

**IMPACT OF JHA PYRAZOLE ON REPRODUCTIVE FAILURE
AND AMINO-ACIDS POOL IN EMBRYOGENESIS OF THE
DESERT LOCUST *SCHISTOCERCA GREGARIA* (FORSKAL)
(ORTHOPTERA: ACRIDIDAE)**

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Abstract

Laboratory evaluations were conducted on newly adult stage (gonadotrophic period) of *Schistocerca gregaria* (Forsk.), treated with pyrazole, an aromatic juvenile hormone analogue to clarify disruption of the embryos growth. Data indicated that a correlation exists between the type and concentrations on proteins and dosed females. Such effect was considered as a juvenilizing action of JHA on these vital metabolites due to their indirect effect on the natural level of juvenile hormone in the treated adults.

Biochemical evidence appears that depression of total protein level 96.3% during last embryonic development stages. Concentrations of the aromatic and non aromatic amino acids such as Alanine , Arginine , Lysine , Proline , Glutamine, Tyrosine, Glycine, Isoleusine, Histidine, Cystin, Glutamic and Phenylalanine, may be limiting factor of the growth of the material tissues during differentiation of organs development in embryos. It appears to be involved more in the metabolism of the embryo and expressed as a dramatic curtailment of growth of embryos.

INTRODUCTION

The development of chemical insecticides that was cholinesterase inhibitors to control population of targeted insects and pests resulted in serious damage to the ecosystem. Therefore, it seems prudent to develop alternative biocontrol strategies that could be drawn upon to counter periodic population outbreaks. One such JHA, pyrazole, was found to be effective under laboratory conditions in reproductive failure and oviposited infertile eggs of crowded *Schistocerca gregaria*. The purpose of the present study is to impose the question what is the role of the juvenile hormone in embryos? Therefore, the study aimed also to demonstrate the existence of biologically active residues at embryonic development stages. Focusing on the metabolic rate of embryogenesis and other two parameters such as embryos growth and their amino-acids content, incentive the need to determine the essential and non-essential amino acids for em-

bryos growth under crowded conditions.

MATERIAL AND METHODS

a. Locust culture: The adults were segregated from the gregarious stock colony of *S. gregaria* at gonatrophic period: a major rise in ecdysteroids, higher juvenile hormone synthetic activity, chorionated oocytes and the rate of vitellogenin synthesis from its onset to day 12 after fledging (Injeyan and Tobe, 1981). They were kept under crowded conditions at a temperature of 30-32°C and a light / dark cycle of 16/8 h, 50% r.h. Rearing conditions were used as described by Harvey (1990) was followed in the present investigation.

b. Juvenile hormone analogue treatment and target stage: Technical grade sample (160 µg/adult female at gonatrophic period) of the JHA (pyrazole) was used, Fig. 1. Fresh solution was prepared in acetone and applied topically in a volume of 10 µl on the venter of the adult abdomen using Hamilton microsyringe (Type 701-NCH). Twenty adults were used in three replicates. Control insects were treated with acetone only. All adults were maintained under the previous rearing conditions to record egg production and hatching rate.

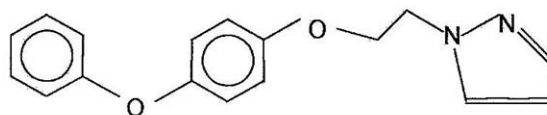


Fig. 1. Pyrazole an aromatic juvenile hormone analogue.

The inhibition percent of egg hatching by JHA and control mortality, which was low were corrected by Abbott's formula (1925). The lethal effect was noted and recorded according to categories of Injeyan *et al.* (1979).

1. Unhatched egg (the chorion did not rupture, dead embryo).
2. First-instar larva successful hatching and intermediate moult, but death within 3 days after hatching.

c. Eggs: Egg pods were dated and transferred within the original vessels into an incubator running at $30\pm 1^{\circ}\text{C}$ and kept at this temperature up till the extraction and clean up from the foam of the pods.

d. Embryonic development and stages: The duration of embryo development under the conditions chosen for the incubation of the eggs required 12-13 days. The embryos staged according to the scheme of Shulov and Pener (1959). The separation of embryos from the yolk was done under a dissecting microscope.

e. The chemical analysis: Main metabolites were quantitatively estimated as a total protein, carbohydrates, lipids and cholesterol in haemolymph, various stages of eggs, ovary and embryo. Yolk separation obtained from dosed females and control. The metabolites were colourimetrically determined pursuing the techniques utilized by Du-bois *et al.* (1956) for carbohydrates determination, Bradford (1976) for protein estimation, Knight *et al.* (1972) for lipid estimation and Richmond (1973) to determine cholesterol.

