Turk J Zool
26 (2002) 229-237

Research Article

Antimicrobial agents: their combined effects on total protein content of the endoparasitoid *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae)

Kemal BÜYÜKGÜZEL
Department of Biology, Faculty of Sciences and Arts, University of Karaelmas, 67100, İncivez, Zonguldak - TURKEY

Received: 15.01.2001

Abstract: The effects of some antibiotics and antifungal agents in combination on the total protein content of young pupae of the hymenopterous endoparasitoid, *Pimpla turionellae* L., were investigated by rearing the larvae aseptically on chemically defined synthetic diets. These effects varied with the kinds and levels of the combination of antimicrobial agents in the diets. The combinations of antibiotics generally increased the total protein content according to their individual levels in the diets. Antifungal agent combinations caused a slight but significant increase in the total protein content. However, some tested combinations of nystatin with methyl p-hydroxybenzoate or cycloheximide significantly decreased the total protein content of the insect. The addition of an antibiotic to the diet in combination with nystatin and methyl p-hydroxybenzoate increased the total protein content. When an antifungal agent was added to the combination of penicillin and streptomycin the protein content decreased. The combinations of antimicrobial agents had an effect on the wet weight of the pupae. It was demonstrated that the total protein content of these pupae varied inversely with changes in their wet weight. It also seems as if increased protein content does not correlate with higher survivorship and life expectancy of this parasitoid.

Key Words: *Pimpla turionellae*, Antimicrobial agents, Total protein, Nutrition

Antimikrobiyal ajanlar: Endoparazitoid *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae)'nın total protein miktarına birlikte etkileri

Özet: Endoparazitoid hymenopter türü, *Pimpla turionellae* L., kimyasal yapısı bilinen sentetik besin ortamlarında aseptik şartlarda beslenerek bazı antibiyotik ve antifungal ajanların kombinasyonlarının böceğin genç puplarının toplam protein miktarına etkileri incelendi. Bu etkiler besindeki antimikrobiyal ajanların kombinasyonlarının çeşidine ve miktarına göre değişmektedir. Antibiyotik kombinasyonlarının çoğu besinde tek başına bulunan miktarlarına göre toplam protein miktarını artırmıştır. Antifungal ajan kombinasyonları ise antibiyotiklere göre fazla olmayan ancak istatistiksel olarak önemli bir artışa sebep olmuştur.Nistatinin metil p-hidroksibenzoat veya sikloheksimid ile kombinasyonu toplam protein miktarını önemli derecede düşürmüştür. Nistatin ve metil p-hidroksibenzoat kombinasyonunu içeren besine bir antibiyotik ilave edilmesi toplam protein miktarını artırmıştır. Penisillin ve streptomisin kombinasyonunu içeren besine bir antifungal ajan ilave edilmesi bu miktarda azalmaya sebep olmuştur. Antimikrobiyal ajanların kombinasyonları aynı zamanda pupların yaş ağırlığı üzerinde etkili olmuştur. Bu pupların toplam protein miktarının yaş ağırlıkları ile ters orantılı olarak değiştiği tespit edilmiştir. Protein miktarındaki artışın bu parazitoid böceğin yaşama ve gelişmesi üzerine olumlu bir etkide bulunmadığı görülmüştür.

Anahtar Sözcükler: *Pimpla turionellae*, Antimicrobial ajanlar, Total protein, Beslenme

Introduction

Many nutritional requirements are common to insects and higher animals. Except for certain nutrients, the qualitative nutritional requirements of insects are more or less similar to those of higher animals (1). Some antimicrobial agents have been used as dietary supplements for promoting the activities and performance of laboratory-reared insects. In recent years some antimicrobial agents, mostly antibiotics, have been fed to insects by adding these agents to their artificial diet or by addition to their natural foods or hosts. There was some work done on silkworm nutrition to improve silk production for industry. Some antibiotics were demonstrated to increase growth and silk production and enhance other some economic traits of a number of silkworm species such as *Philosamia ricini* (Boisd) (2-4) and *Bombyx mori* L. (5). Some antibiotics also increased the incorporation of amino acids into silk proteins

synthesized by *P. ricini* (2). These works indicate that antibiotics exert their positive effects on metabolic activity and growth performance by increasing the protein synthesis of these industrially important insects.

