Egyptian Journal of Biology, 2008, Vol. 10, pp 11-19
© Printed in Egypt. Egyptian British Biological Society (EBBSoc)

The effect of mulberry varieties and nutritional additives on the protein patterns of the silkworm *Bombyx mori*

Somia S El-Akkad [1], Eman MM Hassan [2], Ismail M Abdel-Nabi [3], Abdel-Hamid AI Zannoon [2] & Samy M Zalat [3]

1. Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt.
2. Plant Protection Research Institute, Agriculture Research Center, Cairo, Egypt.
3. Zoology Department, Faculty of Science, Suez Canal University, Ismailiya, Egypt.

Abstract

Two mulberry varieties *Morus alba* var. Kokuso-27 and *Morus indica* var. Kanva-2 were compared with *Morus alba* var. Balady (native) in their effects on the protein banding patterns of 5th-instar larvae of *Bombyx mori.* There was an obvious variation in the number and position of the bands, with many bands specific to a particular treatment. Protein of larvae fed on Kokuso-27 was characterized by the presence of 29 and 10 KDa bands; Kanva-2 produced bands at 251, 74 and 8 KDa; and Balady was characterized by bands at 38 and 11 KDa. When Kokuso-27 was enriched with vitamins C or B, or any of three kinds of bee-honey (clover, cotton and citrus honey) at various concentrations, new protein bands appeared relative to controls. Vitamin C produced bands at 56 and 43 KDa; protein bands at 290, 35 and 7 KDa were present in the control but absent in vitamin B treatments. Feeding with clover honey showed characteristic bands at 303, 49, 44, 37 and 21 KDa which were absent in the control. Cotton honey produced characteristic bands with molecular weights of 160, 52 and 13 KDa. Citrus honey produced bands at 73, 33, 29 and 8 KDa.

Keywords: *Morus alba* var. Kokuso-27, *Morus indica* var. Kanva-2, *Morus alba* native, vitamin C, vitamin B, bee-honey, SDS-PAGE.

Introduction

The silk threads of the cocoons of the silkworm *Bombyx mori* are composed of two major proteins (fibroin and sericin), produced by secretions of the silk glands. The silk gland is divided into three regions: anterior, central and posterior. The posterior silk gland secretes fibroin, while sericin, a glycoprotein which coats fibroin, is secreted by the central silk gland. The fibroin protein is transferred by peristalsis into the central silk gland where it is stored until required for spinning (Shimura 1993).

Mulberry leaves are rich in protein and amino acids, and there is a high correlation between leaf protein levels and the production efficiency of the cocoon shell, i.e. the cocoon shell weight relative to the total amount of mulberry leaves consumed by the silkworm (Machii & Katagiri 1991). It is therefore possible that an increase in the protein level of mulberry leaves may lead to improvements in cocoon productivity.

The nutritional richness of the diet influences the accumulation of storage proteins in the haemolymph of the silkworm larvae. Amounts of storage protein in silkworm larvae fed on a low protein diet were less than those fed on the standard diet, but larvae fed on optimal levels of protein showed higher levels of storage proteins (Nagata & Kobayashi 1990). Seo *et al*. (1985) studied the protein patterns of the haemolymph and various tissues of final-instar larvae and pupae by electrophoresis: 15 protein bands were separated from the haemolymph during metamorphosis and were maintained at a high concentration during the final larval instar, but declined after pupation.

Growth of silkworm larvae was improved significantly on feeding mulberry leaves supplemented with different nutrients including vitamins, sugar and other substances. The total protein content of the silk gland increased, while the water, total lipid and carbohydrate contents decreased (Sarker *et al*. 1995). The feeding of mulberry

* Address for correspondence

leaves dipped in different concentrations of thiamin solutions supplied an additional amount of thiamin to silkworms, and resulted in an increase in the protein content of the fat body and of the haemolymph, but only at low concentrations: at high concentrations the protein content of the fat body was almost unchanged (Nirwani & Kaliwal 1998). L-ascorbic acid (vitamin C) is an important vitamin and is abundant in plant tissues: green leaves have the same amount of ascorbate as chlorophyll (Foyer 1993). Zang & Ma (1991) studied the haemolymph vitamins of fifth-instar silkworm larvae, finding that vitamin concentrations of healthy larvae was greater than that of unhealthy larvae (viral-infected or fluoride-poisoned).

Here we compare the effects of two varieties of mulberry leaves, *Morus alba* var. Kokuso-27 and *Morus indica* var. Kanva-2, with *Morus alba* var. Balady (native) on the protein patterns in larval tissue of the 5th-instar larvae. Then we study the effects of fortifying the Kokuso-27 variety with some nutritional additives (vitamins C and B, clover, cotton and citrus honey).

