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Research Article

Effects of some Antimicrobial Agents on the Total Protein Content of the Endoparasitoid *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae)

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Abstract: The effects of thirteen antimicrobial agents that have different structures and modes of action on the total protein content of pupae of the hymenopterous endoparasitoid, *Pimpla turionellae* L., were investigated by rearing the larvae aseptically on chemically defined synthetic diets. These effects varied according to the their kind and dietary levels. The protein content of the pupae was significantly increased by penicillin, streptomycin, rifampicin, tetracycline hydrochloride, lincomycin hydrochloride, methyl p-hydroxybenzoate, cycloheximide and sodium benzoate, while it was decreased by nystatin. The other tested antimicrobial agents had no significant effects on the total protein content of the insect. Depending on dietary levels, most of the tested agents also had an effect on the body wet weight of the insect. It was demonstrated that pupae from larvae fed on diets with some levels of tested antimicrobial agents had a lower body wet weight but contained more protein content than those of the control diet. The diet with 45 mg of nystatin caused a significant decrease in the wet weight of the pupae and their protein content.

Key Words: *Pimpla turionellae*, antimicrobial agents, total protein, endoparasitoid, insects

Bazı Antimikrobiyal Ajanların Endoparazitoid *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae)'nın Total Protein Miktarına Etkileri

Özet: Endoparazitoid zarkanatlı türü, *Pimpla turionellae* L., kimyasal yapısı bilinen sentetik besin ortamlarında aseptik şartlarda beslenerek farklı yapı ve etkiye sahip olan onüç antimikrobiyal ajanın böceğin pup evresindeki total protein miktarına etkileri incelendi. Bu etkiler antimikrobiyal ajanın çeşidine ve besindeki miktarına göre değişmektedir. Pupların total protein miktarı penisillin, streptomisin, rifampisin, tetrasiklin hidroklorür, linkomisin hidroklorür, metil p-hidroksibenzoat, sikloheksimid, ve sodyum benzoat tarafından önemli derecede artırılırken nistatin ise bu miktarı azaltmıştır.Diğer denenen ajanlar ise böceğin protein miktarına önemli bir etki yapmamıştır.Antimikrobiyal ajanlar aynı zamanda besinsel miktarlarına bağlı olarak böceğin yaş vucut ağırlığı üzerinde etkili olmuştur. Bu ajanların bazı miktarlarını içeren besinler ile beslenen larvaların oluşturduğu pupların kontrol besinindekilere göre daha düşük vucut ağırlığına sahip oldukları ancak bunların daha fazla protein içerdikleri tespit edilmiştir. Nistatinin 45 mg'ının ilave edildiği besin ise pupların yaş ağırlığında ve bunların protein miktarında önemli bir azalmaya neden olmuştur.

Anahtar Sözcükler: *Pimpla turionellae*, antimikrobiyal ajanlar, total protein, endoparazitoid, böcekler

Introduction

The importance of parasitic hymenopterous insects in biological control programs has increased the investigation of their mass-propagation under artificial conditions. Developed chemically defined synthetic diets considerably facilitated such attempts and made it possible to determine the nutritional requirements of these insects (1-9). These diets also provided us with understandings of the effects of dietary ingredients on the total body composition of these insects (10,11). In recent years, some antimicrobial agents have been widely used in these diets to control microbial contamination. Although the effects of these agents on survival and development have been determined in some hymenopterous species (12-15), their effects on the total protein or other biochemical content of these insects have not been investigated.

Hymenopterous insects, like other animals, need protein for the maintenance of the body, as well as for growth and reproduction (16). The protein requirement of the hymenopterous endoparasitoid *Pimpla turionellae* is provided in the form of free amino acids (6). In other words, the insect synthesizes its own proteins from the dietary free amino acids and accumulates them into body tissues. It is obvious that the whole body or tissue protein content of an insect could be considered a valuable criterion for the evaluation of the nutritional importance of any dietary nutrients (17-19). A few studies on the effects of some dietary nutrients on the protein content of some parasitic hymenopterous (11) and dipterous species (20) have been done.

The nutritional importance of antimicrobial agents was already demonstrated in economically important higher animals. Some agents, which are added into their food, promoted their growth and production (21-24). So far, little work has been done on the effects of antimicrobial agents on insects. Most of this work was done on silkworm nutrition to improve silk production and sericulture for industry. For example, some antimicrobial agents caused an improvement in silk production and other economic traits of *Philosamia ricini* (Boisd) (25-27) and *Bombyx mori* L. (28).