Whole eggs of the following stages were quantitatively analyzed: 1) ovarian ripe eggs, taken directly from the oviduct; 2) eggs just laid; 3) eggs after 1 day of incubation stage 1 of Shulov and Pener (1959); 4) after 2 days (stages V-VI); 5) after 3 days (stages VIII-IX); 6) after 6 days (stages XVI-XVII) formation of the embryonic corpora allata and completion of the blastokinesis (katatrepsis); 7) after 9 days (stage XX) the definitive dorsal closure; 8) after 12 days, at the hatching (stage XXIII), the apolysis of the second embryonic cuticle. Homogenates for amino acids determination conducted as Winder and Eggum (1966).

RESULTS AND DISCUSSION

1. Effect of JHA on the mass of the eggs and sterility: Data in table 1. summarized results as follows: the dry weight of eggs was increased by about 31.15% during the first 3 days due to the absorption of water, followed by 46.64% increase during the successive 6 days until hatching due to synthesized metabolism in embryonic cells, but in treated adults of *S. gregaria*, JHA (pyrazole) caused block in weight during development due to the lack of juvenile hormone. The JHA showed an effective suppression (36.8%) in hatching of eggs that were oviposited by treated insects. Ver-

miform larvae, were absent in the native control possibly related to the embryo cuticulinogenesis. Hence, the females were not affected, but the treatment resulted in prothetical metamorphosis of the progeny. Application of JHA at appropriate time can result in disruption of normal development, induction of sterility in female insects and the disruption of embryogenesis probably interferes with the functioning of the embryonic corpora allata and the control of the embryonic ecdysial glands (Menn *et al.*, 1989).

2. Effect of JHA on metabolic rate: It appears that, determination of total protein (listed in Table, 2) during oogenesis in ovaries, the haemolymph of mature females and at various stages of egg development, indicate a lower level of protein ranged between 76.3 and 88.8% in treated ones than in their own control, especially in embryo for about 96.3%. It may due to reduction of vitellogenin and vitellogenesis process. This result confirms those of the experiments done by Girardie *et al.* (1998) who found that a juvenile hormone and LOMOMP (locusta ovarian maturation parsin) induces the appearance of circulating vitellogenin in females and complete vitellogenesis by delays the VgmRNA decay or increases the translation of VgmRNA.

Same pattern with carbohydrate and lipid was found except on day 6 and day 9. Its increasing may due to the depression from fatty metabolism. Similar results were reported for cholesterol. It is well established that, cholesterol may be the biosynthetic precursor of ecdysone hormone in insects, thus our observation confirmed by Scalla and Morgan (1982) by providing evidence of a phase relationship between the activity of the ecdysial glands and the embryonic apolysis, which happened disruption in the hormonal coordination of the ecdysis (failure in ecdysis).

3. Effect of JHA on amino acids: A total of nineteen common amino-acids were found in whole eggs and determined quantitatively, in *S. gregaria*, from ripe oocytes to hatching embryos, the composition of the amino acids listed in Table 1 conversely amino acids concentrations were significantly increased in treated females after depositing until day 6, ranging between 10.6 and 57.6% especially with Glycine 233.33% and Valine 133.64% at just laid eggs, Glycine 433.33% and Methionine 226.67% at day 1 stage I, Isoleucine 975.0% at day2 stage V - VI finally, Glutamic 95.24% and proline 95.0 % at day3 stage VIII-IX. It decreased in treatment for about 47.7% in ripe eggs from oviducts especially with Alanine 59.9% and Tryptophan

55.88%. Since proteins pass from haemolymph pool to metabolized by oocytes, part of yolk protein is formed directly in the female's body and transferred as such to oocytes (Telfer, 1960). Probably, the amino acids do not pass freely between the two pools. A striking decrease of treatment amino-acids content occurs at the second period of incubation, from stages XVI to XXIII (one day before hatching), ranging between 12.5 and 84.7%, especially with Arginine 55.33%, Tyrosine 54.81%, Alanine 54.86%, Glutamic 55.58% and Threonine 54.95%, which occur at day 6 stage XVI-XVII and Histidine 60.32% and Glycine 22.29% at day 9 stage XX, finally, Glutamine 64.10%, Arginine 48.86% and Lysine 46.26% at day 12 stage XX. When histological differentiation is the predominant process such as the definitive dorsal closure is complete or almost completed, their corpora allata were already well developed and completion of the blastokinesis (katatepsis) (Goldsworthy and Wheeler, 1986). It is remarkable that parallels exist between hormonal and metabolism events.