P. turionellae L. (Hymenoptera: Ichneumonidae) is a pupal endoparasitoid of various lepidopterous pest insects, and thus serves as a biological control agent. It can be reared on an artificial diet from egg to adult stage under laboratory conditions (6). A recent study showed that some combinations of antibiotics increased the survival and decreased the development of *P. turionellae* larvae reared on an artificial diet (7). However, a previous study on this parasitic wasp showed that most antibiotics and antifungal agents added in combinations to artificial diet had negative effects on post-larval survival and development, but a combination of nystatin:methyl *p*-hydroxybenzoate caused a significant increase in the survival rate and a decrease in the developmental time of *P. turionellae*. Ternary combinations of these agents also had no adverse effects, and some of them even had positive effects on the survival rate. It was observed that post-larval developmental stages, mostly the pupal stage, were more susceptible to tested combinations of antimicrobial agents than other stages (8). The variable results of this study have sparked important attempts toward investigating the effects of antimicrobial agents on total body composition to optimize the artificial rearing of this parasitoid. Studies on various developmental stages of entomofagous insects suggest that the determination of protein content is an important criterion for evaluating the effects of some dietary supplements (9,10). No information dealing with the effects of antimicrobial agents on the protein content of *P. turionellae* is available. The aim of this study was to investigate the effects of combinations of some antibiotics and antifungal agents on the total protein content in the pupal stage and determine their importance in the artificial mass rearing of the this parasitoid. Thus, I investigated the effects of six antimicrobial agents on total protein content by the addition of penicillin, streptomycin, rifampicin, cycloheximide, nystatin and methyl *p*-hydroxybenzoate in binary and ternary combinations in the larval diet of *P. turionellae*. This study shows that successful artificial mass rearing will require a thorough knowledge about the effects of antimicrobial agents on chemical composition in the different developmental stages of parasitoids.

Materials and Methods

Stock cultures of *P. turionellae* were maintained in the laboratory on the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae), at a temperature of 23±1°C, relative humidity of 75±5% and with 16:8 (LD) h photoperiod. Adults were fed daily with a 50% honey solution and *G. mellonella* pupal 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 utilized from these stocks, sterilization techniques, adding sterilized vitamin-glucose and antimicrobial agent solutions in the diet, dispensing the diets into test tubes, obtaining the sterilized larvae and their inoculation into the diets, and preparation of gelatine capsules for pupation were mainly the same as those described elsewhere (7,8).

Some of the antimicrobial agents used in this work, such as cycloheximide (98%), were obtained from Sigma Chemical Co. (St. Louis, USA). The others were obtained from different firms such as penicillin G (potassium salt, 1600 Units), and streptomycin sulphate (pure,crystalline) from Claus & Huth Ltd. (England). Methyl *p*-hydroxybenzoate (pure, crystalline) was obtained from the Koch-Light Lab. (England), rifampicin (-SV, 95%) came from the Koçak Medicine Co.Ltd. (İstanbul, Turkey), and nystatin (5300 Units) was sourced from the Bristol-Myers Squibb Co. (New Jersey, USA).

Agents tested were expressed as mg/100 ml of the diet. Their desired amounts were dissolved in bidistilled water, then sterilized through a 0.22 mm membrane filter (Sartorius GmbH, Goettingen, Germany, Minisart, NML) and added to 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 that contained a given diet of about 0.5 ml. 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*.

The combined effects of six antimicrobial agents were investigated through the addition of these agents into diets as combinations containing two or three agents

(binary or ternary combinations). At first, both the individual and binary combined levels of each of the antimicrobial agents that either had no apparent effects or had adverse effects on the number of survivors in the pupal stage according to the control diet (without antimicrobial agents) were determined. For the preparation of the tested combinations, these levels were used in different combinations. These individual and binary combined levels were also tested within their own experimental group as controls, in one sense, for investigating how the effects of binary and ternary combinations of antimicrobial agents change respectively. Therefore, extra control diets without antimicrobial agents were not needed for comparison. The experiments were conducted in two series. In the first series, the combinations of two antimicrobial agents were tested to investigate their combined effects on the total protein content of the insect. Their effects were compared to those of individual dietary levels of the combinational antimicrobial agents. The components of these combinations were either antibiotics or antifungal agents. In the second series of experiments, the combined effects of three antimicrobial agents were investigated. These effects were compared with those of binary combined levels of the antimicrobial agents. The ternary combinations were prepared by the addition of antimicrobial agents to the diet with the binary combinations. These ternary combinations consisted of two antibiotics plus an antifungal agent or two antifungal agents plus an antibiotic.

For protein extraction, the young pupae (one-day old) 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 minutes. 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 a 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. The extraction procedure was made according to Plummer (11). The amount of total protein was determined spectrophotometrically (Jenway Ltd., England) at a wavelength of 600 nm with Folin-phenol reagent (Sigma Chemical Co. St. Louis, USA) (12). Bovine serum albumin (Sigma Chemical Co. St. Louis, USA) was used as a standard protein. Protein extraction and determination were done separately from each replication of the experiments.