Materials & Methods

Morus alba var. Kokuso-27 belongs to a group of varieties grown by artificial hybridization. Widely cultivated in Japan, it was produced by artificial hybridization between Naganuma (♀) × Kairyonezumigaeshi (♂). Its leaves are characterized by their shiny dark green colour and being soft for a long period. The variety is drought-resistant, but can also grow in waterlogged ground. The leaves contain 76% water and 24% dry matter, of which 24% is crude protein and 14% sugars (Minamizawa 1997).

Kanva-2 is a diploid variety of *Morus indica.* It is widely cultivated in southern India, whence it was derived (Mysore). The leaf moisture content is 70%, and the 30% dry matter contains 21% protein and 11.5% sugar. It has high rooting ability (80%) and wide adaptability. It is resistant to leaf spot, and moderately resistant to leaf rust and powdery mildew (Datta 2000).

Bombyx mori (9F 7X - Chinese hybrid) were reared under laboratory conditions (26±2°C, 70±5% RH) according to the technique of Krishnaswami (1978). In the first experiment, larvae were reared on fresh mulberry leaves of one of the three varieties with one of three different feeding schedules (two, three or four feeds per day). In the second experiment, larvae were reared on Kokuso-27 enriched with one of: vitamin C (concentrations 0.5, 1.0 or 2%), vitamin B (0.1, 0.2 or 0.4%) or one of three kinds of bee-collected honey (from clover, cotton or citrus flowers) at a concentration of 30, 50 or 70%. The control group had no enrichment. Mulberry leaves were dipped in each material for one minute and left to dry once per day; control leaves were dipped in distilled water. Each group was replicated 3 times.

To extract the tissue proteins, 1.5 ml of the extraction buffer was added per 0.5g fresh weight of tissue of the 5th-instar larva (2-Mercaptoethanol was added to the extraction buffer to provide reducing conditions, inhibiting polypeptide disulfide bonds from forming during aggregation). The silkworm tissue and the extraction buffer were ground together in a mortar using an ice bath to avoid the effects of proteolytic enzymes. The slurry was then centrifuged using a bench centrifuge (Gallenkamp) for 20 minutes at high speed (5000 rpm). The supernatant (extract) was then decanted to another centrifugation tube and kept in a refrigerator until needed.

SDS-polyacrylamide gel electrophoresis has been used widely in many studies to identify variation in protein patterns of many species (Kamikouchi *et al*. 2004). Polyacrylamide gel electrophoresis (PAGE) was used here for the separation of protein subunits and determination of protein molecular weights, as described by Hames (1981)

for a discontinuous buffer system. 50 µl were taken from each sample, mixed with 50 µl marker dye and left in boiling water bath for 5 minutes. 30 µl were taken and loaded into the wells of the prepared gel. Empty wells were filled with 30 µl marker dye, which produces bands with molecular weights of 209, 124, 80, 49, 34, 28, 20, 7 KDa, to mark the movement of the sample through the gel. The circuit was connected at 200 V and 150 mA for 6 hours using scientific vertical electrophoresis. The gel was removed and placed in Coomassie blue stain overnight, removed and placed in destain, which was changed regularly until good clear bands were apparent. The gel was analysed by Gel Documentation Advanced Software (El-Manar Co.).

Results

The electrophoretic pattern of the 5th-instar larval proteins (Figures 1-3) show 46 polymorphic bands with molecular weights ranging from 383 to 8 KDa. Qualitative analysis of proteins exhibited obvious variations in the number and position of bands between the larvae reared on each of the three investigated mulberry varieties. Many bands were found to be specific either to Kokuso-27 or Kanva-2 variety. Protein of larvae feeds on Kokuso-27 was characterized by the presence of 29 & 10 KDa bands. While, in case of Kanva-2 variety 251, 74 & 8 KDa protein bands were observed. Meanwhile, feeding on Balady variety was characterized by the presence of bands with molecular weights 38 & 11 KDa.

The mulberry variety Kokuso-27 was enriched with vitamin C with concentrations 0.5%, 1.0% & 2.0%, vitamin B with concentrations 0.1%, 0.2% & 0.4% and with three kinds of bee-honey (clover, cotton and citrus honey) each with concentrations 30, 50 and 70% and offered to silkworm larvae as nutritional additives. Then larval proteins of *Bombyx mori* in the 5th instar were analyzed by SDS-PAGE compared with the control, and the electrophoretic pattern is shown in figures 4-8. Forty-six polymorphic bands were detected with molecular weight ranging from 360 to 6 KDa. Qualitative analysis of proteins exhibited obvious variations in the number and position of bands from one additive compound to another and the appearance of new protein bands as a result of rearing larvae on these nutritional additives, these bands were absent in the control group; Feeding with vitamin C was characterized by the presence of bands with molecular weight 56 & 43 KDa which were absent in the control. The three concentrations of vitamin B when compared with the control group it is obvious that each concentration has characteristic protein bands were absent in the control. The protein bands with molecular weights 290, 35 & 7 KDa were present in the control and absent in vitamin B treatments. Vitamins C and B share bands with molecular weights of 49, 34, 31, 30, 28 & 25 KDa. Feeding with clover honey when compared with the control it is obvious that the protein bands with a molecular weights 303, 49, 44, 37 & 21 KDa were absent in the control. The cotton honey which represents the second kind of honey show an obvious variation in the number and position of protein bands and when compared with the control group; a protein band with molecular weight 37 KDa was absent in the control. Feeding with cotton honey was characterized among the investigated kinds of honey by the presence of protein bands with molecular weights 160, 52 & 13 KDa. A similar trend was also shown when compared between the citrus honey and its control; citrus honey was characterized by the presence of bands with molecular weights 73, 33, 29 & 8 KDa.

