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Research Journal of Agricultural Sciences
An International Journal

P- ISSN: 0976-1675
E- ISSN: 2249-4538

Volume: 12
Issue: 06

Res. Jr. of Agril. Sci. (2021) 12: 2175–2179

CARAS

Res. Jr. of Agril. Sci. (Nov-Dec 2021) 12(6): 2175–2179
www.rjas.org

Full Length Research Article

Variation in the Aminotransferase Enzymes in Response to the Dietary Supplementation of Amino Acids in the Silkworm Hybrids

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Received: 29 Aug 2021 | Revised accepted: 12 Nov 2021 | Published online: 07 Dec 2021

A B S T R A C T

The dietary nutrition derived from the mulberry foliage is an essential as it regulates the growth and development of the silkworm and hence influences the quality of cocoon produced by them. By and large the nutritional value of mulberry leaves varies in response to both biotic and abiotic factors. Among several dietary supplementations, amino acids play a vital role which regulate metabolism in the silkworm which is essential in their development. In the current investigation, we have analyzed the consequences of dietary supplementation of essential amino acids namely methionine, phenylalanine and valine at different concentrations (0.5, 1.0 and 1.5%) against the alanine and aspartate aminotransferase enzymes activity levels in three different silkworm hybrids viz. FC_1, FC_2 and FC_1 X FC_2. The results obtained revealed that increase in the activity levels of alanine and aspartate aminotransferases has been observed in all the hybrids of the silkworm administered with three amino acids. However, administration of 1.5% of phenylalanine has been proven to be ideal in the enhancement of aminotransferases activity in the fat body tissue and haemolymph of the silkworm hybrids $FC_1 \times FC_2$ followed by FC_2 and FC_1 as compared to control batches. In contrast the lowest aminotransferase activity was expressed by the silkworm hybrids in valine supplemented batches at 0.5% concentration.

***Key words*:** Silkworm, Essential amino acids, Dietary supplementation, Alanine, Aspartate aminotransferase

Since its discovery in 2700 BC till now, sericulture industry has seen several pros and cons with respect to the improvement in the quality of silk derived from the silkworm, *Bombyx mori* L. [1]. The mulberry silkworm is the only sericigenous lepidopteran insect domesticated to obtained economically valuable silk [2]. Silk production is a complex physiological phenomenon which is exclusively regulated by the quality and quantity of leaves consumed by the silkworm. The silkworm, *B. mori* is a monophagous insect that feeds on only mulberry leaves only during larval stages to sustain energy for metamorphosis [3], that further contribute 70 per cent of silk proteins [4]. In general, the quality of mulberry leaves influenced by several factors, including soil type, mulberry variety, irrigation schedule, fertilizer input, spacing, maturity and ecoclimatic variations [5-7] which in turn reflects in the quality of silk produced. Supplementation of nutrients through diet is one of the recent techniques to enhance the economic parameters and biomolecules. For instance, the lepidopteran larvae *Daphnis nerii* fed with tender leaves of *Nerium oleander* not only increase the growth and development but also biomolecules viz., protein, carbohydrates, lipids, amino acids and nitrogen content [8]. Similarly, the dietary supplementation of proteins and amino acids by the mulberry leaves are essential in the silk protein synthesis [9]. On the other hand, the enzymes are organic biocatalyst responsible for proper physiological function and hence directly influence the growth and development of silkworm by regulating over all metabolism [10]. The nutritional scarcity often alters biochemical pathways in turn influences the growth and development of silkworm resulting in decrease in cocoon yield. Hence larvae of silkworm reared with mulberry leaves with adequate quantity of nutrients.

The nutrients such as protein, amino acids, vitamins, carbohydrates, sterols, lipids, minerals and salts, etc., are required by silkworm at optimum level. The supplementations of these nutrients have positive impact on the yield and productivity, but some of them have negative impact for the same. Among nutritional supplementations, amino acid plays a vital role in the synthesis of silk protein and even for growth and development of the silkworm. The silk protein synthesis in the silk gland is regulated by the amino acids concentration of the haemolymph. In addition