Profile analysis of the free amino-acids pools of the treated *S. gregaria* eggs indicated that Alanine and Arginine were the most abundant amino acids and play an important role in somatic growth followed by Histidine, Lysine, Glutamine, Isoleucine, Tyrosine, Proline, Cystine, Glycine, Glutamic and Phenylalanine, which were depressed relative to an array of other amino acids in treatment one.

The data indicate that embryos, were highly susceptible to the residual effect of juvenile hormone analogue on adults *S. gregaria*.

Table 1. Percentage of free amino acids content per egg at various stages of development of *S. gregaria* eggs treated as newly adult females.

Amino acids	Ripe eggs from oviduct			Just laid eggs			Developing eggs at different time of incubation					
	Cont.	Treat	Diff. %	Cont.	Treat.	Diff. %	1 day stage			2 day stage		
							I			V-VI		
Aspartic (ASP)	0.81 ±0.20	0.42 ±0.09	-48.15	1.12 ±0.07	1.26 ±0.05	12.50	Traces	0.39 ±0.10	39.00	1.29 ±0.06	1.41 ±0.03	9.30
Threonine (THR)	0.26 ±0.14	0.15 ±0.05	-44.00	0.43 ±0.10	0.83 ±0.03	93.02	0.46 ±0.06	0.91 ±0.05	97.83	0.54 ±0.26	0.54 ±0.11	27.78
Serine (SER)	0.48 ±0.06	0.25 ±0.03	-47.92	0.69 ±0.07	0.91 ±0.03	31.88	0.92 ±0.17	1.24 ±0.13	34.78	0.96 ±0.08	1.08 ±0.06	12.50
Glutamic (GLU)*	1.17 ±0.07	0.56 ±0.04	-52.14	2.86 ±0.01	2.99 ±0.01	4.55	3.04 ±0.03	3.05 ±0.03	0.33	2.81 ±0.75	3.26 ±0.6	16.01
Proline (PRO)	0.41 ±0.10	0.19 ±0.03	-53.66	0.26 ±0.02	0.50 ±0.02	92.31	0.18 ±0.03	0.45 ±0.04	150.00	0.34 ±0.02	0.37 ±0.03	8.82
Glycine (GLY)	0.22 ±0.03	0.16 ±0.01	-27.27	0.09 ±0.01	0.30 ±0.05	233.33	0.09 ±0.02	0.48 ±0.04	433.33	0.17 ±0.01	0.18 ±0.02	5.88
Alanine (ALA)*	0.44 ±0.03	0.18 ±0.01	-59.90	1.59 ±0.55	1.74 ±0.57	9.43	1.62 ±0.65	1.91 ±0.64	17.90	1.64 ±0.54	1.86 ±0.53	13.41