This similarity between higher animals and laboratory-reared insects, such as silkworms, indicates that antimicrobial agents may also have nutritional importance for the artificial feeding of parasitic hymenopterous insects. This is the most important gap in our understanding of the nutrition of these insects that are utilized as successful biological control agents.

The present study was carried out to determine the effects of thirteen antimicrobial agents on the total protein content of an endoparasitoid hymenopterous insect, *Pimpla turionellae*.

Materials and Methods

Stock cultures of *P. turionellae* were maintained in the laboratory on the greater wax moth, *Galleria mellonellae* at a temperature of 23±1°C, relative humidity of about 75±5% and with 16 h photoperiod. Adults were fed daily with a 50% honey solution and *G. mellonellae* pupae hemolymph.

The synthetic diet described by Yazgan (6) was used for rearing the larvae of *P. turionellae*. The diet contained an amino acid mixture, lipid mixture, an inorganic salt mixture, glucose, RNA and a mixture of water soluble vitamins. The methods used in the preparation of stock solutions of dietary ingredients, a diet from these stocks, sterilization techniques, adding sterilized vitamin-glucose and antimicrobial agent solutions into the diet, dispensing the diets into test tubes, obtaining the sterilized larvae and their inoculation onto diets and preparation of gelatine capsules for pupation were the same as those described elsewhere (15).

Some of the antimicrobial agents used in this work, such as tetracycline hydrochloride, chloramphenicol, erythromycin, trimethoprim, cycloheximide and sodium benzoate, were obtained from Sigma Chemical Co. The others were obtained from different firms such as penicillin and streptomycin from Claus & Huth Ltd., Methyl p-hydroxybenzoate from Koch Light Lab., rifampicin from Koçak Medicine Co., Lyncomycin hydrochloride from Eczacıbaşı Medicine Co., and Cephradine monohydrate and nystatin from Bristol-Myers Squibb Inc.

Agents tested were expressed as mg/100 ml of the diet. The desired amounts were dissolved in bidistilled water, then sterilized through a 0.22 μm membrane filter and added into the diet before gel formation. The volume of the diet was established by adjusting the amount of water in the diet. One newly hatched larva was inoculated into each test tube, which contained a given diet of about 0.5 ml. The larvae used in these experiments were obtained from unfertilized females. In other words, only male larvae were used in these experiments. The experiments were replicated three times with ten larvae per replication. The feeding experiments were done under the same laboratory conditions as mentioned for the stock culture of *P. turionellae*.

For protein extraction, young male pupae (one –day old) that appeared to be the best and the most similar to one another in every respect were taken from each replication of the experiments. The wet weight of these pupae was individually determined. Whole young pupae were ground in 10% trichloroacetic acid solution with a homogenizer (Arthur H. Thomas Co. U.S.A.) for periods of 5 min. Following centrifugation, at a speed of 3500 rpm for periods of 15 min, tissue proteins were precipitated and the supernatant was discarded, and the procedure was repeated three times. Finally, the protein pellet was homogenized in 96% ethyl alcohol then centrifuged at a speed of 3500 rpm and the supernatant was discarded. This procedure for removing tissue lipids was repeated three times. Finally the protein pellet was redissolved in bidistilled water and completed to a certain volume. The extraction procedure was made according to Plummer (29). The amount of total protein was determined spectrophotometrically (Jenway Ltd., England) at a wavelength of 600 nm with Folin-phenol reagent (30). Bovine serum albumin was used as a standard protein. Protein extraction and determination were separately made in the pupae from each replication of the experiments.

The effects of the tested diets with different levels of antimicrobial agents on the total protein content of the insect were measured by determining the average amount and percentage of total protein according to the wet weight of the young pupae of the *P. turionellae*. Data were evaluated by analysis of variance (31). To determine significant differences between means, Duncan's (32) multiple range test was used. Differences were considered significant when F exceeded 0.01.

Results

The effects of the tested levels of thirteen antimicrobial agents, including antibiotics and antifungal agents, on the total protein content of the insect are shown in the Table.