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to this it also helps in maintaining homeostasis and osmoregulation [11]. The concentration of free amino acids depends on the diet provide to silkworm [11], digestion of old cuticle by proteolytic enzymes of the ecdysis fluid [12]. Further, concentration amino acids also determined by nutrition, age and climatic factors [13]. It is well documented that aminotransferase enzymes vary in respect of silkworm breeds and sex and developmental stages [14]. Generally, both essential and non-essential amino acids are required by the silkworm for their normal growth and development. The dietary supplementations of methionine at varied concentrations enhance alanine and aspartate aminotransferase activities in the 5th instar larval stage [15]. Similarly, supplementation of protein based soyabean and lablab bean seed flour elevate the activity of aminotransferase enzymes in the fifth instar silkworm larva [16]. A quite a good number of reports are available on the supplementation of non-essential amino acid in relation to biomolecules as well as economic parameters of the silkworm. However, information available for the same with essential amino acids supplementation is limited. Hence, in the present study we have analyzed the impact of dietary supplementation of three essential amino acid viz. methionine, phenylalanine and valine on the alanine and aspartate aminotransferase enzymes in the popular bivoltine silkworm hybrids FC_1, FC_2 and $FC_1 \times FC_2$ (double hybrid).

MATERIALS AND METHODS

Supplementation of amino acids and rearing of silkworm

The disease free layings of bivoltine silkworm hybrids viz., FC_1, FC_2 and FC_1 X FC_2 (double hybrid) were procured from the NSSO, Mysuru. After black boxing, the silkworm larvae were brushed and reared under laboratory conditions by employing standard rearing technique prescribed by Dandin and Giridhar [17]. The solutions of methionine, phenylalanine and valine were prepared at different concentrations viz. 05, 1.0 and 1.5% by using distilled water. The prepared solutions of respective amino acids were sprayed on ventral surface of mulberry leaf and dried under shade and fed to the silkworms. The silkworms selected for the investigation is divided in to five batches namely, batch I, batch II and batch III were reared on mulberry leaves supplemented with three selected amino acids at 0.5, 1.0 and 1.5% concentrations. Whereas batch IV larvae reared on mulberry leaves sprayed with distilled water (control) and batch V larvae reared on mulberry leaf alone (absolute control). The supplementation of amino acids with mulberry leaf was initiated during fourth and fifth instar larval stages once in a day. Altogether, six rearing trials were conducted with three replications comprising 200 larvae in each treatment. Enzymes analysis were carried out in the haemolymph and fat body tissue of fifth instar 1st, 3rd and 5th day old larva in respective treatments as well as control batches. The fat body tissue homogenate was prepared at 1% concentration (w/v) by using distilled water and content was centrifuged at 3000rpm for ten minutes. Similarly, the haemolymph from larvae of respective treatments was collected by rupturing abdominal legs with a pinch of phenylthiourea as anticoagulant. The fat body tissue homogenate and appropriate diluted haemolymph by distilled water was used for the estimation of aminotransferase enzymes.

Estimation of alanine (ALT) and aspartate aminotransferase (AST) enzymes

The aminotransferase enzymes were estimated by following the method of Reitman and Frankel [18]. For alanine aminotransferase enzyme, 1ml of tissue extract was incubated with 0.5ml of glutamic pyruvate at 37°C for 1 hour followed by adding 0.5ml of 2,4-Dinitrophenylhydrazine and 5ml of 0.4 N NaoH. The colour intensity was measured at 510 nm by using spectrophotometer. For Aspartate aminotransferase, 0.5 ml of glutamic oxalo acetate was used as a substrate. The enzymes activity was expressed in terms of µ moles pyruvate /g protein/hour (ALT) and µ moles oxalo acetate/g protein/hour (AST). The obtained data was analysed by standard deviation method and mean values were expressed.