Cystin (CYS)	0.08 ±0.03	0.04 ±0.02	-50.00	0.35 ±0.01	0.54 ±0.02	54.29	0.16 ±0.01	0.49 ±0.03	206.25	0.16 ±0.01	0.16 ±0.01	0.00
Valine (VAL)	0.37 ±0.05	0.20 ±0.03	-45.95	0.22 ±0.01	0.47 ±0.02	113.64	0.30 ±0.02	0.647 ±0.03	113.33	0.63 ±0.05	0.69 ±0.06	9.52
Methionine (MET)	0.12 ±0.00	0.07 ±0.05	-41.67	0.32 ±0.02	0.52 ±0.03	62.50	0.15 ±0.01	0.498 ±0.03	226.67	0.42 ±0.05	0.45 ±0.06	7.14
Isoleucine (ILU)	0.24 ±0.01	0.13 ±0.01	-45.83	0.30 ±0.02	0.50 ±0.2	66.67	0.25 ±0.01	0.59 ±0.03	136.00	0.04 ±0.01	0.43 ±0.01	975.00
Leucine (LEU)	0.63 ±0.06	0.35 ±0.01	-44.44	0.76 ±0.14	0.94 ±0.14	23.68	0.25 ±0.01	0.64 ±0.02	156.00	1.23 ±0.05	1.39 ±0.05	13.01
Tyrosine (TYR)*	0.37 ±0.02	0.19 ±0.1	-48.65	2.44 ±0.42	2.57 ±0.11	5.33	0.72 ±0.06	1.03 ±0.14	43.06	2.09 ±0.07	2.29 ±0.12	9.57
Phenylalanine (PHE)*	0.27 ±0.01	0.13 ±0.2	-51.85	1.14 ±0.08	1.39 ±0.1	21.39	1.05 ±0.03	1.31 ±0.10	24.76	1.41 ±0.17	1.55 ±0.25	9.93
Histidine (HIS)	0.31 ±0.01	0.15 ±0.01	-51.61	1.49 ±0.01	1.61 ±0.06	8.05	0.66 ±0.05	1.01 ±0.17	53.03	0.91 ±0.07	0.98 ±0.02	7.69
Lysine (LYS)	0.45 ±0.02	0.27 ±0.02	-40.00	Traces ±0.00	0.46 ±0.02	45.00	Traces ±0.00	0.39 ±0.01	38.00	Traces ±0.00	Traces ±0.00	0.00
Arginine (ARG)	0.44 ±0.03	0.22 ±0.03	-50.00	2.07 ±0.01	2.15 ±0.25	3.86	0.56 ±0.06	1.04 ±0.20	85.71	1.87 ±0.06	2.10 ±0.09	12.30
Glutamine (GLU)	1.02 ±0.10	0.63 ±0.01	-38.24	0.78 ±0.05	1.00 ±0.05	28.21	0.62 ±0.03	0.91 ±0.13	46.77	0.72 ±0.03	0.74 ±0.01	2.78
Tryptophan (TRY)	0.34 ±0.05	0.15 ±0.03	-55.88	1.34 ±0.02	1.51 ±0.02	12.69	0.39 ±0.07	0.726 ±0.09	84.62	0.48 ±0.01	0.49 ±0.03	2.08
Egg dry weight (mg)	5.11 ±0.11	3.94 ±0.04	-22.90	10.83 ±0.23	10.47 ±0.59	-3.32	10.20 ±0.2	9.81 ±0.11	-3.82	10.50 ±0.59	12.23 ±1.45	16.29
Total per egg	8.44 ±0.20	4.41 ±0.20	-47.57	18.23 ±0.59	22.19 ±1.42	21.27	11.40 ±0.20	17.69 ±0.33	55.18	18.09 ±0.39	20.01 ±0.74	10.61