Table. The effects of antimicrobial agent levels on the total protein content of *P. turionellae* pupae

Antimicrobial agent levels mg/100 ml diet	Pupal wet weight (mg) (mean* ± S.D.)#		Amount of total protein (mg) (mean* ± S.D.)#		Percentage of total protein per wet weight (mean* ± S.D.)#	
Penicillin						
00 (+)	5.0 ± 0.20	a	1.01 ± 0.04	a	20.5 ± 1.80	a
10	7.7 ± 1.10	b	1.45 ± 0.03	b	19.3 ± 3.10	a
20	7.1 ± 1.30	b	1.48 ± 0.02	b	21.4 ± 3.70	a
30	7.5 ± 0.60	b	1.43 ± 0.01	b	19.1 ± 1.90	a
40	5.0 ± 0.10	a	1.48 ± 0.02	b	29.9 ± 2.00	b
Streptomycin						
00 (+)	7.1 ± 1.3	a	1.39 ± 0.3	a	19.7 ± 1.7	a
10	7.9 ± 0.2	a	1.34 ± 0.1	a	17.0 ± 0.2	a
20	6.0 ± 1.5	a,b	0.94 ± 0.1	a,b	16.2 ± 2.0	a
30	4.1 ± 0.5	b,c	0.80 ± 0.1	b	19.9 ± 1.5	a
40	5.3 ± 0.5	b	1.54 ± 0.2	c	29.9 ± 1.6	b
Rifampicin						
00 (+)	7.2 ± 2.0	a	1.65 ± 0.2	a	24.0 ± 3.8	a
2.5	6.7 ± 1.5	a	2.53 ± 0.4	b	38.3 ± 5.1	a,b
5.0	6.9 ± 1.2	a	2.76 ± 0.2	b	40.6 ± 5.0	a,b
7.5	6.5 ± 1.5	a	2.75 ± 0.1	b	44.0 ± 7.1	b
10.0	4.4 ± 0.4	b	2.76 ± 0.4	b	62.1 ± 1.2	c
Tetracycline						
00 (+)	9.3 ± 0.60	a	1.38 ± 0.10	a	14.8 ± 0.40	a
15	7.7 ± 1.70	a	1.27 ± 0.10	a	16.9 ± 2.20	a
30	5.6 ± 1.60	b	2.57 ± 0.20	b	48.8 ± 9.50	b
45	4.5 ± 0.20	b	1.27 ± 0.10	a	28.3 ± 1.50	a,c
60	4.9 ± 0.30	b	1.90 ± 0.04	b,c	38.6 ± 1.20	b,c
Lincomycin						
00 (+)	8.2 ± 0.40	a	1.66 ± 0.04	a	20.3 ± 1.70	a
15	5.7 ± 0.80	b	1.59 ± 0.05	a	28.4 ± 4.90	a
30	5.4 ± 0.70	b	1.51 ± 0.04	a	28.5 ± 3.20	a
45	3.3 ± 0.20	c	1.45 ± 0.04	a	44.6 ± 1.50	b
60	5.3 ± 0.80	b	1.02 ± 0.03	a	31.1 ± 4.90	a
Methyl p-HB						
00 (+)	5.0 ± 0.40	a	1.38 ± 0.07	a	27.8 ± 3.20	a
2.5	4.7 ± 0.50	a	2.25 ± 0.01	b	48.2 ± 5.10	b
5.0	3.5 ± 0.60	a	2.26 ± 0.20	b	65.7 ± 7.70	b
7.5	9.4 ± §		3.56 ± §		37.9 ± §	
10.0	6.9 ± §		2.70 ± §		38.8 ± §	

Table. (Continued)

