 48	23, 48	23, 48	23, 48
	P	0.000	0.000	0.000	0.000	0.000

Table 6. ANNOVAs of the differences among fatty acid classes for each testing group in *A. galleria* and parasitized host larva (PHL).

Stages/PHL	Statistics	Control	0.1 mg/ml	0.5 mg/ml	0.75 mg/ml	1.0 mg/ml
Parasitoid Larvae	F	68.150	49.365	49.525	218.460	246.233
	df	2, 6	2, 6	2, 6	2, 6	2, 6
	P	0.000	0.000	0.000	0.000	0.000
Parasitoid Pupae	F	31.263	75.943	206.014	163.919	517.057
	df	2, 6	2, 6	2, 6	2, 6	2, 6
	P	0.001	0.000	0.000	0.000	0.000
PHL	F	7.175	19.007	9.311	45.188	1.141
	df	2, 6	2, 6	2, 6	2, 6	2, 6
	P	0.026	0.003	0.014	0.000	0.380

Table 7. ANNOVAs of 5-Aza-dC-related changes for fatty acid classes in *A. galleria* and parasitized host larva (PHL).

Stages/PHL	Fatty acid classes	F	df	P
Parasitoid Larvae	SFAs	1.119	4, 10	0.400
	UFAs	1.457	4, 10	0.286
	PUFAs	0.571	4, 10	0.690
Parasitoid Pupae	SFAs	3.325	4, 10	0.056
	UFAs	2.451	4, 10	0.114
	PUFAs	0.855	4, 10	0.522
PHL	SFAs	2.768	4, 10	0.087
	UFAs	1.617	4, 10	0.245
	PUFAs	0.602	4, 10	0.670

A. galleriae and PHL lacked some of fatty acids including especially those of 20 carbons. Ogg *et al.* (1993) and Uscian *et al.* (1992) stated that C_{20} components could be missed while analyzing lipid samples on gas chromatography with flame ionization detection. Therefore, using FID could be the reason for the absence of these fatty acids in this study. Although I could not observe here, we had found $C_{14:0}$, $C_{14:1}$, $C_{15:0}$, $C_{18:3}$, $C_{20:2}$, $C_{21:0}$, $C_{22:1}$, and $C_{22:2}$ fatty acids in our previous studies (Nurullahoğlu *et al.*, 2004; Uçkan *et al.*, 2009) for both host and parasitoid species. The most important reason for these differences may be the use of synthetic diet to feed the host species in this study, unlike our previous studies using natural blackened comb for nourishing (Nurullahoğlu *et al.*, 2004; Uçkan *et al.*, 2009). Fatty acid composition of an insect can reveal metabolic pathways and the differences in insect feeding can cause a different distribution in the fatty acid content. Since the life of parasitoids depend on host species, their fatty acid composition could be affected by the feeding conditions of host indirectly. It seems that our data show fourteen different fatty acids for PHL in all experimental groups but seven for parasitoid larvae are agree with this assumption. It is known that accumulation or consumption of fatty acids could be examined in the stress conditions (Hoch *et al.*, 2002). On the other hand, re-occurrence of certain fatty acids in the pupal stage suggests that the adverse effect of 5-Aza-dC on the larval stage seems to be compensated.

The fatty acids have a variety of function such as sources of energy in the form of triacylglycerols and are structural components of membranes in insects (Downer, 1985). The distribution of fatty acids alters greatly based on different factors such as nutrition and development (Ogg and Stanley-Samuelson, 1992; Bozkuş, 2003; Çakmak *et al.*, 2007). We had shown before that each developmental stage of *A. galleriae* had a different fatty acid distribution (Nurullahoğlu *et al.*, 2004; Uçkan *et al.*, 2009). The results of this study support this statement because of the differences of larval and pupal fatty acids. There are also a lot of studies showing insects having different fatty acids in different stages and sexes (Ogg and Stanley-Samuelson, 1992; Bashan *et al.*, 2002; Bozkuş, 2003; Çakmak *et al.*, 2007). Having various functions, the rate of fatty acids would be expected to fluctuate according to physiological needs of an insect. In earlier studies we have found that all stages and sexes of *A. galleriae* and PHL contained 10-24 carbon fatty acids (Nurullahoğlu *et al.*, 2004; Uçkan *et al.*, 2009). Similarly, I detected 14-24 carbon fatty acids for same stages in this study. It is known that carbon number within this range is characteristic for parasitic hymenopteran species (Thompson and Barlow, 1974). Our early results (Nurullahoğlu *et al.*, 2004; Uçkan *et al.*, 2009) and current findings, which were showing the major proportion of fatty acids was palmitic, palmitoleic, stearic, oleic, and linoleic acids, were also in agreement with those reported for other parasitoid species (Bracken and Barlow, 1967; Thompson and Barlow, 1974). All these data support the generalization about insects that the major fatty acid component is usually C_{16} and C_{18} saturated and unsaturated fatty acids (Candy and Kilby, 1975). I found that the most abundant fatty acid of PHL was palmitic acid ($C_{16:0}$) in all control and experimental groups (except for 0.5 mg/ml dose). In contrast, palmitoleic acid ($C_{16:1}$) was the major fatty acid in abundance in *A.*

grisella larvae (both parasitized and nonparasitized) according to our previous data (Nurullahoğlu *et al.*, 2004). Using a synthetic diet instead of natural blackened comb for feeding the host insect might be the reason of this difference. Moreover, oleic acid ($C_{18:1}$) was considerably the most abundant fatty acid in all stages/sexes of *A. galleriae* even if the parasitoid was grown in different host insects (Nurullahoğlu *et al.*, 2004; Uçkan *et al.*, 2009). In this study, I also showed that oleic acid constituted the major proportion of fatty acids in the larval stage of *A. galleriae* unlike to pupal stage containing palmitic acid at the highest rate. This difference between larva and pupa may result from the use of 5-Aza-dC and synthetic nutrients simultaneously. More definitive conclusions on this subject can be obtained by the use of natural blackened comb and synthetic diet at the same experimental design.

