ARTIFICIAL DIET FOR REARING *TRICHOGRAMMA* WASPS (HYMENOPTERA: TRICHOGRAMMATIDAE) WITH EMPHASIS ON PROTEIN UTILIZATION

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ABSTRACT

Trichogramma wasps are tiny hymenopterous egg parasitoids widely used in biological control programs worldwide. The huge quantities of insects necessary for inundative releases are mainly produced on factitious hosts like *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae), or on silkworms. In order to simplify production, increase its flexibility, and potentially reduce cost, studies on artificial media for the development of the parasitoids have been ongoing for many years. Some successes were obtained, mainly with artificial media containing insect extracts such as pupal hemolymph from Lepidoptera. To define new artificial media devoid of insect components or improve the performances of existing ones, a better knowledge of parasitoid nutrition would be useful. Proteins are key components in artificial media, and research was conducted on *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) to better understand the nutritional value of proteins by investigating to what degree they are assimilated by the insect.

A method was developed for studying the assimilation of these nutrients by the preimaginal stages of *T. pretiosum* based on adding a mixture of free 14C-radiolabelled amino acids to the medium to be tested. The basic composition of the medium already included proteins, and proteins to be tested were also added. Amino acid analyses were performed on medium (for free and protein amino acids) and on *T. pretiosum* grown in the medium (for protein amino acids). For each radiolabelled amino acid, comparison of the specific activity in total amino acids in *T. pretiosum* pupae with the specific activity in free and protein amino acids in the medium, allowed us to determine the degree and the means by which the protein was utilized.

We showed that the proteins included in the hemolymph-based medium, as well as casein added at final concentrations of 1.6 or 3.2 %, were completely assimilated. This protein, incorporated into the hemolymph-based medium to increase its protein content, led to im-

proved body composition and some development parameters of *T. pretiosum*. Even media containing hemolymph could be improved by protein addition because of the relatively low content of proteins in the hemolymph. The addition of 3.2% casein increased the protein content of *T. pretiosum* pupae by 25% and normal adult emergence yield by 40%.

INTRODUCTION

Oophagous Hymenoptera of the genus *Trichogramma* are used in many countries in biological control programs to regulate pest populations (mainly lepidopteran species) (Li 1994; Parra and Zucchi 2004). These parasitoids are generally reared in factitious host eggs, the most common belonging to Lepidoptera like *Ephestia kuehniella* Zeller, *Corcyra cephalonica* (Stainton) (Pyralidae), *Sitotroga cerealella* (Olivier) (Gelechiidae) or silkworms, but their multiplication on a large scale remains expensive. This limitation to their use can be overcome by the possibility of artificial rearing systems. Studies have been conducted in different countries on *in vitro* rearing of egg parasitoids for many years. Presently, different kinds of artificial media are available enabling immature development of many species of *Trichogramma*. The best results have been obtained with media mainly composed of insect-derived elements such as hemolymph, body, or egg juices, but media without insect additives have also been tested with some success (Consoli and Parra 1997; Grenier 1994; Grenier *et al.* 1995; Thompson 1999; Thompson and Hagen 1999). In these latter media, one of the main concerns is protein supply, and this is true even with artificial media containing insect hemolymph, which is usually poor in protein content compared to lepidopteran eggs.

This work was conducted in order to define artificial diets for *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) that are more suitable for the development of this oophagous parasitoid, based upon a better knowledge of the nutritional value of proteins and of their utilisation by larvae. The assimilation of the proteins was evaluated by adding a mixture of radiolabelled amino acids to the medium. In addition, a hemolymph-based medium, also supplemented with proteins, was tested for *Trichogramma* development. Assimilation and development tests were performed with the basic medium and with casein supplementations.

MATERIALS AND METHODS

Stock cultures of a thelytokous strain of *T. pretiosum* originating from Uruguay, were maintained on *E. kuehniella* eggs killed by UV irradiation. Adults were fed on a diluted honey solution (30% in water). For experiments, rearing was conducted in 1 litre-glass jars (10 cm diameter, 16 cm high) with the proportion of one female for 10 host eggs glued on cardboard. Climatic conditions were 23 ± 0.5 °C, 75 ± 5 % R.H., and a 16:8 h light-dark regime.