Cycloheximide			
00 (+)	7.0 ± 0.10 a	2.00 ± 0.10 a	28.5 ± 1.50 a
0.002	4.4 ± 0.50 b	2.17 ± 0.06 b	50.0 ± 5.90 b
0.004	3.6 ± 0.20 b	1.93 ± 0.02 a	53.2 ± 3.00 b
0.006	6.8 ± §	1.22 ± §	18.0 ± §
0.008	3.3 ± §	1.55 ± §	48.2 ± §
Sodium benzoate			
00 (+)	7.6 ± 1.00 a	2.13 ± 0.05 a	28.5 ± 3.60 a
2.5	6.2 ± 0.70 a	1.81 ± 0.04 a	29.7 ± 2.50 a
5.0	5.9 ± §	1.52 ± §	25.7 ± §
7.5	5.4 ± 0.60 a,b	2.27 ± 0.10 c	42.9 ± 6.00 b
10.0	3.5 ± 0.70 b	1.28 ± 0.10 a,b	37.9 ± 6.80 a
Nystatin			
00 (+)	8.6 ± 1.90 a	2.45 ± 0.20 a	30.1 ± 8.20 a
15	6.2 ± 1.20 b	1.70 ± 0.08 b	28.2 ± 4.50 a
30	7.6 ± 0.40 a	2.05 ± 0.03 a	27.2 ± 2.40 a
45	5.1 ± 0.80 b	0.59 ± 0.01 c	11.8 ± 2.00 b
60	9.1 ± 1.20 a	2.94 ± 0.03 a	32.9 ± 4.80 a
Chloramphenicol			
00 (+)	7.6 ± 0.90 a	2.44 ± 0.03 a,b	32.8 ± 4.00 a
15	8.5 ± 0.30 a	1.78 ± 0.08 a	20.9 ± 1.40 a
30	4.0 ± 0.10 c	1.49 ± 0.30 a	37.6 ± 6.40 a
45	6.1 ± 1.30 a,b	2.50 ± 0.10 a,b	43.9 ± 13.90 a
60	7.3 ± 0.70 a	1.96 ± 0.02 a,b	27.1 ± 2.70 a
Cephradine monohydrate			
00 (+)	9.4 ± 0.30 a	1.58 ± 0.05 a	16.7 ± 0.60 a
15	6.6 ± 0.60 b	1.27 ± 0.07 a	19.4 ± 0.90 a
30	8.6 ± 1.60 a	1.42 ± 0.03 a	17.2 ± 3.60 a
45	5.4 ± 0.50 b	1.27 ± 0.09 a	23.5 ± 2.90 a
60	6.3 ± 1.00 b	0.98 ± 0.01 a,b	16.0 ± 2.80 a
Erythromycin			
00 (+)	6.1 ± 0.60 a	1.67 ± 0.03 a	27.8 ± 3.60 a
15	5.3 ± 0.90 a	1.40 ± 0.02 a	27.2 ± 5.00 a
30	7.7 ± 0.30 a,b	1.41 ± 0.10 a	18.4 ± 1.20 a
45	5.5 ± 0.20 a	1.75 ± 0.00 a	31.8 ± 1.80 a
60	8.9 ± 1.10 b	1.65 ± 0.04 a	18.9 ± 2.40 a
Trimethoprim			
00 (+)	8.3 ± 1.20 a	1.49 ± 0.10 a	18.3 ± 2.10 a
15	7.9 ± 0.80 a	1.53 ± 0.30 a	19.4 ± 2.00 a
30	7.3 ± 0.00 a	1.16 ± 0.04 a,b	15.9 ± 0.50 a
45	7.5 ± 0.80 a	1.91 ± 0.02 a	25.7 ± 3.20 a
60	7.7 ± 0.50 a	1.55 ± 0.10 a	20.2 ± 1.30 a

* Average of 3 replicates
\# Values followed by the same letter are not significantly different from each other, P > 0.01
\+ Control diet
§ From two replicates

The highest level of penicillin increased the percentage of total protein when compared to the control (without penicillin), but the amount of total protein per mg of the young pupae was significantly increased by all the tested levels of these agents.

As seen in the Table, the diet with 40 mg of streptomycin caused a significant increase in the percentage of total protein. At 30 mg, it had no significant effects on this percentage but significantly decreased the amount of total protein.

The percentage of total protein was regularly increased through increasing the dietary levels of rifampicin. There were significant differences only between the diets with higher levels (7.5 mg and 10 mg) of rifampicin and the diet without this antibiotic. All tested levels of this agent caused a significant increase in the amount of total protein.

A dietary level of 30 mg of tetracycline had the most positive effect on the amount and the percentage of total protein when compared to other tested levels of this agent. This level caused an approximate two-fold increase in the amount and an approximate three-fold increase in the percentage of total protein.

The diet containing 45 mg of lincomycin significantly increased the percentage of the total protein when compared to the control diet. But none of the tested levels of this agent affected the amount of the insect's total protein.

Methyl p-hydroxybenzoate, at a level of 2.5 mg, had no significant effect on the percentage of the total protein per wet weight of the pupae, but caused a significant increase in the total protein amount of the insect. The diet with 5 mg of this antifungal agent significantly increased both the amount and percentage of total protein.