RESULTS AND DISCUSSION

Alanine (ALT) and aspartate aminotransferase (AST) enzymes activity

The silkworm larvae supplemented with essential amino acids at varied concentration recorded variations with respect to alanine and aspartate aminotransferase activity levels in fat body and haemolymph of fifth instar larva in the silkworm hybrids. The highest activity of ALT was noticed in the haemolymph of fifth instar 1st day followed by 3rd day and 5th day (387.05, 383.93 and 381.06 µ moles pyruvate /g protein/h) at 1.5% phenylalanine in the larvae of $FC_1 \times FC_2$, FC_2 and FC_1, respectively over distilled water control batch (379.03, 378.00 and 373.93 µ moles pyruvate /g protein/h) and absolute control batch (376.02, 373.91 and 369.92µ moles pyruvate /g protein/h). The lowest ALT activity was registered in larvae supplemented with 0.5% valine in fifth instar 5th day of FC_1, (311.01 µ moles pyruvate/h), FC_2 (315.04 µ moles pyruvate /h) and $FC_1 \times FC_2$ (318.02 µ moles pyruvate/h) over control batches (Table 1). Similar trend was also observed in the batches of silkworm larvae reared on methionine and valine. The highest activity of ALT was noticed in the fat body of fifth instar 1st day followed by 3rd day and 5th day (353.29, 350.51 and 346.52 µ moles pyruvate /g protein/h) at 1.5% phenylalanine in the larvae of $FC_1 \times FC_2$, FC_2 and FC_1, respectively when compared to distilled water control batch (344.43, 343.42 and 339.65 µ moles pyruvate /g protein/h) and absolute control batch (341.73, 339.43 and 335.45 µ moles pyruvate /g protein/h). The lowest ALT activity was observed in 0.5% valine supplementation in fifth instar 5th day larvae of FC_1, (280.90µ moles pyruvate/h), FC_2 (285.12µ moles pyruvate /h) and $FC_1 \times FC_2$ (287.88µ moles pyruvate/h) as against control batches. This type of result was also observed in the batches of silkworm reared on methionine and valine supplementation.

The maximum activity of AST was registered in the haemolymph of fifth instar 3rd day followed by 5rd day and 1th day (169.56, 164.00 and 159.71 µ moles oxalo acetate/g protein/h) at 1.5% phenylalanine in the larvae of $FC_1 \times FC_2$, FC_2 and FC_1, respectively as against distilled water control batch (160.19, 159.15 and 147.20 µ moles oxalo acetate/g protein/h) and absolute control batch (155.63, 147.79 and 144.67 µ moles oxalo acetate/g protein/h). The minimum activity of AST was observed at 0.5% valine supplementation in fifth instar 1st day larvae of FC_1, (102.66 µ moles oxalo acetate/h), FC_2 (112.65 µ moles oxalo acetate/h) and $FC_1 \times FC_2$ (116.39 µ moles oxalo acetate/h) when compared to distilled water supplemented batch and

untreated batch (Table 2). Similar results were also noticed in the batches of silkworm supplemented with methionine and valine. The highest activity of AST was noticed in the fat body of 5th instar 3rd day followed by 5th day and 1st day (126.40, 123.18 and 122.29 µ moles oxalo acetate/h) at 1.5% phenylalanine in the larvae of $FC_1 \times FC_2$, FC_2 and FC_1, respectively when compared to distilled water control batch (117.25, 113.10 and 110.19 µ moles oxalo acetate/h) and absolute control batch (113.92, 110.78 and 109.18 µ moles oxalo acetate/h). The lowest AST activity was observed in 0.5% valine supplementation in 5th instar 1st day larvae of FC_1, (72.13µ moles oxalo acetate/h), FC_2 (77.26 µ moles oxalo acetate/h) and $FC_1 \times FC_2$ (79.57 µ moles oxalo acetate/h) as against control batches. Similar trend was also observed in the batches of silkworm larvae reared on methionine and valine supplementation.

Table 1 Effect of mulberry leaf fortified with amino acids on alanine aminotransferase activity in silkworm hybrids