Continued Table 1.

Amino acids	developing eggs at different time of incubation											
	3 day stage VIII-IX			6 day stage XVI-XVII			9 day stage XX			12 day stage XXIII		
	Cont.	Treat.	Diff. %	Cont.	Treat.	Diff. %	Cont.	Treat.	Diff. %	Cont.	Treat.	Diff. %
Aspartic (ASP)	1.31 ±0.05	1.95 ±0.03	48.85	2.61 ±0.21	1.24 ±0.01	-52.49	1.54 ±0.32	1.43 ±0.15	-7.14	1.09 ±0.02	1.31 ±0.01	20.18
Threonine (THR)	0.42 ±0.13	0.62 ±0.07	47.62	0.91 ±0.20	0.41 ±0.12	-54.95	0.60 ±0.20	0.53 ±0.19	-11.67	0.19 ±0.12	0.55 ±0.06	189.47
Serine (SER)	0.59 ±0.09	0.91 ±0.07	54.24	1.19 ±0.05	0.60 ±0.04	-49.58	0.66 ±0.11	0.68 ±0.13	3.03	0.30 ±0.19	0.68 ±0.20	126.67
Glutamic (GLU)*	2.64 ±0.03	3.73 ±0.03	41.29	5.38 ±0.01	2.39 ±0.02	-55.58	3.39 ±0.01	2.83 ±0.01	-16.52	1.78 ±0.02	1.48 ±0.01	-16.85
Proline (PRO)*	Traces	0.96 ±0.01	95.00	1.30 ±0.81	0.62 ±0.06	-52.31	0.90 ±0.03	0.80 ±0.01	-11.11	0.53 ±0.02	0.36 ±0.01	-32.08
Glycine (GLY)*	0.34 ±0.02	0.51 ±0.03	50.00	0.75 ±0.13	0.35 ±0.09	-53.33	0.67 ±0.02	0.52 ±0.01	-22.29	0.41 ±0.03	0.34 ±0.01	-17.07
Alanine (ALA)*	0.72 ±0.25	1.07 ±0.41	48.61	1.44 ±0.13	0.65 ±0.08	-54.86	0.92 ±0.13	0.84 ±0.11	-8.70	0.73 ±0.21	0.50 ±0.18	-31.51
Cystin (CYS)*	0.09 ±0.01	0.17 ±0.02	88.89	0.19 ±0.03	0.10 ±0.02	-47.37	0.11 ±0.12	0.12 ±0.13	-9.09	0.08 ±0.03	0.05 ±0.03	-37.50
Valine (VAL)	0.63 ±0.03	0.94 ±0.02	49.21	1.24 ±0.31	0.58 ±0.26	-53.23	0.81 ±0.11	0.687 ±0.08	-16.05	0.40 ±0.03	0.41 ±0.04	2.50
Methionine (MET)	0.20 ±0.03	0.28 ±0.01	40.00	0.38 ±0.05	0.18 ±0.03	-52.63	0.24 ±0.01	0.22 ±0.01	-8.33	0.08 ±0.04	0.13 ±0.10	62.50
Isoleucine (ILU)*	0.41 ±0.02	0.61 ±0.03	48.78	0.80 ±0.03	0.37 ±0.02	-52.50	0.50 ±0.01	0.45 ±0.02	-10.00	0.43 ±0.03	0.27 ±0.01	-37.21
Leucine (LEU)	1.03 ±0.71	1.55 ±0.09	50.49	2.04 ±0.12	0.94 ±0.03	-53.92	1.18 ±0.07	1.09 ±0.09	-7.63	0.43 ±0.03	0.50 ±0.02	16.28
Tyrosine (TYR)*	0.69 ±0.03	0.97 ±0.08	40.59	1.35 ±0.07	0.61 ±0.02	-54.81	0.91 ±0.01	0.76 ±0.03	-16.48	0.70 ±0.02	0.44 ±0.01	-37.14
Phenylalanine (PHE)*	0.40 ±0.03	0.60 ±0.03	50.00	0.91 ±0.03	0.43 ±0.01	-52.75	0.60 ±0.02	0.52 ±0.03	-13.33	2.09 ±0.05	1.68 ±0.02	-19.62
Histidine (HIS)*	0.46 ±0.01	0.67 ±0.07	45.65	0.86 ±0.01	0.47 ±0.01	-45.35	0.63 ±0.02	0.25 ±0.01	-60.32	0.16 ±0.01	0.13 ±0.02	-18.95
Lysine (LYS)*	0.77 ±0.01	1.14 ±0.52	48.05	1.60 ±0.05	0.74 ±0.02	-53.75	1.11 ±0.07	0.91 ±0.07	-18.02	3.48 ±0.09	1.87 ±0.06	-46.26
Arginine (ARG)*	0.79 ±0.01	1.16 ±0.64	46.84	1.50 ±0.01	0.67 ±0.02	-55.33	1.00 ±0.01	0.84 ±0.02	-16.00	0.88 ±0.03	0.45 ±0.01	-48.86
Glutamine (GLU)*	0.63 ±0.02	1.23 ±0.73	95.24	1.99 ±0.02	1.30 ±0.01	-34.67	0.67 ±0.01	0.66 ±0.01	-1.49	0.78 ±0.03	0.28 ±0.01	-64.10
Tryptophan (TRY)	0.20 ±0.07	0.35 ±0.09	75.00	0.93 ±0.13	0.62 ±0.12	-33.33	1.01 ±0.01	1.165 ±0.01	14.85	0.08 ±0.01	0.31 ±0.06	287.50
Egg dry weight (mg)	11.40 ±0.74	11.37 ±0.58	-0.26	19.14 ±0.39	13.52 ±0.09	-29.36	25.12 ±1.50	13.74 ±0.95	-45.30	19.38 ±1.2	9.00 ±0.08	-53.56
Total per egg	12.30 ±0.65	19.39 ±0.74	57.64	25.75 ±0.00	13.25 ±0.04	-48.54	17.46 ±0.07	15.28 ±0.00	-12.49	14.61 ±0.06	12.24 ±0.04	-16.22

Table 2. Metabolic parameters in the females and during embryogenesis development of *S. gregaria* treated as newly adults.