The host-parasitoid interaction is an ideal model for studying how chemicals affect the life of an organism depending on another one. Aside from the effects of development, it is known that fatty acid compositions of whole insect change in response to the change in dietary conditions (Stanley-Samuelson *et al.*, 1988). Certain of the parasitoid wasps appear to match their fatty acid profiles almost the same of the host (Nurullahoğlu *et al.*, 2004; Uçkan *et al.*, 2009). Thus, chemicals could cause changes in the metabolite composition of the parasitoid species indirectly through host physiology (Sak *et al.*, 2006). Eicosenoic acid ($C_{20:1}$), one of the six dominant fatty acid determined in PHL constituted above 4% of the fatty acid composition in control groups. However, the ratio of this acid fell below 4% at 0.1 mg/ml dose and could not be detected at higher doses of 5-Aza-dC. Similarly, eicosenoic acid did not be observed in both control and experimental groups except for 0.1 mg/ml doses for parasitoid larvae feeding with host sources. Thus, the results showed that 5-Aza-dC caused a prominent reduction in the ratio of eicosenoic acid in the larval stage of parasitoid species. Moreover, arachidic acid ($C_{20:0}$) arose to the fifth most dominant fatty acid level at all doses of 5-Aza-dC instead of eicosenoic acid in both PHL and parasitoid pupae. At the same time, the ratio of eicosenoic acid down to a very low value, such as 1%, at 0.1 mg/ml dose in pupae and could not be detected at higher doses of 5-Aza-dC as in PHL. It seems that the negative effects of 5-Aza-dC also continued in the later developmental stages of *A. galleriae*. The situation of reduction and then total destruction of eicosenoic acid may be attributed to the 5-Aza-dC-induced adverse effect in diet quality resulting in an intervention of adequate food supply from the host (Uçkan and Ergin, 2002; Uçkan *et al.*, 2007). The changes in the value of eicosenoic acid mentioned above and the considerable rising of arachidic acid in PHL and parasitoid pupae may indicate that fatty acid composition could be rearranged during different developmental stages of an insect as a result of chemical-induced stress. It is known that insect lipids and fatty acids are affected by a number of neuroendocrinological, physiological and environmental influences, which are closely interrelated (Downer, 1985) and insects are able to modify their fatty acid compositions to suit the physiological requirements for overcoming the stress conditions.

Unsaturated fatty acids constituted the major proportion of fatty acids except for one case, whereas PUFAs were the smallest in all stages/PHL regardless of 5-Aza-dC

treatment. Our results are fully consistent with our previous study (Nurullahoğlu *et al.*, 2004) concerning fatty acid classes. On the other hand, SFAs were the most dominant fatty acids in parasitoid larva, pupa, and PHL in *A. galleriae-G. mellonella* (Uçkan *et al.*, 2009) system. This difference can be attributed to the use of different insect species as a host and may be related to variance among insect species. Unlike to single fatty acids, 5-Aza-dC did not cause significant changes in the percent of different fatty acid classes for all stages/PHL. In the matter of lipid metabolism at this point, it is not possible to state that 5-Aza-dC causing irreversible adverse effect on insects examined here. Poirier *et al.* (2014) demonstrated that 5-AzaC selectively and strongly reduced the expression of key genes that regulate lipid metabolism unlike 5-Aza-dC. Thus, insignificant changes in the ratio of total lipid, total fatty acids, and fatty acid classes might be resulted from inefficiency of 5-Aza-dC on special genes controlling the lipid metabolism. When evaluating the issue from another angle, the crucial changes in the ratio of linolenic, lignoceric, eicosenoic, and arachidic acids indicate the effectiveness of 5-Aza-dC on lipid metabolism of *A. galleriae* and PHL. However, insignificant differences in fatty acid classes bring to mind that these insects may be changing the synthesis and the degradation pathways in metabolism in order to protect the total fatty acid composition in body content. The author is currently evaluating the effect of 5-Aza-dC on the lipid and fatty acids of *A. galleriae* adults and on the biological parameters of *A. grisella*. The findings of how 5-Aza-dC affects the lipid and fatty acid content of parasitoid adults will make this assumption more reasonable. Besides, considering the effects of the drug on DNA, the results obtained from this study requires a more global perspective of metabolism eliminating the harmful effects of 5-Aza-dC in *A. galleriae* and *A. grisella*; thus it is necessary to integrate the possible effects of 5-Aza-dC on protein, carbohydrate, and also other substrate levels and to recognize how one metabolite impinges upon another.

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