INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

Metamorphic changes in the profiles of transdeamination parameters in the intersegmental muscle of the silkworm, *Bombyx mori.*

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ABSTRACT

Changes in the profiles of transdeamination parameters (Eg. free amino acids, aminotransferases and glutamate dehydrogenase activity) were analyzed in the intersegmental muscle (ISM) of thoracic and abdominal segments of *Bombyx mori* during pupal-adult metamorphosis. The growth trends in the levels of free amino acids (FAA) and activity levels of aspartate aminostransferase (AAT), alaninine aminotransferase (AlAT) and glutamate dehydrogenase (GDH) indicates that transdeamination pathway undergoes stage-specific, region-specific and sex-specific quantitative changes in *B. mori* during metamorphosis. Firstly, the pathway seems to be active during the early and later phases of pupal life and in the adult stage, indicating the fact that the metamorphic energy demands of larval-pupal and pupal-adult transitional stages are met through gluconeogenesis. Secondly, this process starts early in the abdominal muscle and late in the thoracic muscle, reflecting the fact that the transdeamination process is more active in the rapidly degenerating tissues compared to those of slower ones. Thirdly, the transdeamination activity, mediated by AlAT and GDH takes preponderance in males, while that mediated by AlAT assumes significance in females. The preponderance of GDH activity in males indicates that alpha ketoglutarate is used as a substrate for sperm production, sperm motility and successful mating, which in turn facilitates higher choice for fertilization leading to higher fecundity and viable productivity. Further, the findings indicate that most of the higher energy demands for the expression of male sex in *B. mori* are met through enhanced levels of GDH.

Keywords: Aminotransferases, *Bombyx mori*, Free amino acids, Glutamate dehydrogenase, Transdeamination.

INTRODUCTION

The insect metamorphosis is a sequentially ordered energy-intensive process involving histolysis, histogenesis, differentiation and morphogenesis of larval and pupal tissues to support the re-architecture of organs in the adult[1]. It has been demonstrated that some larval muscles, which have no role in the pupal and adult stages undergo degeneration and disappear, finally giving place for the development of new muscles that have pre-destined roles in the later stages of life[2, 3]. The intersegmental muscle (ISM) is one important tissue that undergoes remarkable transitional changes in form and function during metamorphosis in *B. mori*. Essentially, these changes include the disintegration of abdominal muscle and reformation of thoracic muscle. With a rich source of over 258 proteins, the ISM plays a vital role in energy metabolism by performing a variety of functions including respiration, thermiogenesis, circulation of body fluids, body movements, maintenance of posture, oviposition, copulation, locomotion, jumping movements, stabilizing joints etc[4-5].

Though, most of the energy requirements are met from the dietary reserves obtained during the larval stage, it is most unlikely to cater to the diverse needs of organogenesis, characteristic of the subsequent pupal and adult stages[6-7]. Obviously, the silkworm has to rely on alternative energy sources for the successful completion of metamorphosis. Since, the pupal stage represents the non-feeding stage; the silkworm makes good its energy requirements by mobilizing them internally from the adjacent tissues that are subjected to histolysis under the impact of increased proteolytic activity[8]. The free amino acid pool, generated from proteolysis has been identified as one of the major candidates for energy turnover during metamorphosis[9-11]. More importantly, the energy requirements of metamorphosis are met from this pool through transdeamination, a dynamic metabolic process triggered under the influence of aminotransferases and glutamate dehydrogenase[12-13]. The present study intends to assess the role of transdeamination in energy mobilization by analyzing changes in free amino acid (FAA) reserves and in the activity levels of aspartate amino transferase (AAT), alanine amino transferase (AlAT) and glutamate dehydrogenase during pupal-adult metamorphosis in *B. mori*.