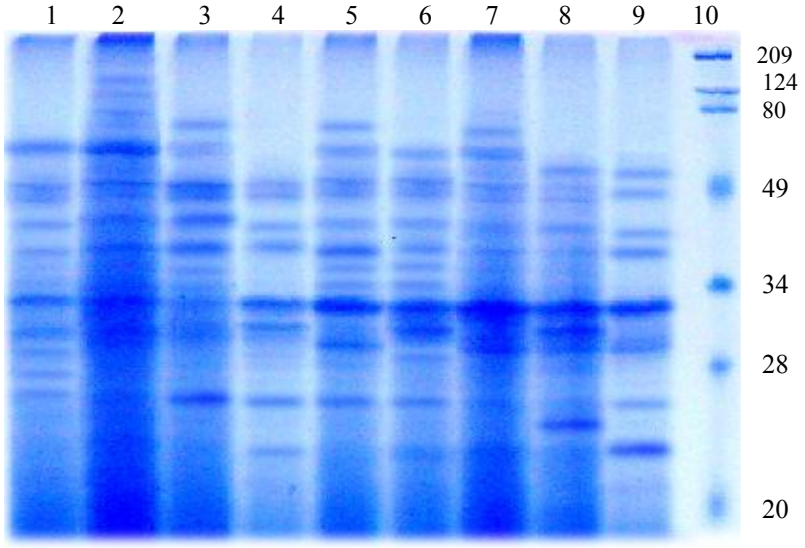

Figure 1: Protein banding patterns of 5th instar larvae reared on the Kokuso-27 variety of mulberry leaves. **Lanes 1-3:** 2 feeds/day; **4-6:** 3 feeds/day; **7-9:** 4 feeds/day. **10** = Marker: 209, 124, 80, 49, 34, 28 & 20 KDa.

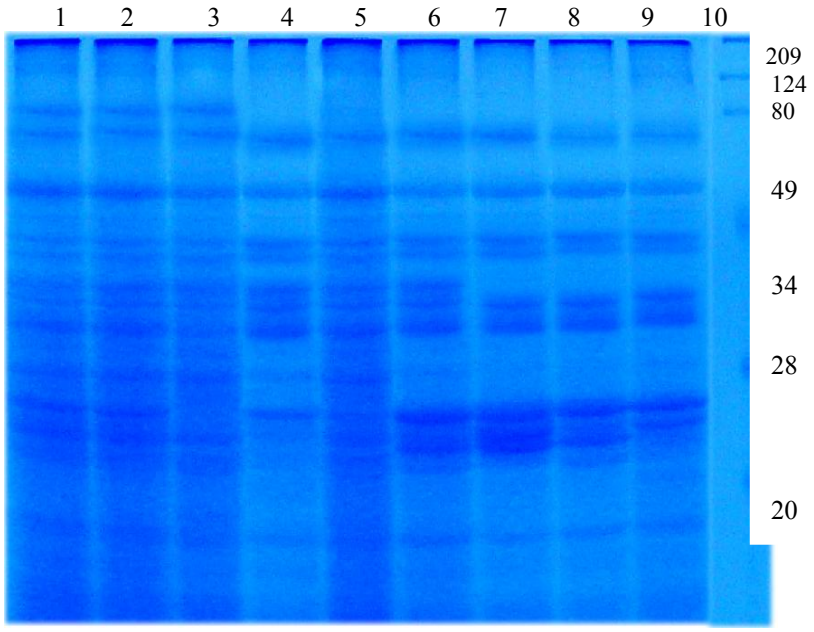

Figure 2: Protein banding patterns of 5th instar larvae reared on the Kanva-2 variety of mulberry leaves. **Lanes 1-3:** 2 feeds/day; **4-6:** 3 feeds/day; **7-9:** 4 feeds/day. **10 =** Marker: 209, 124, 80, 49, 34, 28 & 20 KDa.