The effects of the tested diets with different combinations of the antimicrobial agents on the total protein content of the insect were measured by determining the average amount in mg and the percentage of the total protein according to the wet weight of the young pupae of *P. turionellae*. Data were evaluated by analysis of variance (ANOVA) (13). To determine significant differences between means, Duncan's (14) multiple range test was used. Differences were considered significant when *F* exceeded 0.01.

Results and Discussion

The results of this work showed that the antimicrobial agents in different combinations added to the diets had noticeable positive effects on the total protein content of an endoparasitoid *P. turionellae* pupae compared to their individual dietary components. However, a few combinations containing two or three agents were demonstrated to have negative effects on the total protein content of the insect. These effects varied in response to the kinds and dietary levels of the combinational agents.

The diets containing different combinations of the two antibiotics remarkably increased the total protein percentage of the pupae. For example, a combination of penicillin with streptomycin (10:20 mg) significantly increased the total protein content to a level as high as 61.1% when compared to those tested alone with penicillin and streptomycin. Moreover, another tested combination of penicillin and streptomycin (20:20 mg) significantly increased the total protein percentage two-fold according to these agents alone. The other binary combinations of antibiotics such as penicillin:rifampicin (20:2.5 mg), streptomycin:rifampicin (20:2.5 mg) significantly raised the total protein percentage to 54.9% and 61.7% respectively according to the individual dietary levels of the experimental group. Feeding some antibiotics, including streptomycin, procaine penicillin, oxytetracycline, chloramphenicol and gentamycin, to larvae were effective in improving growth and other

economic traits such as the weight of the larval and pupal cocoon shell and the silk production of the various silkworm species, *Philosamia ricini* (Boisd) (2-4) and *Bombyx mori* L. (5). It was already established that some antibiotics alone or in combination added into the food of some domesticated breeding animals enhanced their performance and the productivity. These agents were demonstrated to have positive effects on growth and performance by increasing feed consumption, facilitating the intestinal absorption of nutrients and reducing the protein digestion of these animals (15-18).

There are some reports about the effects of certain antifungal agents used alone on the growth, survival and reproduction of some hymenopterous insects, mostly from the Trichogrammatid species (19-21). No work has been done on their individual or combined effects on body composition of these insects. This work demonstrated that tested antifungal agent combinations exerted variable effects on the total protein content of the pupae. For example, a combination of nystatin and methyl *p*-hydroxybenzoate (30:2.5 mg) caused a significant increase in the protein content from a percentage of 27.3 to 39.7% when compared to those of the individual dietary level of nystatin. The effect of this combination was similar to that of methyl *p*-hydroxybenzoate by itself at a level of 2.5 mg. A similar result was obtained from a combination of methyl *p*-hydroxybenzoate and cycloheximide (2.5:0.008 mg). This combination had no effects according to 2.5 mg of methyl *p*-hydroxybenzoate alone but compared to 0.002 mg of cycloheximide it significantly increased the total protein from a percentage of 43.5 to 67.4% (Table 2). The results show that tested antifungal agent combinations cause a smaller but significant increase in the total protein content according to their individual dietary levels. The amount of the total protein in the pupae may be elevated as a result of increased food consumption and also the intake of raw material and energy sources for protein synthesis. Nutrient intake, particularly that of amino acids, is the primary stimulus for protein synthesis in higher animals (22). Streptomycin was demonstrated to have positive effects on the food consumption of the *Pimpla* larvae (23). This antibiotic, and perhaps the others in the tested combinations, may have provided the efficient availability of amino acids for protein synthesis. It was demonstrated that the excessive supply of amino acids led to an increase in the protein synthesis of some young vertebrates (24, 25). However, some combinations of nystatin with methyl *p*-hydroxybenzoate (45:2.5 mg) dramatically decreased the percentage of total protein to a level as low as 12.9% when compared to individually tested levels of these antifungal agents. A significant decrease in the total protein from a percentage of 44.7 to 21.3% was obtained by the addition of 30 mg of nystatin to the diet with 0.002 mg of cycloheximide. These combinations of antibiotics or antifungal agents may have contributed to the nutritional value of the diet. This suggestion is consistent with the view that physiological responses of an insect to dietary changes depend mainly on their nutritional value (26). Zucoloto (27) reported that insects select a diet or host that will provide the best development on the basis of its nutritional value. As in the binary combinations of antifungal agents, the tested combinations of three antimicrobial agents had variable effects on the percentage of the total protein of the insect. The addition of nystatin or methyl *p*-hydroxybenzoate to the diet containing penicillin:streptomycin combination caused a significant decrease in the total protein content from a percentage of 56.6 to 37.8 and 25.2% respectively. When penicillin or streptomycin was added to the combination of nystatin:methyl *p*-hydroxybenzoate, the total protein content was increased from 13.9 to 38.5% and 31.2% respectively. Furthermore, the data shows that the penicillin:rifampicin:nystatin combination may substitute for the penicillin:rifampicin combination in the larval diet of *P. turionellae*, as both of these combinations enabled the pupae to synthesize protein by ≈ 49% (Table 3).