Hybrids	Amino acids	Concentrations (%)	Haemolymph			Fat body		
			1st day	3rd day	5th day	1st day	3rd day	5th day
FC_1	Methionine	0.5	375.98±0.52	343.96±1.83	311.79±2.40	341.48±0.82	310.16±2.14	281.99±1.65
		1.0	378.96±0.51	347.01±0.53	314.91±0.70	343.44±0.17	311.71±0.57	284.07±0.88
		1.5	380.11±0.49	348.02±1.57	315.95±1.82	345.50±0.51	313.85±0.73	285.88±0.67
	Phenylalanine	0.5	379.06±0.52	347.01±2.59	315.02±0.95	344.52±0.04	313.18±1.58	285.00±1.36
		1.0	379.95±1.70	347.88±2.08	316.03±1.82	345.52±0.44	313.89±1.17	285.88±2.07
		1.5	381.06±1.29	349.04±0.84	316.86±0.75	346.52±1.60	315.02±0.84	287.18±1.63
	Valine	0.5	375.04±2.08	342.89±1.46	311.01±1.48	340.53±1.19	308.93±1.19	280.90±1.18
		1.0	377.73±0.99	345.99±0.78	313.90±1.13	342.41±0.44	311.00±0.45	282.96±0.29
		1.5	377.73±1.53	345.99±0.93	313.90±2.48	342.41±1.11	311.00±0.42	282.96±0.94
FC_2	Methionine	0.5	380.17±1.13	348.19±1.10	315.97±0.53	345.38±1.04	313.84±0.54	285.94±2.19
		1.0	380.93±1.19	348.92±1.71	317.04±0.50	346.47±1.22	314.91±0.77	286.89±1.15
		1.5	381.02±1.79	349.10±0.87	316.88±2.31	347.52±0.87	315.94±0.66	287.96±1.13
	Phenylalanine	0.5	381.98±0.90	349.79±2.26	318.08±0.83	347.51±2.04	315.96±0.84	288.01±2.29
		1.0	383.01±0.94	350.89±0.77	319.27±1.30	349.59±0.06	318.06±1.50	290.00±1.28
		1.5	383.93±1.78	351.92±1.33	320.02±0.45	350.51±0.82	319.03±0.44	291.04±0.52
	valine	0.5	379.00±0.84	346.86±1.25	315.04±0.95	344.52±1.76	313.07±2.37	285.12±1.79
		1.0	380.01±0.86	347.95±1.28	316.09±0.90	345.52±1.03	313.77±1.54	285.94±1.12
		1.5	380.00±1.77	348.08±1.36	316.21±0.71	345.50±1.15	313.87±1.36	286.04±1.14
$FC_1 \times FC_2$	Methionine	0.5	382.01±0.96	350.03±1.29	318.05±1.80	347.51±0.48	315.91±0.29	288.03±1.29
		1.0	383.03±0.96	351.05±0.96	318.87±1.18	348.73±0.97	317.05±1.80	289.09±0.45
		1.5	385.12±0.44	352.90±0.80	321.04±0.50	350.52±0.46	318.98±1.72	291.12±0.61
	Phenylalanine	0.5	383.97±0.73	351.75±0.61	320.06±0.79	349.39±0.56	318.00±0.89	290.00±1.35
		1.0	386.05±0.86	353.96±0.92	321.99±0.90	351.51±0.50	319.87±1.60	291.97±2.00
		1.5	387.05±4.78	355.04±1.32	322.87±1.59	353.29±1.03	321.87±0.78	294.01±1.28
	Valine	0.5	382.02±1.03	349.78±1.56	318.02±1.83	347.51±1.19	315.90±0.78	287.88±1.15
		1.0	382.98±0.94	350.98±1.16	319.02±1.79	348.54±1.00	316.93±2.51	288.92±1.19
		1.5	383.96±0.87	351.95±0.89	320.15±1.61	349.57±0.08	317.96±0.81	289.88±1.04
Control (Distilled water)		FC_1	373.93±0.14	341.95±2.31	310.04±0.50	339.65±0.75	308.00±1.21	279.89±2.56
		FC_2	378.00±0.04	345.91±2.31	313.89±2.48	343.42±0.82	311.88±0.75	284.04±0.50
		$FC_1 \times FC_2$	379.03±2.05	347.02±2.29	314.89±0.77	344.43±0.69	313.08±0.81	285.05±1.80
Control (Absolute)		FC_1	369.92±1.61	338.03±1.03	305.99±0.50	335.42±1.10	303.99±0.40	275.86±1.18
		FC_2	373.91±2.70	342.03±3.52	310.05±0.49	339.43±1.19	307.73±0.56	279.89±1.13
		$FC_1 \times FC_2$	376.02±0.58	343.89±2.06	311.90±0.33	341.73±0.59	309.94±0.70	281.88±0.76

The biochemical parameters are considered as an ideal indicator aid in the breeding of plants and animals [19]. Like other lepidopteran insects, silkworm is known to contain unusually large amounts of free amino acids [20-21]. Insect metamorphosis is a dynamic process involving both histogenesis and histolysis [11]. The amino acid pool in silkworm is derived both from proteins through histolysis and from non-protein sources like carbohydrates and lipids through *de novo* synthesis. Continuous increase in the levels of free amino acids following amino acid supplementation with leaf attributable to the synthesis of amino acids from non-protein sources like glucose and fatty acids [22]. Given the importance of silkworm, it is presumed that amino acids are crucial for the synthesis of fat body particularly, during larval-pupal transition. Moreover, amino acids are mobilized from other tissues into silk gland and fat body via the haemolymph [23].