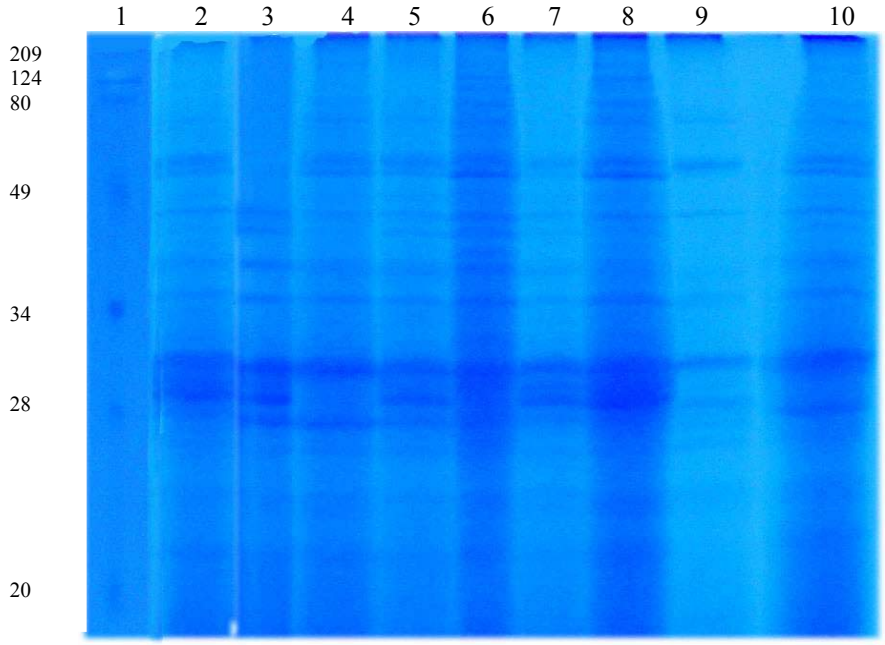

Figure 3: Protein banding patterns of 5th-instar larvae reared on the Balady variety of mulberry leaves. **Lane 1=** Marker: 209, 124, 80, 49, 34, 28 & 20 KDa. **Lanes 2-4:** 2 feeds/day; **5-7:** 3 feeds/day; **8-10:** 4 feeds/day.

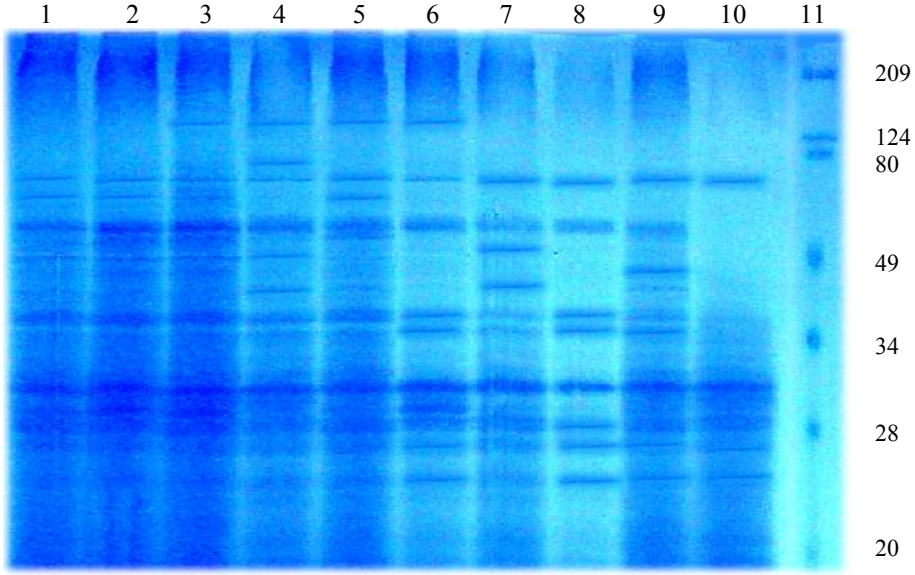

Figure 4: Protein banding patterns of 5th-instar larvae reared on Kokuso-27 enriched with Vitamin C. **Lanes 1-3:** concentration 0.5%; **4–6:** concentration 1.0%; **7–9:** concentration 2.0%; **10:** control. **11** = Marker: 209, 124, 80, 49, 34, 28 & 20 KDa.