These variations in the effects of the combinations containing two and/or three agents may be attributed to a possible interaction between combinational agents. Interactions between antimicrobial subtances may affect the feeding rate and activity of the larvae. Singh and House (28) stated that antimicrobial agents in the diets deterred feeding activities owing to olfactory or gustatory stimuli. It is noteworthy that synergistic interaction was obtained from penicillin:streptomycin (10:20 mg) and streptomycin:rifampicin (20:2.5 mg) combinations (Table 1). The percentage of total protein caused by these combinational antibiotics was greater than the sum of those of each of the agents when used alone for feeding *Pimpla* larvae. However, when the larvae were fed with the diet containing 45 mg of nystatin plus 2.5 mg of methyl *p*-hydroxybenzoate, the

Table 1. The effects of some antibiotics alone and in binary combination on the total protein content of the *P. turionellae* pupae.

Levels of antibiotics (mg/100 ml diet)		Wet weight mg (mean ± S.D.)[a]	Content of total body protein mg (mean ± S.D.)[a]	Content of total body protein % (mean ± S.D.)[a]
Penicillin:streptomycin				
10	-	6.6 ± 0.50a	1.46 ± 0.04a	22.4 ± 2.30a
-	20	5.8 ± 0.20a	1.11 ± 0.03a	19.2 ± 1.30a
10	20	4.9 ± 0.80a	2.98 ± 0.40b	61.1 ± 3.70b
10	40	7.3 [c]	2.80 [c]	42.8 [c]
20	20	5.3 ± 0.90a	2.06 ± 0.02c	40.4 ± 5.90c
Penicillin:rifampicin				
10	-	6.4 ± 0.40a	1.71 ± 0.20a	26.8 ± 3.70a
-	2.5	6.5 ± 0.40a	2.36 ± 0.20b	36.5 ± 3.30a,b
10	2.5	6.5 [c]	1.15 [c]	17.7 [c]
10	10	-	-	-
20	2.5	5.0 ± 1.10a	2.70 ± 0.05b	54.9 ± 10.30c
Streptomycin:rifampicin				
20	-	5.8 ± 0.20a	1.10 ± 0.04a	19.1 ± 1.30a
-	2.5	6.7 ± 0.30a	2.15 ± 0.10b	32.1 ± 0.60a
20	2.5	4.3 ± 0.50b	2.60 ± 0.05c	61.7 ± 6.20b
20	10.0	4.0 [b]	2.84 [b]	62.5 [b]
40	2.5	4.0 [c]	1.60 [c]	40.9 [c]

[a]Three replicates. Values followed by the same letter are not significantly different from each other, P > 0.01
[b]From one replicate
[c]From two replicates

Table 2. The effects of some antifungal agents alone and in binary combination on the total protein content of the *P. turionellae* pupae.

Levels of antifungal agents (mg/100 ml diet)		Wet weight mg (mean ± S.D.)[a]	Content of total body protein mg (mean ± S.D.)[a]	Content of total body protein % (mean ± S.D.)[a]
Nystatin:methyl *p*-HB				
30	-	7.4 ± 0.30a	2.00 ± 0.06a	27.3 ± 1.40a
-	2.5	4.6 ± 0.40b	2.10 ± 0.08a	46.0 ± 5.00b
30	2.5	9.5 ± 0.70c	3.80 ± 0.04b	39.7 ± 2.00b
30	10	8.4 [c]	2.69 [c]	32.3 [c]
45	2.5	9.6 ± 0.80c	1.23 ± 0.01c	12.9 ± 0.30c
Nystatin:cycloheximide				
30	-	7.8 ± 0.20a	1.96 ± 0.10a	25.3 ± 2.40a
-	0.002	4.9 ± 0.30b	2.16 ± 0.10a	44.7 ± 4.40b
30	0.002	7.3 ± 1.00a	1.53 ± 0.05a	21.3 ± 2.50a
30	0.008	7.8 [c]	1.43 [c]	19.2 [c]
45	0.002	8.3 [b]	2.26 [b]	27.2 [b]
Methyl *p*-HB:cycloheximide				
2.5	-	4.5 ± 0.40a	2.30 ± 0.07a	51.5 ± 3.60a,b
-	0.002	5.1 ± 0.10a	2.22 ± 0.20a	43.5 ± 3.30b
2.5	0.002	6.0 [b]	2.75 [b]	45.8 [b]
2.5	0.008	4.2 ± 0.90a	2.76 ± 0.20b	67.4 ± 10.80a
10	0.002	-	-	-

[e]Three replicates. Values followed by the same letter are not significantly different from each other, P > 0.01
[b]From one replicate
[c]From two replicates
methyl *p*-HB: methyl *p*-hydroxybenzoate

Table 3. The effects of combinations containing two and three antimicrobial agents on the total protein content of *P. turionellae* pupae.