In general, the aminotransferase enzymes are responsible for the inter conversion of non-essential amino acid to form aspartate and glutamate which is essential in the growth of the development of the silkworm [20]. ALT and AST are existed in mitochondrial as well as cytosol fractions of the cell. There is a relation exist between transaminase reaction and mitochondrial integrity [24]. The ALT and AST are the enzymes which transfer of amino group from one amino acids to keto acid to form another amino acid without the liberation of ammonia. The alanine and aspartate aminotransferases which function as a strategic link between the carbohydrate and protein metabolism and their activity is strongly influenced by several pathological and physiological conditions [25]. The increase in enzyme activities could indicate the active transamination reaction in both tissues either for structural organization of amino acids, incorporation of ketoacids into TCA cycle to favour

gluconeogenesis or energy products. The AST activates ketoacids a general index of amino acids breaks down and ALT marks mobilization of amino acid into TCA cycle [26-27]. The result obtained from the present investigation revealed that increase both alanine and aspartate aminotransferase activity levels in all three amino acids supplementation maximum being in 1.5% of phenylalanine in the haemolymph of $FC_1 \times FC_2$, FC_2 and FC_1 hybrids and this type of trend was also observed in fat body tissue. It is presumed that increase in the enzyme activities is due to exogenous supplementation of amino acids increases the transaminases reaction and thereby responsible for maintaining amino acid pool in haemolymph. Moreover, haemolymph serves as a body fluid helpful in exchange of metabolites in varies tissues and also mobilized pyruvic acid which is necessary compound for Krebs cycle. However, the activity level was found lower in fat body tissue which is main site for intermediary metabolism and decrease in both enzymes activity is due to lower transaminases reaction. The ALT activity was maximum fifth instar 1st day old larvae and gradually the decrease in 3rd and 5th day larvae supplemented with amino acids as compared to control batches. It clearly indicated that transaminases reaction decrease with the advancement of age (towards spinning stage). On the other hand, AST activity was highest in fifth instar 3rd day followed by 5th and 1st day larvae the supplemented with amino acids against control batches. It is evident from the result that higher transaminases activity was noticed in middle of fifth instar larval stage (3rd day) and sharp decline in the activity in 5th day. Presumably, the fluctuating levels of amino acids in *B. mori* reflect general changes in the metabolism during metamorphosis. The increase in amino acid pool at late fifth instar is reflective of the initiation of proteolytic activity, which is the characteristic feature of the pupal stage. The elevation in the activity levels of ALT and AST in tissues at beginning of the fifth instar and of the silk gland at the late fifth instar is indicative of increased amino acid turnover and glutamate formation. The decrease in their activity in the haemolymph and fat body at the late fifth instar is suggestive of their low utilization, thereby increasing the free amino acid concentration in these tissues.

Table 1 Effect of mulberry leaf fortified with amino acids on alanine aminotransferase activity in silkworm hybrids