Both the amount and percentage of the total protein of the young pupae were significantly increased by 0.002 mg of cycloheximide when compared to the control diet. A dose of 0.004 mg caused a significant increase only in the percentage of total protein.

Among the tested levels of sodium benzoate, only a level of 7.5 mg had significant positive effects on the amount and percentage of total protein.

Nystatin was the only antimicrobial agent that had the most negative effects on both the amount and percentage of total protein. The diet with 45 mg of nystatin decreased total protein from an amount of 2.45 mg and a percentage of 31.1 in the control diet, to 0.59 mg and 11.8% respectively. The lowest level of nystatin agent caused a significant decrease in the amount of total protein alone.

None of the tested levels of chloramphenicol, cephradine, erythromycin and trimethoprim affected the amount or percentage of total protein in the insect

Discussion

The results of this work show that the tested antimicrobial agents had noticeable effects on the total protein content of the insect according to type and dietary levels. In general, this work also showed that most of these agents had positive effects on total protein content. The tested antimicrobial agents can be classified into three groups according to their effects on the percentage of the total protein in pupae of the insect. The first group of agents, such as penicillin, streptomycin, rifampicin, tetracycline hydrochloride, lyncomycin hydrochloride, methyl p-hydroxybenzoate, cycloheximide and sodium benzoate, had positive effects on the total protein content of the insect. The second group, which consisted of only nystatin, showed negative effects. The third group, including chloramphenicol, erythromycin, cephradine monohydrate and trimethoprim, had no effect on the total protein content. However, there were some exceptions in their effects from the standpoint of the mg amount of the total protein of the insect. For example, nystatin also had negative effects on the amount of total protein. Some tested levels of streptomycin decreased the amount of total protein in the pupae. Lyncomycin was an ineffective agent in this regard. Without regard to these differences, the total protein content of the insect was generally increased by most of the tested antimicrobial agents.

The effects of various dietary nutrients on protein content have already been demonstrated in the larval stages of some holometabolic entomophagous insects (11,20). The metabolic process varies with each of the developmental stages of holometabolous insects (33). Because of the high metabolic activity and cellular differentiation (34), the pupal stage may also be of critical importance in understanding the nutritional requirement of these insects. For example, Sulanç et al.

(11) demonstrated that the *P. turionellae* larvae fed on a synthetic diet (control diet) had a low protein content as a percentage. However, the present work with *P. turionellae* showed that the pupae from the larvae fed on this synthetic diet had a noticeably higher protein percentage. Similarly, the pupae of the same insect were previously demonstrated to have a higher glycogen percentage than those of the prepupae (10). For these reasons, the pupal stage was considered to be suitable for determining the effects of antimicrobial agents on the total protein content of the insect.

In general, except for nystatin, most of the tested antimicrobial agents had positive effects on the total protein content of the insect. Nystatin is a polyene antifungal agent that binds itself to sterols on eucaryotic cell membranes and disturbs their function (35). A recent study showed that nystatin also had an effect on the tissue distribution of plasma lipoprotiens in humans (36). The negative effects of this agent on the total protein content of the insect may be attributed to its destructive effects on the cell membrane permeality of the insect. It was demonstrated that some antibiotics, such as polymyxin B, caused changes in biological activities by inhibiting cell membrane function in higher animals (37).

The effects of antimicrobial agents have extensively been investigated on other lower eucaryotic organisms such as yeast (38-41), protozoan parasites (42-46, 47) and higher eucaryotic organisms including humans (48-53). These attempts to determine the effects of antimicrobial agents on eucaryotic organisms showed that these agents generally had adverse effects on protein synthesis. However, little information was known about their effects on insects, which are an important member of the eucaryotic group. For example, a eucaryotic protein synthesis inhibitor cycloheximide, which was injected into tobacco hornworm, *Manduca sexta* (54) and pea aphid, *Acyrthosiphon pisum* (55) inhibited some proteins involved in a certain metabolic process in these insects. However, our feeding experiments with cycloheximide showed that this agent caused an increase in the total protein content of *P. turionellae*. These results indicate that the effects of antimicrobial agents vary according to the kind of insect and even the method of application of the agents to the insect. The effects of antimicrobial agents also varied with their dietary levels. For example, 30 mg of streptomycin caused a significant decrease in the amount of total protein, but its highest level it actually increased this amount when compared to the control diet. These changes in the effects of the tested levels of an agent may be a result of different factors, such as the amount of the diet eaten by the larvae, the absorption rate of the agents from the gut and their interaction with dietary nutrients (15,56).