The method of investigation was based on the adding of a mix of 14C-labelled amino acids (aa) to the artificial medium in which the *Trichogramma* larvae were grown. The specific activity of each labelled aa is defined as the radioactive activity of the aa in counts per min / mg (cpm/mg) divided by the concentration of the aa in nmol/ mg. We analyzed free and protein aa in the medium, but only the protein aa in the insect body, considering that i) if the

Trichogramma larvae do not digest and assimilate the proteins in the medium (utilization of free aa only), the specific activity of the aa in the insect body will be the same as the specific activity of the free aa of the medium, ii) if the *Trichogramma* larvae completely digest and assimilate the proteins in the medium, the specific activity of the aa in the insect body will be the same as the specific activity of the total aa of the medium, iii) if the *Trichogramma* larvae partly digest and assimilate the proteins in the medium, the specific activity of the aa in the insect body will be intermediate between the specific activity of the free and total aa of the medium.

Artificial host eggs made of a polyethylene film (15 µm thick) in the form of hemispherical cupules were filled with artificial medium (about 5 µl) used as the diet for larval development. Each rearing device contained 30 cupules arranged as a 6 x 5 matrix. The experiments were conducted under aseptic conditions as described earlier (Grenier 1994; Grenier and Liu 1990; Grenier *et al.* 2002). Climatic conditions were the same as for the stock culture.

The basic artificial medium contained pupal hemolymph from *Mamestra brassicae* L. (Lepidoptera: Noctuidae) (40%), hen's egg yolk (20%), semi-skimmed cow's milk (20%), Neisenheimer salt solution (10%) and distilled water (10%). Besides this medium, two other media enriched with casein (BDH) at two concentrations (final concentrations in the medium of 1.6 or 3.2%) were used for investigating protein assimilation.

The experimental process consisted of incorporating into the media a radiolabelled aa solution of a 14C-protein hydrolysate containing Ala, Arg, Asp, Glu, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Tyr, and Val (Sigma). This labelled medium was distributed in 4 out of 30 cupules of each matrix, the remaining cupules being filled with the same medium without radiolabelled aa. Analyses were performed on the medium (free and total aa). *T. pretiosum* females were allowed to lay eggs for 24 hours. After the larvae had completed development, the pupae were analysed for total aa. Each experimental condition was replicated three times.

For total aa analysis of media and pupae, all samples were hydrolysed under nitrogen in HCl vapour at 120°C for 24 hours using a Pico-Tag work station (Waters, St. Quentin Les Yvelines, France). Along with 2-(beta)-mercaptoethanol (4%) to preserve sulphur-containing aa, 200 µl of 6N HCl were placed in the hydrolysis tank. After hydrolysis, 10 nmol of glucosaminic acid per mg of sample were added as an internal standard. The samples were dried under vacuum in a Speedvac apparatus (Savant Instrument Inc., Farmingdale, New York) and taken up with 0.05 M lithium-citrate buffer (pH 2.2). Samples were submitted to ion exchange chromatography in an automatic amino acid analyser (Beckman 6300, Roissy, France). Amino acids were detected by the ninhydrin reaction, identified by their retention time and wavelength ratio, and quantified by their absorption at 570 nm (440 nm for proline). For each condition, 3 to 5 replicates were analysed. Free aa of media were analysed by the same procedure without hydrolysis, but after precipitation of the proteins by TCA (trichloro acetic acid, final concentration 5%) followed by the elimination of TCA and lipids by chloroform extraction. Again, 3 to 5 replicates were analysed.