MATERIAL AND METHODS

The Pure Mysore x CSR_2 hybrid strain of silkworm *Bombyx mori* was selected as the test species and its pupal and adult stages were used in the present investigation. Earlier, its larvae were reared under standard environmental conditions of 28^o C, 85% RH and fed with M_5 variety of mulberry leaves, 5 times a day at 6 A.M, 10 A.M, 2 P.M, 6 P.M and 10 P.M throughout the larval period[14]. Biochemical assays were carried out on the intersegmental muscle of thoracic and abdominal segments. The muscle tissue was collected by the mid-dorsal dissection of the pupal and adult bodies in *Bombyx* Ringer[15]. The Free amino acid levels were estimated by the method of Moore and Stein[16] in 5% homogenate of the muscle tissue in 10% trichloroacetic acid. The activity levels of aspartate (AAT) and alanine (AlAT) amino transferases were estimated by the method of Reitman and Frankel[17] in 5% homogenate in distilled water, while the glutamate dehydrogenase (GDH) activity was estimated by the method of Lee and Lardy[18] in the 5% homogenate in ice-cold 0.25 M sucrose solution. The experimental data were statistically analyzed using online software (www.graphpad.com/ quick calcs/ index cfm/) / (www.percent – change com/index php) and MS Excel platforms and the growth trends in the levels of the free amino acids and enzymes activities were analyzed in terms of compound periodical growth rates (CPGRs) as given by Sivaprasad[19].

RESULTS AND DISCUSSION

During starvation and non-feeding conditions, most of the energy requirements of the body are met from the gluconeogenesis[20]. In this process, some of the glucogenic amino acids are converted to carbohydrates through transdeamination, an inherent pathway mediated by three enzymes; aspartate aminotransferase (AAT) alanine aminotransferase (AlAT) and glutamate dehydrogenase (GDH). In a process called transamination, the aminotransferases (AAT and AlAT) catalyze the transfer of amino group of one amino acid to a keto acid, resulting in the formation of L-glutamate, which then acts as an amino group donor for biosynthetic pathways or for excretion pathways that lead to the elimination of nitrogenous wastes. Glutamate dehydrogenase (GDH), the mitochondrial matrix enzyme, causes the conversion of glutamate to alpha-keto glutarate, during which energy is released. The alpha–keto glutarate, so formed enters the citric acid cycle for the synthesis of glucose[21-22]. Since, *B. mori* remains in a state of starvation during pupal and adult stages, the changes in the levels free amino acids (FAA), together with those of AAT, AlAT and GDH are considered valuable parameters of transdeamination and its contribution to energy pool during insect metamorphosis. The findings of the present study on these parameters are presented in the Table 1 and Figures 1 to 2.

Free amino acids: The free amino acid (FAA) pool in the silkworm is contributed by the proteolysis of disintegrating tissues such as the muscle, silk gland and the gut[11, 23]. Besides acting as the precursors for proteins, they play essential role in the synthesis of fats and vitamins. Additionally, the FAA pool is a rich source of aspartate, alanine and glutamate that participate in transdeamination and energy production[24]. Obviously, their levels in the silkworm muscle vary as a function of metamorphic needs and demands. The growth trends in the FAA pool, analyzed in terms of compound periodical growth rates (CPGRs) revealed that their levels in the intersegmental muscle (ISM) undergo region-specific quantitative changes in the thorax and abdomen (Table 1). Initially, their levels grew @23.8% per day in the thorax and @9.2% in the abdomen, indicating their availability for transdeamination. Though, the increasing trend in their levels continued @2% per day in thorax, it actually declined @15.8% per day in the abdomen during the latter half of the pupal life (i.e., from day 5 to day 9). Interestingly, the declining trend continued through the late pupal and adult stages, reflecting their active utilization in during pupal-adult transitional period (Table 1). Interestingly, their utilization is more predominant in females (86.6%) compared to that in males (76.8%).

AAT activity: The activity levels of AAT projected fluctuating trends during metamorphosis. The enzyme activity showed contrary growth trends during the first half of the pupal life (i.e. from day 1 to day 5). While, it declined @14% per day in the thoracic muscle, it actually rose @ 11% per day in the abdominal muscle. Thereafter, during the latter half of the pupal life, the enzyme activity was slightly elevated @3% per day in each of the thoracic and abdominal muscles and maintained a static trend during the transition from pupal to adult stage in males. But, interestingly, the AAT activity recorded an elevation (+22%) in males, but slumped (-22%) in females (Table 1).

AlAT activity: The activity levels of alanine amino transferase were similar to those of AAT and projected contrasting trends in the thoracic and abdominal muscles. While the enzyme activity showed a declining trend in the thoracic ISM, it rose consistently in the abdominal ASM during pupal development. During this period, the enzyme activity declined @15.9% per day during the first five days and @10% in the next four days. During pupal-adult transition, the AAT activity increased by 12.5% in males and 18.8% in females in the thoracic muscle and increased by 9.3% in males and declined by 28% in females (Table 1).