Another significant result from this work is the difference in the effects of a tested antimicrobial agent on the average amount and percentage of total protein in the pupae. For example, 0.004 mg of cycloheximide had no effect on the mg amount of total protein, but it increased the percentage of this total protein when compared with the control diet without cycloheximide. This tested level of cycloheximide also significantly decreased the wet weight of the insect. Similar results were obtained with some tested levels of streptomycin and nystatin. These results indicate that antimicrobial agents might have an effect on total protein percentage in conjuction with the wet weight of the pupae. These agents may influence the total body water composition of this insect. Water constitutes a large amount of the total weight of most insects (57). The increase in the percentage of total protein caused by this tested level of cycloheximide (0.004 mg) does not show a real increase in the total protein content of the insect. This increase in the percentage of total protein may be the result of a decrease in the total water content of the pupae. On the other hand, most of the tested antimicrobial agents, at certain levels, caused a significant decrease in the body wet weight of the pupae but increased the total protein content, in amount and percentage, of these pupae. However, 45 mg of nystatin significantly decreased both the body wet weight of the pupae and their protein content. Some levels of agents, such as chloramphenicol and erythromycin, caused a significant alteration in the wet weight but had no effects on the amount or the percentage of the total protein of the insect. These results show, as we suggested above, that the tested antimicrobial agents may influence the percentage of total protein depending on their effects on the wet weight of the insect. Nutritional balance is of importance for the efficient metabolic activities of insects (17). For example, in *A. housei* the amount of body protein varied with the balance of nutrients in the diet (58). The differences in the effects of a tested antimicrobial agent on both average amount and percentage of total protein may also be the result of a nutrient imbalance caused by these agents in the diets.

Among tested antimicrobial agents, only rifampicin had the most positive effects on the total protein content of the *P. turionellae*. In a previous work, it was demonstrated that rifampicin had adverse effects on the survival and development of the same insect (15). These results clearly indicate that in addition to the effects of antimicrobial agents on survival and development, their effects on the total protein content of the insect may also be taken into account for the evaluation of the nutritional value of antimicrobial agents for insect nutrition. In this regard, some attempts have been made to investigate the effects of some dietary nutrients on the total protein content of some parasitic dipterous (20) and hymenopterous insects (11).

This work showed that some antimicrobial agents caused a significant increase in the total protein content of *P. turionellae*. These agents may provide some precursor residues for amino acid synthesis of the insect. Most of the antimicrobial agents, especially antibiotics, contain residues that may be derived from amino acids (59). On the other hand, this increase in total protein content is probably a result of increased amino acid avaliability for protein synthesis. It was shown that tetracycline caused an increase in the incorporation of amino acids into silk proteins produced by *Philosamia ricini* (25). Streptomycin, procaine penicillin and tetracycline had positive effects on silk production and some other economic traits of *Philosamia ricini* (Boisd) (26, 27) and *Bombyx mori* L. (28). Similar positive effects of some antimicrobial agents were already demonstrated in economically important higher animals, such as domesticated farm ones. Antibiotics added into their foods increased the food consumption, body weight and growth rate of young animals (21-23). It was also demonstrated that certain antibiotics increased amino acid availability for protein synthesis, protein retention, and the conservation of energy during food digestion in higher animals (24).

The results of this work showed that antimicrobial agents can be used as a dietary additive in the nutrition of parasitic hymenopterous insects, which are potential biological control agents, though their type and levels should be determined with caution. Rifampicin and cycloheximide produced pupae with very low wet weight but a much higher amount of protein compared to the controls. In addition to these agents, most other tested agents at certain levels had a similar effect on the total protein content of the insect. This elevated protein content can serve as a compensatory reserve in the emerging of rigorous adults with high performance for biological control programs. This work may also prove of great value in better understanding the protein requirement of the pupae and in improving the protein sources of existing artificial diets for the larvae of this insect. It was known that the rate of protein synthesis during development is controlled by the neuroendocrine system in conjunction with a stimulatory factor appearing following the feeding of insects(60). Thus, the association between increased protein content and some of the tested antimicrobial agents raised the possibility that these agents could trigger the release of a factor stimulating protein synthesis. This factor may increase the incorporation of amino acids into the proteins of the insect depending on increased food consumption by the antimicrobial agents.

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