Biological (parasitism, adult emergence rate, normal adult rate) and biochemical data (pupal body composition in aa) of *Trichogramma* reared in the different media were compared. The diets were prepared and the experiments were performed as described above, but

no radiolabelled aa were added. The degree of parasitism was measured by the number of eggs laid per cupule. The percentage of emergence was evaluated by dividing the number of cupules per box producing adults by the total number of cupules x 100. The percentage of normal adults was calculated by dividing the number of adults with normal wings and abdomen by the total number of adults per box x 100. The compositions in aa were expressed in nmol/mg of fresh pupal weight.

RESULTS AND DISCUSSION

ASSIMILATION

The quantity of labelled aa represented 1% of the quantity of the free amino acids in the medium, and thus was not intended to modify the original balance in aa. The external contamination of the pupae grown in labelled medium, checked by washing them several times, was negligible. The feces, rejected just after the emergence by the adults obtained from *E. kuehniella* eggs, were collected on a glass tube and analysed for aa presence. They contained mainly ammonium and only very small quantities of aa (0.52 nmol / *Trichogramma* vs. 10-30 for body content according to their size and consumed food).

In the three media, the specific activity for all free aa was quite high (up to 15000 cpm/nmol), while the specific activity for total (free and protein) aa in the pupal body was lower (less than 1000 cpm/nmol) (Fig. 1). The specific activities of the aa in the pupal body were quite similar to the specific activity of the total aa of the medium for most of the amino acids, mainly essential ones. The lower amounts of labelled total aa observed in pupal body compared to media, for some aa (Thr, Ser, Glu, Gly, and mainly Pro and Ala) could be explained by the importance of the intermediate metabolism in which these energetic aa are implicated. These differences were greater in control medium and lower in medium with 3.2% of casein, showing a better efficiency of protein utilisation in the latter medium. For essential basic aa (Lys, His, Arg), the proteins did not seem to be completely assimilated, because the values for total aa content of pupae were slightly higher than those for the media. Nevertheless, the differences were very small.

In the control medium as well as in media with casein added, all the proteins present were almost completely digested and assimilated. Subsequently, the effect of adding casein was tested on biological and biochemical parameters.

DEVELOPMENT IN MEDIA

Female wasps readily laid eggs inside artificial host eggs (Fig. 2). The parasitization rate, measured as the mean number of eggs laid per artificial host egg (cupule) was not significantly modified when 1.6% casein was added to the control medium (139.1 vs. 146.9), but was significantly reduced with 3.2% casein (111.6). Free aa are usually known as egg laying stimulants (Xie *et al.* 1991), thus this lower parasitization rate was possibly due to a reduction of the relative concentration in aa resulting from the addition of pure casein. Larvae successfully developed and after excreting a black substance turned into pupae (Fig. 2).