Levels of antimicrobial agents (mg/100 ml diet)				Wet weight mg (mean ± S.D.)[a]	Content of total body protein	
					mg (mean ± S.D.)[a]	% (mean ± S.D.)[a]
pen. :	Strep.:	Nys.:	methyl *p*-HB			
10	20	-	-	5.5 ± 0.40a	3.80 ± 0.06a	56.6 ± 3.10a
-	-	45	2.5	9.3 ± 0.60b	1.29 ± 0.03b	13.9 ± 0.60b
10	20	30	-	6.9 ± 0.80c	2.61 ± 0.50c	37.8 ± 2.60c,d
10	20	-	2.5	8.8 ± 0.90b	2.21 ± 0.20b	25.2 ± 0.30e
10	-	45	2.5	6.9 ± 0.50c	2.65 ± 0.20d	38.5 ± 2.00c
-	20	45	2.5	5.1 ± 1.50a	1.59 ± 0.50c	31.2 ± 0.90d,e
pen. :	rif. :	Nys.:	cycl.			
20	2.5	-	-	5.1 ± 1.00a	2.47 ± 0.30a	49.2 ± 4.40a
-	-	30	0.002	7.5 ± 0.90b	1.46 ± 0.03b	19.8 ± 1.50b
20	2.5	30	-	5.9 ± 1.80a,b	2.69 ± 0.40a	48.4 ± 8.70a
20	2.5	-	0.002	4.6 ± 0.30a	1.76 ± 0.20a,b	38.0 ± 2.20a,c
10	-	30	0.002	7.3 ± 1.40b	1.65 ± 0.30b	22.9 ± 0.90b
-	2.5	30	0.002	7.3 ± 0.60b	2.12 ± 0.20a,b	28.9 ± 1.50b,c

[a]Three replicates. Values followed by the same letter are not significantly different from each other, P > 0.01
pen.:penicillin, rif.:rifampicin, nys.:nystatin, cycl.:cycloheximide, strep.:streptomycin, methyl *p*-HB:methyl *p*-hydroxybenzoate

total protein content was decreased from a percentage of 46 to 12.9% (Table 2). Similarly, it has been demonstrated that the negative effects of some antibiotics on the protein synthesis of various parasitic protozoa were increased by their combination with other antibiotics used to treat the infections of these parasites in some higher animals (29-31). Therefore, it is reasonable to suggest that the kinds and levels of combinational antimicrobial agents should be carefully chosen when they are used in the feeding of the insect. Feeding some antimicrobial agent combinations to larvae also affected the wet weight of the pupae. The total protein content of these pupae appeared to vary inversely with changes in their wet weight. For example, combining 20 mg of streptomycin with 2.5 mg of rifampicin significantly decreased the average wet weight of the pupae, but these pupae contained high protein by ≈ 3-fold (Table 1). When 30 mg of nystatin was added to the diet with 0.002 mg of cycloheximide, the pupal wet weight was significantly increased from a weight of 4.9 mg to 7.3 mg, but the percentage of the total protein was significantly decreased from 44.7 to 21.3% (Table 2). Moreover, some tested combinations had no significant effect on the wet weight of the pupae, but they had an effect on the total protein content (Table 1). These different results were also obtained with the tested combinations of three antimicrobial agents (Table 3). Such differences in pupal wet weight may be the result of the changed water content of the insect. These antimicrobial agents in the diet may influence the water content of the pupae. The larvae may balance their water content by increased water uptake from the diet and/or by reduced water loss (32). A work with *Locusta migratoria* L. demonstrated that experimentally increased hemolymph osmotic pressure may increase the amount of the water obtained from food (33). Changes in water content, which constitues a large amount of the total body composition of immature stages (34), usually have an effect on the wet weight and thus on the metabolic activity of the insects. Young organisms are known to have a higher water content and a lower ash content than adults (35). Statistical analysis of the data showed the difference in the effects of some combinations of antimicrobial agents on the average amount in the mg and the percentage of the total protein per wet weight. For example, a combination of nystatin with methyl *p*-hydroxybenzoate (30:2.5 mg) significantly increased the wet weight from an amount of 4.6 mg in the diet with 2.5 mg of methyl *p*-hydroxybenzoate alone to 9.5 mg. This combination also caused a significant increase in the amount of total protein from 2.10 mg to 3.80 mg, but it seems to have had no significant effects on the percentage of the total protein of the insect. Although the addition of 0.002 mg of cycloheximide to the diet with 30 mg of nystatin significantly decreased the amount of total protein from 1.96 mg to 1.53 mg, it did not cause