GDH activity: The glutamate dehydrogenase activity showed declining growth trends during the first half of the pupal development and elevatory trends during the latter half of the pupal life and during the pupal-adult transition. In the thoracic ISM, its activity declined @32.5% per day during the first five days of pupal life and raised @5.6% per day during the latter half of the pupal life. Interestingly, the GDH activity rose manifold during the pupal-adult transition in males and just by 80% in females. At the same time, in the abdominal ISM, the enzyme activity decreased @22.1% during the first five days of pupal life, but increased just @2.3% during the next four days of pupal life. As in the thoracic ISM, the enzyme activity recorded a CPGR of 393% in males and 86.6% in females (Table 1).

The positive and negative growth trends in the levels of FAA, AAT, AlAT and GDH reflect stage-specific, region-specific and sex-specific occurrence of transdeamination during the metamorphosis of *B. mori*.

1. Firstly, the transdeamination pathway seems to be in place during the early and later phases of pupal development and adult life, compared to that of mid-pupal life. Because of this reason, the activity levels of all the three enzymes, which maintained a higher profile during the early pupal stage actually declined in the mid and late pupal stages and increased in the adult stages (Figs 1 and 2). The fluctuations in their levels reflect concomitant trends in gluconeogenesis and energy production[2, 12]. More importantly, the transamination-triggered gluconeogenesis appears to be the major source of energy during the larval-pupal and pupal-adult transitional stages of metamorphosis in silkworm. Thus, the additional energy requirements of body reorganization and reformation during insect metamorphosis are met through gluconeogenesis predominantly, if not exclusively.
2. Secondly, transamination begins early in the abdominal ISM, compared to that of thorax as reflected in the higher growth trends in the activity levels of AAT (-14% in thorax and +11% in abdomen), AlAT (-16% in thorax and +9% in abdomen) and GDH (-32.5% in thorax and -22% in abdomen) in the abdominal muscle compared to that of thorax (Figs. 1and 2). As reported in our earlier findings, the transdeamination seems to be more active in degenerating tissues compared to those of reforming ones[25]. Obviously, the rapidly degenerating abdominal intersegmental muscle contributes the much needed amino acid reserves for transdeamination, which apparently meets the short-term energy requirements in all abdominal segments. Conversely, the low or no transdeamination activity in the thoracic muscle is attributable to active utilization of FAA in the protein synthesis that characterize the growth and development musculature associated with wings and legs in adult flies. Nevertheless, considerable part of the amino acid pool from the disintegrating thoracic ISM, is apparently diverted via transdeamination for meeting immediate energy requirements.
3. Thirdly, the study points out that the transdeamination activity, mediated by AlAT and GDH takes preponderance in males, while that mediated by AlAT assumes significance in females (Figs. 1 and 2) This is evidenced by corresponding elevations in the activity levels of all the three enzymes, with concomitant falls in the levels of FAA during pupal-adult transition. More importantly, the transdeamination, mediated by GDH seems to be more advanced in males (355 to 393%), compared to that in females (80 to 86%), while the role of AAT and AlAT seems to be minimal during this transition period (- 6.5% to +12.5% in males) and females (-53% to +21% in females). The preponderance of GDH activity in males substantiates that alpha ketoglutarate is

used as a substrate for sperm production, sperm motility and successful mating, which in turn facilitates higher choice for fertilization leading to higher fecundity and viable productivity[9,12,26]. Obviously, higher energy demands for the expression of male sex in *B. mori* are met through enhanced levels of GDH. This is further supported by the fact that the genes responsible for glycolytic pathways are down regulated, while those of others are induced during pupation and mating[27]. Probably, the induced genes belong to the group of transdeamination and this requires elucidation.