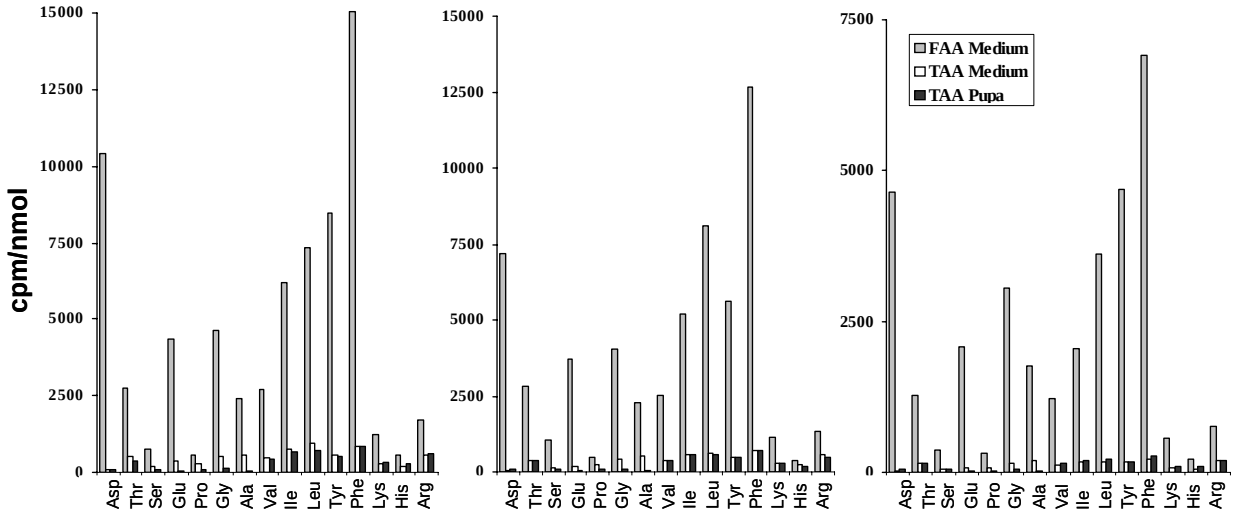

Figure 1. Specific activities (cpm/nmol) of free (FAA Medium) and total amino acids (TAA Medium) in the basic artificial medium as control, in this basic medium supplemented with 1.6 or 3.2% of casein, and of the total amino acids (TAA Pupa) in *Trichogramma pretiosum* pupae grown in these three media.

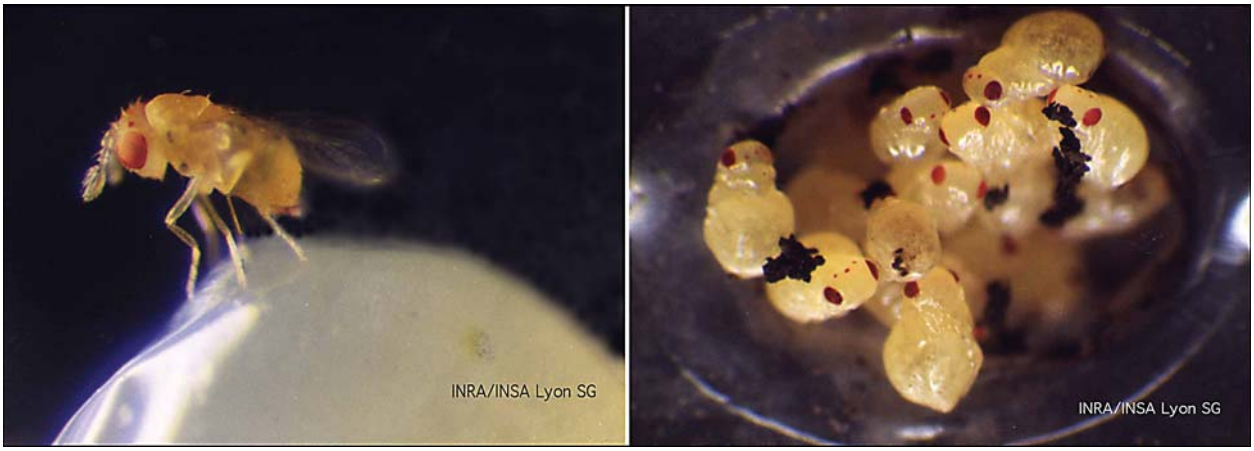

Figure 2. *Trichogramma* female laying eggs inside an artificial host egg (left); *Trichogramma* pupae grown in an artificial host egg (right). Photos: INRA/INSA de Lyon, Simon Grenier. UGA1390018, UGA1390019

Adult production (emergence rate or normal adult rate) was increased when casein was added either at 1.6 or 3.2% (Fig. 3). The lower emergence rate with 3.2% casein compared to 1.6% casein could be explained by the lower parasitization observed in the 3.2% casein medium: if the number of larvae in a cupule is too low, the larvae will become bloated and no further development can occur (Grenier *et al.* 1995). The percentage of normal adults was the highest in medium with 3.2% casein, probably in correlation with a higher amount in aa content of the pupae. The total aa content was 672.3 ± 38.0, 729.3 ± 28.0, and 839.6 ±36.4 nmol/mg for pupae grown in basic medium, and in medium with 1.6% or 3.2% of casein, respectively. The highest value for total aa content of pupae obtained in medium with 3.2% casein was lower than the control values obtained with pupae grown in *E. kuehniella* eggs (88.7 vs. 118.4 expressed in ng/µg), and also than the value (128.1 ng/µg) found for *Trichogramma dendrolimi* Matsumura grown in *E. kuehniella* eggs (Grenier *et al.* 1995).