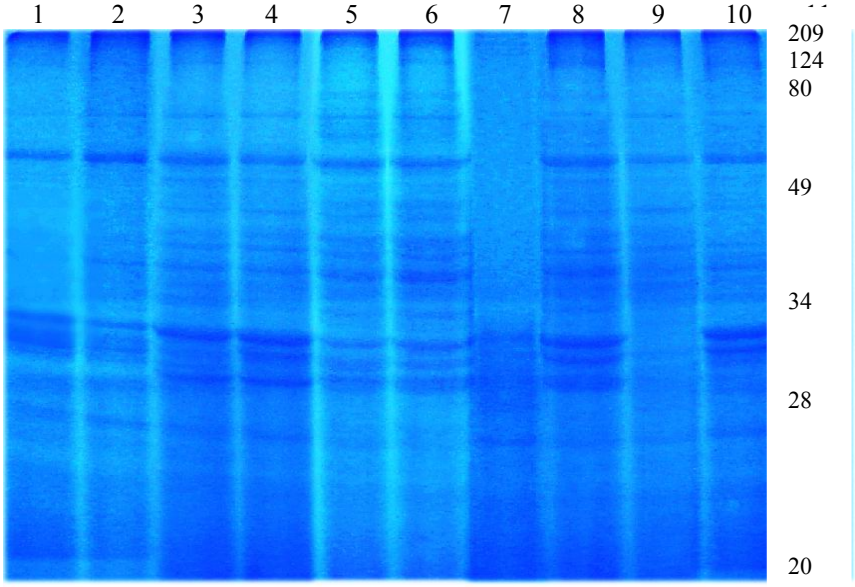

Figure 5: Protein banding patterns of 5th instar larvae reared on Kokuso-27 enriched with Vitamin B. **Lanes 1-3:** concentration 0.1%; **4–6:** concentration 0.2%; **7–9:** concentration 0.4%; **10:** control. **11** = Marker: 209, 124, 80, 49, 34, 28 & 20 KDa.

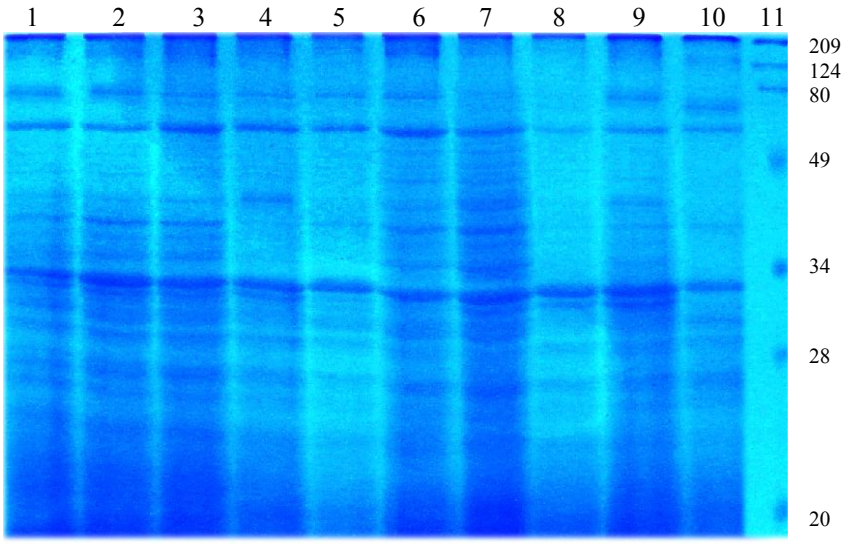

Figure 6: Protein banding patterns of 5th-instar larvae reared on Kokuso-27 enriched with Clover honey. **Lanes 1- 3:** concentration 30%; **4–6:** concentration 50%; **7–9:** concentration 70%; **10:** control. **11** = Marker: 209, 124, 80, 49, 34, 28 & 20 KDa.

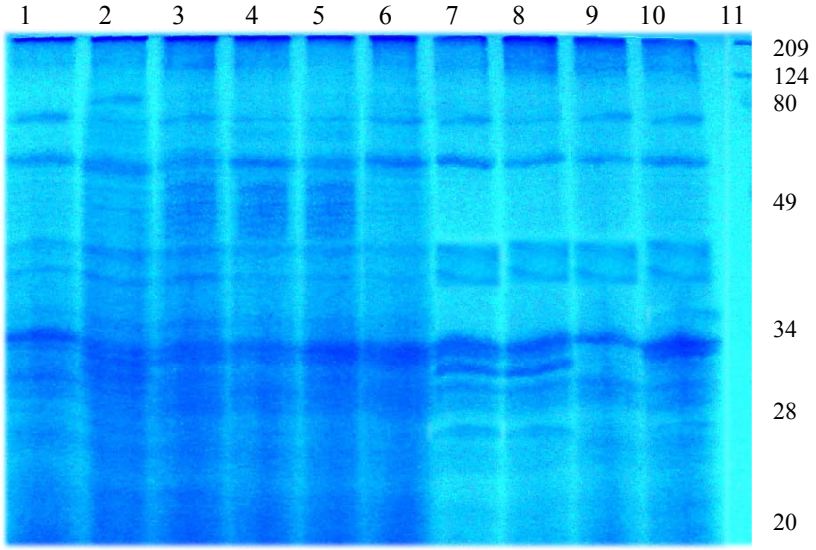

Figure 7: Protein banding patterns of 5th-instar larvae reared on Kokuso-27 enriched with Cotton honey. **Lanes 1-3:** concentration 30%; **4–6:** concentration 50%; **7–9:** concentration 70%; **10:** control. **11** = Marker: 209, 124, 80, 49, 34, 28 & 20 KDa.