a significant decrease in the percentage of the total protein of the insect (Table 2). These differences indicate that the total protein content varies in relation to the wet weight. This may be attributed to the effects of the combinational agents on the water content of the pupae. Boza et al. (36) reported that body wet weight changes were not related to the amount of food consumed or its nutritional value, but may result from variations in the water content depending on the feeding habits of some higher animals. They also observed a high level of protein in the liver and muscle in short-term starved animals that was linked with water loss. In this study, the protein content of the pupae was generally found to increase with a decrease in body wet weight or conversely it was decreased with an increase in wet weight. Similarly, the adults of *Pimpla turionellae* fed with a heavy metal contaminated diet had a higher water content with decreased protein content when compared to control groups (37). These results suggest that variations in wet weight may be dependent not only on the possible effects of the combinational antimicrobial agents on total water content, but also on the contribution of the amount of the total protein synthesized.

Variations in total protein content may raise the possibility that tested combinations of antimicrobial agents exert direct effects on *P. turionellae*. The fact that this parasitoid lacks endosymbiotic organisms (Ş. Y. Unpublished data) supports this suggestion, as endosymbionts provide a potential target for the control of most insects, especially homopterous ones, with antibacterial agents (38). These may have been effective on dietary nitrogenous nutrients, mostly amino acids, needed for building the proteinaceous tissue of the insect. The protein requirement of *P. turionellae* is provided in the form of free amino acids in the diet (6). Asepsis was maintained for this study by means of normal bacteriological techniques. Therefore, this study is concerned only with the dietary implications of combinations of antimicrobial agents for practical purposes rather than with their microbial activity. These combinations tested may be used for the control of bacterial and fungal co-contaminations that occur during the artificial rearing of *Pimpla* larvae under laboratory conditions. Further investigations are desirable to clarify this point.

The generally tested combination of antimicrobial agents increased the total protein content of *P. turionellae* pupae. This may be important for the nutrition of the insect when it converts dietary nutrients with high efficiency to body materials. However, it may not be important when considered in terms of the artificial mass rearing of this parasitoid for augmentative biological control programs. For example, a combination of streptomycin and rifampicin (20:2.5 mg) significantly increased the total protein in the pupal stage from 19.1 to 61.7%. However, the same combination significantly decreased the survival in this stage from 40.7 to 25.0% (8). This previous study also showed that the nystatin:methyl *p*-hydroxybenzoate combination (45:2.5 mg) produced a yield of 73.1% pupae, and 57.7% adults, and also decreased the developmental time of the insect. However, this combination significantly decreased the amount (1.23mg) and percentage of total protein (12.9%) in this study. It seems that survivorship varies inversely with changes in the total protein content of the pupae. Consequently, decreased pupal survival may be attributed to the increased accumulation of protein in the tissues of the pupae. This may be a reasonable suggestion, because a study demonstrated that high doses of some proteins in diets displayed significant toxicity and growth inhibition in *Acyrthosiphon pisum* (Harris) (39). These antimicrobial agents may also have an effect on protein retention in the pupal stage through to adult emergence, as in the higher economically important animals (18). This study clearly shows that an optimum body composition would be important for normal survival and development to adult emergence as well as a balanced diet. In this respect, diets with ternary combinations of antimicrobial agents seem to have optimum effects on the total protein content of the insect (Table 3). The addition of nystatin at a level of 30 mg to a combination of penicillin and rifampicin (20:2.5 mg) had no significant effect on the wet weight of pupae or their total protein content. However, this formulation also significantly increased pupal and adult survival from 16.7 to 54.2% and 45.8% respectively (8). Therefore, to use data concerning only survival and development sometimes may be insufficient to ascertain the nutritional suitability of antimicrobial agents or their usefulness in the artificial mass rearing of parasitoids. However, a comparison of total protein content in different developmental stages may be of great importance to evaluate the effects of antimicrobial agents on this parasitic wasp. Similarly, total protein content was determined to be a valuable criterion for evaluating the

qualitative and quantitative nutritional requirements of *P. turionellae* (10). An important area for future research would be to determine the protein content of this parasitoid reared on natural hosts for comparison with that of individuals reared on artificial diets with these combinations of antimicrobial agents, and to mimic the protein content found in parasitoids that were reared naturally. This would improve their initial attack an hosts in nature and consequently contribute to successful biological control. However, investigations are in progress to determine the effects of antibiotics on protein buildup in each developmental stage of *P. turionellae* reared on natural hosts.