Hybrids	Amino acids	Concentrations (%)	Haemolymph			Fat body		
			1st day	3rd day	5th day	1st day	3rd day	5th day
FC_1	Methionine	0.5	107.56±1.95	152.69±0.56	127.10±0.55	77.62±0.17	116.43±0.96	96.26±0.84
		1.0	108.53±0.98	153.42±0.56	128.02±0.60	78.65±1.33	117.39±0.67	97.21±0.58
		1.5	109.66±1.67	154.70±1.58	129.17±1.17	79.57±0.87	118.28±0.25	98.19±1.50
	Phenylalanine	0.5	110.65±1.71	155.75±0.76	130.15±0.48	80.65±0.78	119.38±0.75	99.27±0.24
		1.0	112.54±1.75	157.63±0.79	132.08±0.97	81.50±0.53	120.22±1.07	100.20±0.65
		1.5	114.65±0.35	159.71±1.95	134.26±1.32	83.62±1.01	122.29±1.09	102.26±0.52
	Valine	0.5	102.66±0.53	147.78±1.58	122.16±0.86	72.13±0.74	110.86±1.46	90.73±0.59
		1.0	104.59±1.01	149.80±0.62	124.16±2.15	74.58±0.54	113.31±1.10	93.16±0.60
		1.5	106.62±1.01	151.74±0.62	126.16±2.15	76.37±0.54	115.09±0.65	94.98±0.60
FC_2	Methionine	0.5	114.57±1.47	159.58±1.52	134.07±0.43	79.65±1.19	118.42±0.33	98.29±0.55
		1.0	116.66±1.82	161.75±1.38	136.06±0.86	80.53±1.63	119.25±1.52	99.05±0.50
		1.5	118.65±2.05	163.70±1.14	138.13±1.97	81.27±0.51	119.98±0.52	99.86±0.68
	Phenylalanine	0.5	117.69±1.43	162.79±0.69	137.16±0.31	82.68±0.40	121.41±1.15	101.38±1.04
		1.0	118.55±0.82	163.65±0.32	138.05±1.00	83.53±0.88	122.25±0.78	102.09±1.36
		1.5	119.16±1.21	164.00±0.75	138.70±0.55	84.46±0.26	123.18±1.15	102.74±0.74
	valine	0.5	112.65±0.80	157.73±0.32	132.17±0.83	77.26±0.35	115.96±0.60	95.95±1.06
		1.0	113.60±0.63	158.75±0.74	133.10±0.82	78.63±0.46	117.37±1.22	97.25±0.62
		1.5	114.57±0.50	159.70±0.83	134.06±1.00	79.09±0.98	117.74±0.67	97.62±1.11
$FC_1 \times FC_2$	Methionine	0.5	119.57±0.44	164.71±0.57	139.05±1.00	80.99±0.40	119.70±0.77	99.76±0.73
		1.0	120.26±1.48	165.36±0.91	139.77±2.15	81.21±0.57	119.97±1.79	99.89±0.57
		1.5	121.41±0.81	166.37±0.77	140.77±0.28	81.56±0.50	120.20±0.53	100.26±0.61
	Phenylalanine	0.5	120.65±1.18	165.78±1.04	140.16±0.39	85.60±0.05	124.24±0.28	104.18±0.87
		1.0	122.56±0.85	167.74±0.72	142.04±1.03	86.25±0.60	124.97±0.98	104.94±1.20
		1.5	124.57±1.26	169.56±1.37	143.96±0.46	87.66±1.16	126.40±0.85	106.28±0.64
	Valine	0.5	116.39±1.23	161.35±0.72	135.76±0.26	79.57± 0.19	118.41±1.27	98.16±0.40
		1.0	118.71±0.73	163.67±4.93	138.06±1.83	81.24±0.58	120.05±0.86	100.01±0.82
		1.5	119.45±1.22	164.64±0.56	139.10±0.90	82.14±0.94	120.89±0.21	100.75±0.73
Control (Distilled water)		FC_1	102.05±1.42	147.20±1.01	121.56±0.48	71.46±0.18	110.19±0.96	90.07±0.84
		FC_2	114.08±1.00	159.15±0.53	133.56±1.80	74.45±0.51	113.10±0.06	93.06±2.54
		$FC_1 \times FC_2$	115.05±0.96	160.19±2.15	134.56±1.32	78.56±0.81	117.25±0.51	97.21±0.95
Control (Absolute)		FC_1	99.53±0.38	144.67±0.82	119.07±1.33	70.45±0.64	109.18±1.00	89.21±1.62
		FC_2	102.55±0.56	147.79±0.70	122.14±0.87	72.05±0.56	110.78±0.33	90.65±0.63
		$FC_1 \times FC_2$	110.56±1.02	155.63±0.78	130.02±0.32	75.19±0.83	113.92±0.57	93.80±0.61

CONCLUSION

From the aforementioned investigation it could be concluded that the overall result of the present study inferred that fortification of mulberry leaf with phenylalanine, methionine and valine at 1.5% enhance alanine and aspartate aminotransferase activity levels in the hybrids $FC_1 \times FC_2$, FC_2 and FC_1.

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