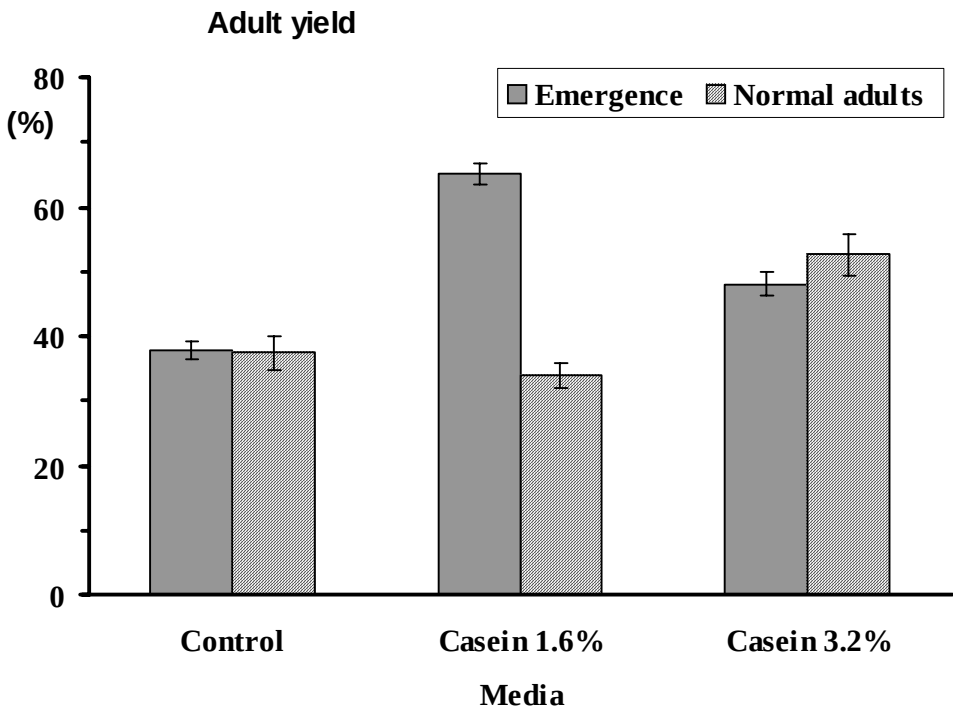

Figure 3. Percentages of emergence and normal adult rates of *Trichogramma pretiosum* on the basic artificial medium, and on the basic artificial medium supplemented with 1.6 or 3.2% casein. Means are given with their SE.

CONCLUSIONS

It was demonstrated that the principle of studying the assimilation rate of proteins can be applied successfully to tiny endoparasitoid insects such as *Trichogramma* species (pupal weight around 30 µg). The results revealed a complete utilisation of the proteins for essential aa, and showed the high level of implication in intermediate metabolism for the other aa.

The methodology, although quite complex and difficult to perform, was shown to be efficient. Through several experiments it appeared that the *Trichogramma* larvae completely assimilate all the proteins present inside the basic medium. Also the casein added into the medium was completely assimilated at the tested concentrations of 1.6 or 3.2%. Thus, casein could be used in artificial media to increase the protein content and improve the performance of the basic medium.

For further experiments, different proteins should be tested at various concentrations to enlarge the spectrum of the components to be used in artificial media. Experiments using this method could also be conducted on *Trichogramma* strains harbouring or not *Wolbachia*, a symbiont inducing thelytokous parthenogenesis in *Trichogramma*, to elucidate the possible role of this symbiotic rickettsia in the digestive physiology of the host. Artificial media could be used not only for production purposes, but also as a powerful tool to study the physiology of immature parasitoids, particularly endoparasitoids, by simplification of their environment (Grenier 2000), as shown again in this study.

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