Acknowledgments

I am grateful to Professor Ş. Yazgan for his helpful advice and criticism throughout this work.

References

1. House, H.L. Nutrition of natural enemies, pp 151-182, *In* R.L Ridgway and S.B. Vinson [eds], Biological Control by Augmentation of Natural Enemies, Plenum Publ. Corporation. 1977.
2. Eid, M.A.A., El-Nakkadi, A. and Saleh, M.A. Functional adaptation of silk glands after administration of antibiotic to larvae Philosamia ricini (Boisd). Insect Sci. Its Application. 10: 139-143. 1989.
3. Govindan, R., Magadum, S.B., Krishna, B.G. Magadum, V.B., Devaiah M.C. and Narayanaswamy, T.K. Effect of fortification of streptomycin and procaine penicillin on economic traits of eri silkworm. Curr. Res. 19: 196-197. 1990.
4. Govindan, R., Magadum, S.B., Krishna, B.G., Magadum V.B. and Narayanaswamy, T.K.. Effect of antibiotics chloramphenicol and oxytetracycline on economic traits of eri silkworm [Samia cynthia ricini] Curr. Res. Universtiy of Agricultural Sciences, Bangalore. 22: 11-12. 1993.
5. Saha, A.K., Rahaman, M.S., Saha, B.N., Rahman, M. and Das, B.K.. Effects of antibiotics on the growth and rearing performance of silkworm, Bombyx mori L. J. Asiatic Soc. Bangladesh Sci. 21: 289-292. 1995.
6. Yazgan, Ş. A meridic diet and quantitative effects of tween 80, fatty acid mixture and inorganic salts on development and survival of the endoparasitoid Pimpla turionellae L. Z. Ang. Entomol. 91: 433-441. 1981.
7. Büyükgüzel, K. Positive effects of some gyrase inhibitors on survival and development of Pimpla turionellae L. (Hymenoptera: Ichneumonidae) larvae reared on an artificial diet. J. Econ. Entomol. (in press). 2001.
8. Büyükgüzel, K. and Yazgan, Ş. Combinational effects of some antimicrobial agents on the survival and development of the endoparasitoid Pimpla turionellae L. (Hymenoptera: Ichneumonidae). Commun. Fac. Sci. Univ. Ank. Series C. 48: 1-14. 1999.
9. House, H.L. and Barlow, J.S. The effects of a new salt mixture developed for Agria affinis (FALLÉN) (Diptera: Sarcophagidae) on the growth rate, body weight, and protein content of the larvae. J. Insect Physiol. 11: 915-918. 1965.
10. Sulanç, M., Emre, İ. and Yazgan, Ş. İnorganik tuzların erkek Pimpla turionellae L. (Hymenoptera: Ichneumonidae) larvalarının gelişmelerine ve sentezledikleri protein miktarına kalitatif etkileri. Tr. J. Zoology. 16: 92-100. 1992.
11. Plummer, D.T. An introduction to practical Biochemistry. McGraw-Hill Book Comp. U.K. 1971.
12. Lowry, O.H., Rosebrough, N.I. Farr, A.L. and Randall, R.J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 19: 265. 1951.
13. Snedecor, G.W. and Cochran, W.G. Statistical methods. 6th ed., Iowa State University, Press. Ames. 1967.
14. Duncan, D.B. 1955. Multiple Range and Multiple F tests. Biometrics. 11: 1-14.
15. Patrick, H. and Schaible, P.J. Poultry: Feeds and Nutrition Vol. I and II, 2nd ed. 668 pp. 1980.
16. Forbes, A.B., Wilkinson, J.I.D. Hope, E.J. and Rawlinson, P. Effects of actaplanin on fertility in dairy cows. Proceedings, Sixth International Conference on Production Disease in Farm Animals. Belfast, U.K. 47-50. 1986.
17. Ensminger, M.E., Oldfiled, J.E. and Heinemann, W.W. Feed & Nutrition. 2nd ed. The Ensminger Publ. Company, Clovis, California, USA. 1990.
18. Fiems, L.O., Boucque, C.V. and Cottyn, B.G. Effect of feed antibiotic on animal performances, meat quality and health in ruminants, and on aspects of environmental pollution. Scientific conference on growth promotion in meat production, Proceedings Brussels, Belgium, 151-167. 1996.
19. Xie, Z.N., Nettles Jr., C.W., Morrison, R.K. K. Irie and Vinson, S.B. Three methods for the *in vitro* culture or Trichogramma pretiosum Riley. J. Entomol. Sci. 21: 133-138. 1986.
20. Grenier, S. and Liu, W.H. Antifungals: Mold control and safe levels in artificial media for Trichogramma [Hymenotpera: Trichogrammatidae]. Entomophaga. 35: 283-291. 1990.
21. Grenier, S. and Liu, W.H. Mold control and safe levels of antifungals in artificial media for egg parasitoids (Hymenoptera). Les colleques de l'INRA. 56: 141-144. 1991.