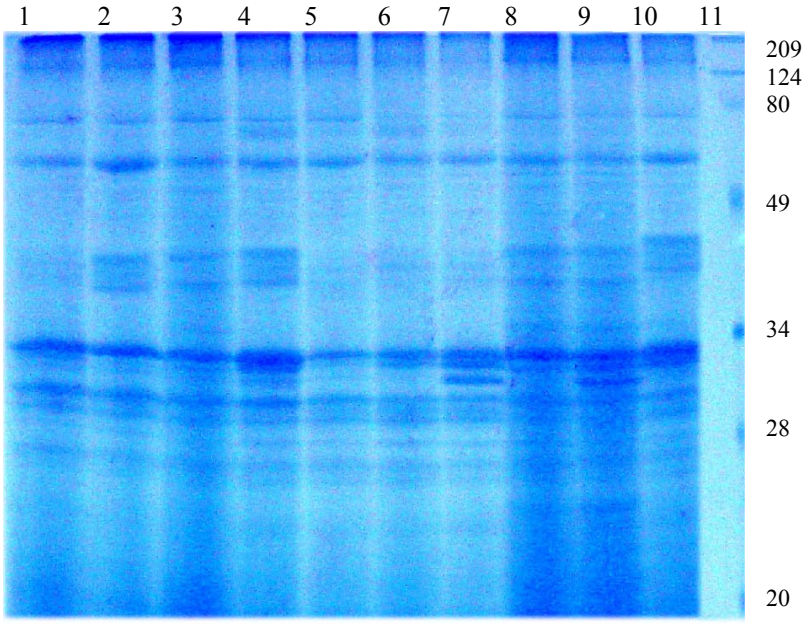

Figure 8: Protein banding patterns of 5th-instar larvae reared on Kokuso-27 enriched with Citrus honey. **Lanes 1-3:** concentration 30%; **4–6:** concentration 50%; **7–9:** concentration 70%; **10:** control. **11** = Marker: 209, 124, 80, 49, 34, 28 & 20 KDa.

Discussion

Qualitative analysis of silkworm proteins showed obvious variations in the number and position of bands when fed different mulberry varieties: Kokuso-27 was characterized by 29 and 10 KDa bands absent in other varieties; Kanva-2 was characterized by 251, 74 and 8 KDa bands; and Balady variety was characterized by 38 and 11 KDa bands.

Mahmoud (2000) and Ashour (2005) postulated that *Morus alba* var. Kokuso-27 contains comparatively more crude protein, soluble sugar, starch, moister and fat than *M. alba* var. *morittiana* and *M. alba* var. *rosa***.** The appearance of particular bands when larvae are fed a certain variety that disappear with another variety may be explained by varietal differences in crude protein content. The greater utilization of exogenous proteins may be due to high activities of amylase and protease in the haemolymph and midgut tissue, resulting in the production of more silk (LakshmiKumari *et al*. 1997). Thus variable bands might be due to upregulation of digestive (amylase) and oxidizing (succinate dehydrogenase) enzymes to help utilize more exogenous food materials, ultimately leading to more production. Hisashi (2001) studied varietal differences in the leaf-protein profiles of 17 diploid ($2n$ = 28) varieties of mulberry and found close correlations between inter-varietal similarity.

Larvae reared on Kokuso-27 leaves enriched with vitamin C were characterized by the presence of 56 and 43 KDa bands absent in the control group. 290 and 7 KDa bands were present in the control but absent in larvae fed vitamin-B enriched leaves. Variation in protein quality may arise from two possibilities. Firstly, the phagostimulant action of vitamins C and B increases food consumption and conversion, leading to increased amounts of proteins/amino acids and other nutrients contributing to extra growth of silkworm. This hypothesis is supported by the fact that silk content increases when ascorbic acid is added to the diet (Sarker *et al*. 1995). Vitamin C also aids in detoxification of various metabolic or tissue toxins and acts as strong antioxidant, increasing protein synthesis. Egg production increases on rearing on mulberry leaves supplemented with thiamine (vitamin B_1) (Nirwani & Kaliwal 1998). A second hypothesis is the action of vitamin B as a coenzyme in amino-acid and nucleic-acid metabolism. Activity levels of enzymes in the midgut, fat body tissues and silk glands increase as levels of dietary vitamin B_6 increase (Horie & Nakamura 1986).