CONCLUSION

During pupal-adult metamorphosis, the silkworm, *Bombyx mori* undergoes great many changes in form and behaviour before attaining adulthood and sexual maturity. Since, the pupal and adult stages represent non-feeding stages; the silkworm obtains its energy requirements by suitably modifying its metabolism. Transdeamination is one such process during which glucose is synthesized from the glucogenic amino acids emanating from the proteolysis of larval tissues. The larval intersegmental muscle is one such tissue that undergoes total transformation through histogenesis and histolysis and contributes the much needed reserved pool of free amino acids required for gluconeogenesis. In this process, the glucogenic amino acids are subjected to transdeamination by active participation of aspartate aminotransferase (AAT) alanine aminotransferase (AlAT) and glutamate dehydrogenase (GDH). All these parameters show stage-specific, region-specific and sex-specific variation in their profiles during metamorphosis. The transdeamination process seems to be active in the abdominal muscle compared to that of the thorax and appears to be the major source of energy during larval-pupal and pupal-adult transitional stages. More importantly, the transdeamination, mediated by AlAT and GDH seems to takes precedence in males compared to females, indicating their active role in male sex maturation, sperm production and copulation. The study opens new avenues for exploratory investigations in the energetics of insect metamorphosis.

ACKNOWLEDGEMENTS

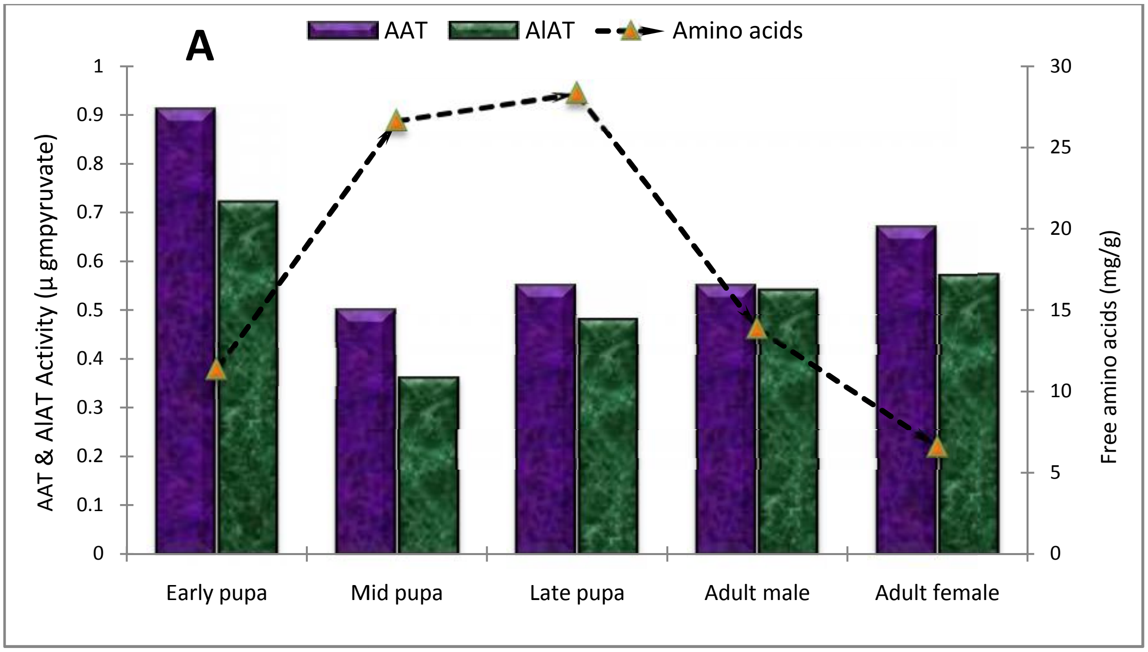
The author thanks the Department of Science and Technology, New Delhi, for providing financial assistance for this research project.

Table 1
Changes in the levels of free amino acids, AAT, AlAT and GDH activity in the Intersegmental muscle of *Bombyx mori* during pupal- adult metamorphosis.