22. Davis, T.A., Burrin, D.G., Fiorotto, M.L., Reeds, P.J. and Jahoor, F. Roles of insulin and amino acids in the regulation of protein synthesis in the neonate. J. Nutr. 128: 347-350. 1998

23. Büyükgüzel, K. and Yazgan, Ş. Bazı antibiyotiklerin endoparazitoid Pimpla turionellae L. (Hymenoptera: Ichneumonidae)'nin yaşama ve gelişimine etkileri. Tr. J. Zoology, 20: 1-7. 1996.

24. Watt, P.W., Corbett, M.E. and Rennie, M.J. Stimulation of protein synthesis in pig skeletal muscle by infusion of amino acids during constant insulin availability. Am. J. Physiol. (Endocrinol Metab.) 263: 453-460. 1992.

25. Liechty, E.A., Boyle, D.W., Moorehead, H., Auble, L. and Denne, S.C. Aromatic amino acids are utilized and protein synthesis is stimulated during amino acid infusion in the ovine fetus. J. Nutr. 129: 1161-1166. 1999.

26. Slansky, F. Jr. and Scriber, J.M. 1985. Food consumption and utilization, pp 87-163. *In* G.A. Kerkut and L.I. Gilbert [eds], Comprehensive Insect Physiology, Biochemistry and Pharmacology, Pergamon Press, Oxford.

27. Zucoloto, F.S. Feeding habits of Ceratitis capitata (Diptera: Tephritidae): Can larvae recognize a nutritionally effective diet? J. Insect Physiol. 33: 349-353. 1987.

28. Singh, P. and House, H.L. Antimicrobial agents: Their detrimental effects on size of an insect. Agria affinis. Can. Entomol. 102: 1340-1344. 1970.

29. Gershon, P.D. and Howells, R.E. Combination of antibiotics erythromycin and tetracycline with three standard antimalarials against Plasmodium falciparum in vitro. Ann. Trop. Med. Parasitol. 78: 1-11. 1984.

30. Gingras, B.A. and Jensen, J.B. Activity of azithromycin (cp-62, 993) and erythromycin against chloroquine-sensitive and chloroquine-resistant strains of Plasmodium falciparum in vitro. Am. J. Trop. Med. Hyg. 47: 378-382. 1992.

31. Gingras, B.A. and Jensen, J.B. Antimalarial activity of azithromycin and erythromycin against Plasmodium berghei. Am. J. Trop. Med. Hyg. 49: 101-105. 1993.

32. Rivers, D.B. and Yoder, J.A. Site-specific of parasitism on water balance and lipid content of the parasitic wasp Nasonia vitripensis (Hymenoptera: Pteromalidae). Eur. J. Entomol. 93: 75-82. 1996.

33. Raubenheimer, D. and Gäde, G. Compensatory water intake by locusts (Locusta migratoria): Implications for mechanisms regulating drink size. J. Insect Physiol. 39: 275-281. 1993.

34. Hadley, N.F. Water relation of terrestrial arthropods. Academic Press, New York, 356 pp. 1994.

35. Forbes, G.B. Body composition: Overview: J. Nutr. 129: 270-272. 1999.

36. Boza, J.J., Moënnoz, D., Vuichoud, J., Jarret, A.R., Gaudard-de-Weck D., Fritsché, R., Donnet, A., Schiffrin, E.J., Perruisseau, G. and Ballèvre, O. Food deprivation and refeeding influence growth, nutrient retention and functional recovery of rats. J. Nutr. 129: 1340-1346. 1999.

37. Ortel, J. Effects of lead and cadmium on chemical composition and total water content of the pupal parasitoid, Pimpla turionellae. Entomol. exp. appl. 59: 93-100. 1991.

38. Costa, H. S., Thomas, J. H. and Nick, C.T. Effects of antibacterial materials on Bemisia argentifolii (Homoptera:Aleyrodidae) oviposition, growth, survival, and sex ratio. J. Econ. Entomol. 90:333-339. 1997.

39. Rahbe, Y. and Febvay, G. Protein toxicity to aphids: an in vitro test on Acyrthosiphon pisum. Entomol. Exp. Appl. 67:149-160. 1993.