Enriching Kokuso-27 leaves with honey produced new bands absent in the control, with qualitative differences among honey types. This might be caused by the presence of water-soluble fractions including protein, gluconic acid and ascorbic acid and perhaps the combined activities of enzymes as glucose oxidase, catalase and peroxidase in honey which prevent tissue destruction and help protein catabolism (Nagata & Kobayashi 1990). Only 40-65% of the total nitrogen in honey is protein, and some nitrogen resides are amino acids (Lee *et al*. 1990). Most of the amino acids are essential to life and must be obtained in the diet, and honey contains 11-21 free amino acids (Sinha & Sinha 1991). Proline, glutamic acid, alanine, phenylalanine, tyrosine, leucine and isoleucine are the most common, with proline predominating. Nour *et al*. (1997) concluded that propolis in honey has an anabolic effect on *B. mori* larvae, and the present work is consistent with this because honey resulted in a marked increase of haemolymph protein bands.

We conclude that rearing silkworm with good mulberry varieties rich in protein is highly beneficial to protein synthesis in larval tissues. Furthermore, fortification of mulberry leaves with either vitamins C or B, or with different kinds of bee-honey, precursors of protein, is recommended because they enhance protein metabolism.

Acknowledgements

We wish to thank our colleagues in the Biotechnology Research Center and Zoology Department, Faculty of Science, Suez Canal University for laboratory assistance.

References

Ashour A (2005) Silkworm feeding trials for evaluating the varietal effect of three mulberry leaves on the silkworm growth and cocoon yield quality. *Egypt Journal of Agricultural Research* 83(3): 1043-1049

Datta RK (2000) Mulberry for animal production: mulberry cultivation and utilization in India. *Central Sericultural Research and Training Institute, Central Silk Board, Srirampura, Mysore. Agriculture Department, FAO Animal Production and Health Paper* 147.

Foyer C (1993) Ascorbic acid. pp. 31-58 in RG Alscher & JL Hess (eds) *Antioxidants in Higher Plants.* CRC Press, Boca Raton.

Hames BD (1981) An introduction to poly-acrylamide gel electrophoresis. pp. 1-91 in D Hames & Richwool D (eds.) Practical approach. IRL Press, Oxford.

Hisashi H (2001) Varietal differences of leaf protein profiles in mulberry. *Phytochemistry* 21(7): 1513-1518

Horie Y & Nakamura M (1986) Effect of dietary pyridoxine on alanine and aspartate aminotransferases in the silkworm, *Bombyx mori* (Lepidoptera: Bombycidae). *Applied Entomology & Zoology* 21(1): 164-170

Kamikouchi A, Morioka M & Kubo T (2004) Identification of honey bee antennal proteins expressed in a sex- and /or caste-selective manner. *Zoological Science* 21(1): 53-62

Krishnaswami S (1978) New technology of silkworm rearing. *Bulletin of the Central Sericultural Research & Training Institue, Mysore* 2: 1-10

LakshmiKumari B, Ananthanarayana SR & Jayaprakash K (1997) Effect of radiation on the activity of digestive enzymes in the silkworm *Bombyx mori* L. *Sericologia* 37(2): 221-228

Lee CY, Smith NL, Underwood BA & Morse RA (1990) Honey protein from different bee species in relation to apple juice clarification activity. *American Bee Journal* 130(7): 478-479

Machii H & Katagiri K (1991) Varietal differences in nutritive values of mulberry leaves for rearing silkworms. *JARQ* 25: 202-208

Mahmoud S (2000) Feeding effect of different mulberry varieties on silkworm, *Bombyx mori* L. *Egyptian Journal of Applied Science* 15(6): 253-261

Minamizawa K (1997) Moriculture: science of mulberry cultivation. CRC Press. 169 pp.

Nagata M & Kobayashi J (1990) Effect of nutrition on storage protein concentrations in the larval haemolymph of the silkworm, *Bombyx mori. Journal of Sericultural Science, Japan* 59: 469-474

Nirwani RB & Kaliwal BB (1998) Effect of thiamine on commercial traits and biochemical contents of the fat body and haemolymph in the silkworm, *Bombyx mori* L. *Sericologia* 38(4): 639-646

Nour ME, El-Maasarawy SA & Mahmoud S (1997) Propolis in the nutrition of the silkworm *Bombyx mori* L.: influence on biology, silk yield and biochemical changes in haemolymph. *Proceedings of the 1st International Conference of Silk "ICSAI".*

Sarker A, Haque M, Rab M & Absar N (1995) Effect of feeding mulberry (*Morus* sp.) leaves supplemented with different nutrients to silkworm (*Bombyx mori* L.). *Current Science* 69(2): 185-188

Seo EW, Yun CY, Kang CS & Kim HR (1985) A study on the protein pattern of haemolymph during last instar larval and pupal stages of *Bombyx mori*. *Entomological Research Bulletin* 11: 153-163

Shimura K (1993) Physiology and biology of spinning in *Bombyx mori*. *Experientia* 39: 441-450

Sinha RP & Sinha PN (1991) A study on the biochemical composition of fresh and stored mustard pollen and honey. *Apicata* 26 (2): 38-44