Parameters	Total protein µg/mg			Total lipid µg/mg			Cholesterol µg/mg			Carbohydrates µg/mg		
	Cont.	Treat	Diff. %	Cont.	Treat	Diff. %	Cont.	Treat	Diff. %	Cont.	Treat	Diff. %
Haemolymph (female)	175.03 ±6.00	19.69 ±0.39	-88.75	64.53 ±6.23	63.98 ±1.39	-0.85	423.81 ±15.93	809.52 ±8.75	-97.89	157.02 ± 5.9	18.96 ± 1.03	-87.93
Ovary	368.10 ±9.7	21.50 ±0.74	-94.16	3.09 ±0.12	3.16 ±0.42	2.27	2.38 ±0.11	25.00* ±2.64	950.42	812.20 ±15.65	21.10 ±0.39	-97.40
Embryo at day 9	324.70 ±14.15	12.10 ±2.13	-96.27	20.99 ±2.46	14.34 ±2.13	-31.68	3.57 ±0.07	79.76* ±6.25	2134.17	56.80 ±4.03	45.50 ±1.79	-19.89
Yolk at day 9	3.20 ±0.09	5.20 ±0.11	62.50	14.41 ±0.39	29.47* ±0.33	104.51	120.24 ±4.41	139.29 ±8.22	15.84	11.60 ±1.45	13.90 ±2.13	19.83
First day egg	1.43 ±0.05	4.04* ±0.03	182.50	2.50 ±0.26	2.83 ±0.09	13.20	2.38 ±0.07	traces ±0.00	-99.96	570.60 ±6.13	30.40 ±1.5	-94.67
Third day egg	13.84 ±0.69	13.23 ±0.74	-4.41	26.78 ±1.42	31.50 ±1.39	17.63	826.98 ±8.03	745.37 ±9.60	-9.87	38.20 ±2.99	31.40 ±1.50	-17.80
Six day egg	15.21 ±1.01	15.85 ±0.97	4.21	14.54 ±1.45	42.70 ±1.49*	193.67	860.50 ±8.22	882.50 ±6.36	2.56	63.30 ±1.81	34.50 ±1.70	-45.50
Twelve day egg	5.65 ±0.00	17.03* ±1.02	201.42	13.41 ±1.42	0.45 ±0.01	-96.64	351.32 ±6.25	872.60 ±5.62	148.43	85.20 ±2.04	53.90 ±7.63	-36.74
Thirteen day egg (hatching)	69.11 ±4.37	16.38 ±1.04	-76.30	108.60 ±6.36	26.76 ±1.42	-75.36	428.77 ±4.42	899.95 ±4.03	109.89	23.20 ±1.42	62.00* ±5.86	167.24

Data are presented as mean ± standard deviation

*: Significant at the level 1%.

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تأثير منظم النمو الحشرى بيرازول على فشل التناسل وتكون
الأحماض الأمينية في أجنة الجراد الصحراوي شستوسركا جرجاريا
(مستقيمت الاجنحة - اكريديدى)

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عوملت الحشرات الكاملة للجراد المصرى بمركب بيرازول سطحياً بمعدل ١٦٠ ميكروجرام لكل حشرة ، ثم تم تقدير تأثير هذا المشابه الهرمونى على كل من البروتين الكلى ، الكربوهيدرات ، الليبيدات والكلوستيرول فى دم الحشرات الكاملة وأثناء تطور الاجنة وكذلك فى الملح والجنين كل على حدة فى الفترة المتأخرة من نمو الجنين . كذلك تم تقدير النسبة المئوية لتركيز الأحماض الأمينية خلال فترات نمو وتطور الأجنة.

أوضحت نتائج المعاملة حدوث إنخفاض فى مستوى البروتين بشكل عام وصل إلى ٩٦,٣٪ فى اليوم الثانى عشر من نمو الجنين. كذلك حدوث انخفاض فى الأحماض الأمينية التى واكبت العمليات الحيوية للتخليق بنسب تتراوح بين ١٢,٥٪ إلى ٨٤,٧٪ بالمقارنة بغير المعامل ، ولوحظ أن بعض الأحماض الأمينية مثل جلوتاميك أسيد ، اسبارتيك أرجانين ، فينيل الأنين ، الأنين ، هسثادين ، جليسين ، تريبتوفان، ثيرونين ، أرجانين ، ليسين ، برولين ، جلوتامين ، ايزوليوسين والتيروزين تلعب دوراً هاماً فى تخليق بعض الأعضاء بالجنين وخاصة جهاز الغدد الصماء مما يؤدى إلى فشل فى التطور والموت للأجنة.