Day	Statistical tool	Free Amino acids *(mg / gm wt)*		AAT (µ m of pyruvate / mg protein / h)		AlAT (µ m of pyruvate / mg protein / h)		GDH (µ m of pyruvate / mg protein / h)	
		Thorax	Abdomen	Thorax	Abdomen	Thorax	Abdomen	Thorax	Abdomen
Early Pupa (Day 1)	Mean	11.34	19.14	0.91	0.54	0.72	0.46	0.82	0.38
	S.D	± 0.97	± 0.58	± 0.033	± 0.014	± 0.05	± 0.02	± 0.033	± 0.02
Mid Pupa (Day 5)	Mean	26.65	27.2	0.50	0.83	0.36	0.64	0.17	0.14
	S.D	± 1.95*	± 1.82*	± 0.039*	± 0.036*	± 0.01*	± 0.009*	± 0.015*	± 0.01*
	CPGR (%)	23.81	9.18	-13.90	11.35	-15.91	8.61	-32.52	-22.09
Late Pupa (Day 9)	Mean	28.34	16.24	0.55	0.93	0.48	0.75	0.20	0.15
	S.D	± 1.63**	± 0.54*	± 0.017**	± 0.02*	± 0.02*	± 0.006*	± 0.004*	± 0.01**
	CPGR (%)	2.07	-15.79	3.23	3.86	10.06	5.43	5.57	2.33
Adult Male	Mean	13.88	8.09	0.55	0.87	0.54	0.82	0.91	0.74
	S.D	± 0.33*	± 0.17*	± 0.012**	± 0.022*	± 0.02**	± 0.013*	± 0.013*	± 0.004*
	CPGR (%)	-51.02	-50.18	0.00	-6.45	12.50	9.33	355.0	393.33
Adult Female	Mean	6.59	2.18	0.67	0.44	0.57	0.54	0.36	0.28
	S.D	± 0.48*	± 0.24*	± 0.021*	± 0.005*	± 0.02**	± 0.010*	± 0.015*	± 0.02*
	CPGR (%)	-76.75	-86.58	21.82	-52.69	18.75	-28.00	80.00	86.6

**Statistically significant (P<0.001), ** Statistically not significant.*

Each value in the above table, expressed as mg / g wt. of tissue in case of FAA and µ moles of pyruvate formed / mg protein / hour in case of AAT, AlAT, GDH, *represents the mean ± standard deviation (SD) of four separate observations. For each observation tissue from 10 to 15 animals were pooled. The compound periodical growth rates (CPGR) were computed separately from day1to day 5, from day 5 to day 9 of pupal life and from day 9 of pupa to adult stage.*



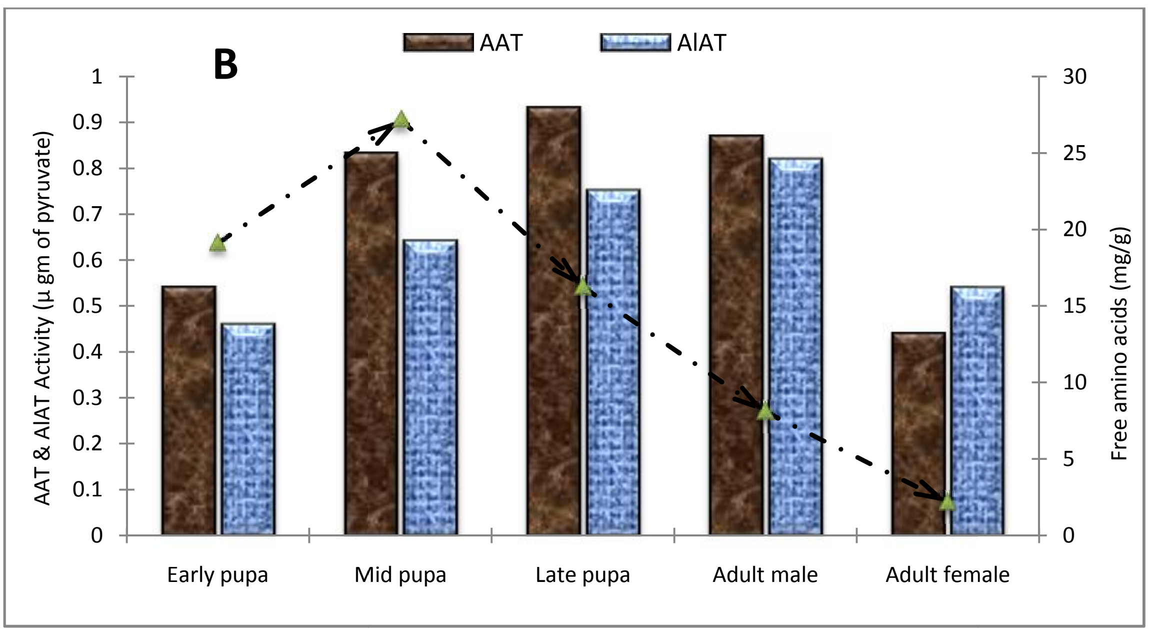


Fig. 1
Changes in the levels of AAT, AlAT activity and FAA in the intersegmental muscle of thorax (A) and abdomen (B) in *Bombyx mori* during pupal-adult metamorphosis.