Zang RC & Ma ZC (1991) A study on vitamins in the haemolymph of fifth instar larvae of *Bombyx mori*. *Acta Entomologica Sinica* 34 (4): 433-437

الملخص العربي

تأثيرات بعض أصناف ورق التوت وبعض الإضافات الغذائية علي الشرائح البروتينية لديدان الحرير "*بومبيكس موراي*"

سمية صلاح الدين العقاد[1] ، إيمان محمود محمد حسان [٢]، إسماعيل محمد عبد النبي[3] ، عبد الحميد على إبراهيم زنون[٢]، سامى محمد زلط [3]

١- قسم النبات، كلية العلوم، جامعة عين شمس، القاهرة، مصر.
٢- معهد بحوث وقاية النباتات، مركز البحوث الزراعية، القاهرة، مصر.
٣- قسم الحيوان، كلية العلوم، جامعة قناة السويس، الإسماعيلية، مصر.

أجريت هذه الدراسة لبيان تأثير صنفين من أصناف ورق التوت وهما: الصنف الياباني (Kokuso-27) والصنف الرومي (Kanva-2) علي شرائح البروتين في أنسجة ديدان الحرير ومقارنتهما بالصنف المحلي المنتشر في مصر (Balady)، لتعيين الصنف الأفضل وكذلك نظام أو عدد الوجبات اليومية الأكثر فاعلية في تغذية اليرقات، حيث تم تقسيم اليرقات ثلاث مجموعات، وتم تغذية كل مجموعة بعدد وجبات مختلف (مرتان و ثلاثا و أربع مرات يوميا) وذلك لكل صنف. وقد أدى التحليل الكهربي النوعي للبروتين في أنسجة اليرقة في العمر الخامس إلي تباين واضح في عدد ومواضع شرائح البروتين بين الأصناف قيد الدراسة وظهور شرائح بروتينية مميزة لكل صنف وكذلك لكل نظام غذائي. حيث تميز الصنف الياباني بظهور الشرائح البروتينية ذات الأوزان الجزيئية ٢٩ & ١٠ كيلو دالتون في بروتين اليرقة، بينما تميز الصنف الرومي بالشرائح البروتينية ٢٥١، ٧٤ & ٨ كيلو دالتون، أما التغذية بالصنف البلدي فقد تميزت بظهور الشرائح البروتينية ذات الأوزان الجزيئية ٣٨ & ١١ كيلو دالتون. ثم تم تزويد ورق التوت الياباني الذي ثبت تميزه وارتفاع قيمته الغذائية في التجربة الأولى ببعض الإضافات الغذائية بتركيزات مختلفة؛ حيث تم استخدام فيتامين سي بثلاث تركيزات (٠.٥%، ١.٠% و ٢.٠%) و فيتامين بي المركب بثلاث تركيزات (٠.١%، ٠.٢%، ٠.٤%) و تم استخدام ثلاثة أنواع من عسل النحل (عسل البرسيم و القطن و الموالح) بثلاث تركيزات أيضا (٣٠%، ٥٠% ، ٧٠%) وتمت دراسة تأثير هذه الإضافات (مجموعات الاختبار) ومقارنتها بمجموعة الكنترول وذلك لبيان تأثير هذه الإضافات الغذائية علي شرائح البروتين في أنسجة يرقات العمر الخامس. وقد أدى التحليل الكهربي إلي تباين واضح في عدد ومواضع شرائح البروتين بين الإضافات الغذائية قيد الدراسة مع ظهور شرائح بروتينية مميزة لكل معاملة غير متواجدة في مجموعة التحكم نتيجة للتغذية بهذه الإضافات. حيث ظهرت الشرائح البروتينية ذات الأوزان الجزيئية ٥٦ & ٤٣ كيلو دالتون في بروتين اليرقات المغذاة بورق التوت المدعم بفيتامين سي، أما الشرائح البروتينية ٢٩٠، ٣٥ & ٧ كيلو دالتون فقد ظهرت في مجموعات التحكم بينما اختفت من معاملات فيتامين بي. وقد أظهرت التغذية بعسل البرسيم الشرائح البروتينية ٣٠٣، ٤٩، ٤٤، ٣٧ & ٢١ كيلو دالتون والتي اختفت من مجموعة التحكم. وقد تميز عسل القطن عن أنواع العسل الأخرى بشرائح البروتين ذات الأوزان الجزيئية ١٦٠، ٥٢ & ١٣ كيلو دالتون، وعسل الموالح تميز بالشرائح البروتينية ٧٣، ٣٣، ٢٩ & ٨ كيلو دالتون. وهذا يوضح وجود تحسن في تكوين البروتين وكذلك الحالة الحيوية لليرقات وبالتالي زيادة جودة خيط الحرير وزيادة إنتاجه التي تعتبر الهدف الأساسي لهذه الدراسة.