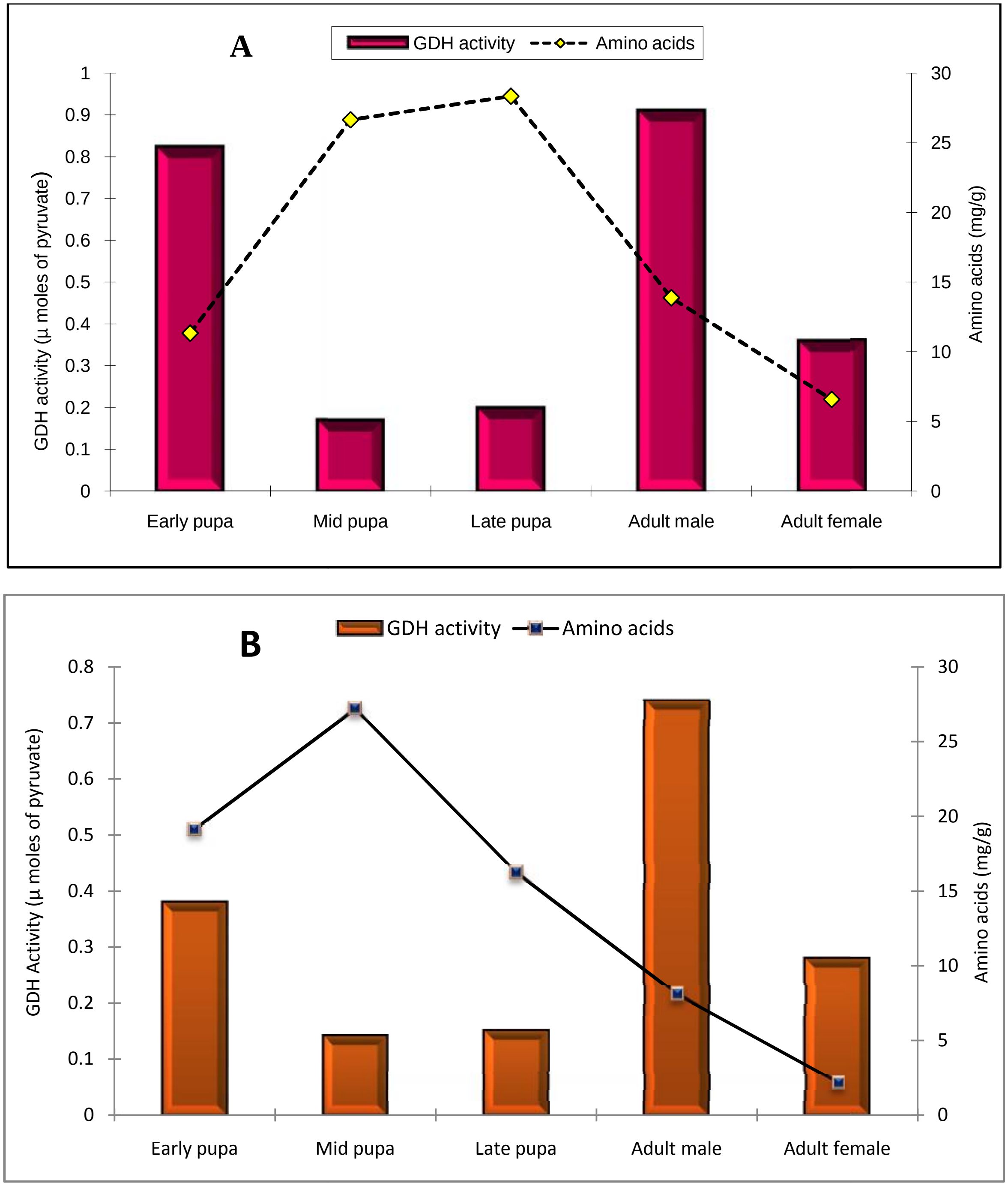


Fig. 2
Changes in the levels of GDH activity and FAA in the intersegmental muscle of thorax (A) and abdomen (B) of *Bombyx mori* during pupal-adult metamorphosis.

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