

Effect of In-Ovo inoculation with royal jelly on weight gain of chicken embryo**O. A. Aljumaili****Technical Institute of Al-Anbar****Abstract**

Three groups of fertilized chicken eggs (10 eggs per group) were injected at 12 days of incubation; the first group were injected with diluted royal jelly (with a phosphate buffer solution at 1:1) in amount of 0,05ml in to the allantoic fluid. Second group were injected into the yolk sac with 0.05 ml of diluted royal jelly, while third group left as a control group which were divided into two groups: control 1 were injected with phosphate buffer solution in allantois and control 2 were injected with phosphate buffer solution in the yolk sac. Eggs were weighed before and after injection. We concluded that injection via allantoic have significant ($P < 0.05$) effect on hatched chick as compared to other group.

حقن الهلام الملكي في أجنة الدجاج وتأثيره على وزن الأجنة

عدي عبد الرزاق الجميلي

معهد التقني / الأنبار

الخلاصة

تم حقن ثلاث مجموعات من بيض الدجاج المخصب (10 بيضات في كل مجموعة) في اليوم 12 من الحضانة، حقنت المجموعة الأولى مع غذاء ملكات النحل المخفف (بنسبة 1:1 مع محلول الملح الدارري الفوسفاتي) وبكمية 0.05 مل في سائل الألتويس. أما المجموعة الثانية حقنت في كيس الصفار مع 0.05 مل من غذاء ملكات النحل المخفف، بينما المجموعة الثالثة تركت كمجموعة السيطرة التي قسمت إلى مجموعتين: تم حقن الـ (C1) مع محلول الملح الدارري الفوسفاتي (C2) حقنت بمحلول الملح الدارري الفوسفاتي في كيس الصفار. وتم وزن البيض قبل وبعد الحقن. واستنتجت من هذا العمل أن الأجنة التي حقنت في سائل الألتويس حققت زيادة معنوية ($P < 0.05$) في وزن الأفرخ الفاقسة مقارنة مع بقية المجموعات.

Introduction

Honey is processed into Royal jelly (RJ) in the hypopharyngeal and mandibular endocrine glands of nurse bees between 5 and 15 days old. Queen and Worker bees have the same DNA. An unknown component in RJ silences a specific methyltransferase gene which adds methyl tags to DNA in the larvae destined to be workers (1). RJ composed of water-67%, proteins-13%, sugars-11% fructose 6%, glucose 4%, sucrose 1%, fatty Acids 5%, minerals 1% Ca, Cu, Fe, Mg, Mn, Na, K, Zn, Si, 7-9 different sterols-sitosterol, cortisol, cholesterol, phospholipids from which cell walls are made, 5 glycolipids which provide energy, 4 vitamins in RJ : Only trace or insignificant amounts of vit B12, vit C, and lipid soluble vitamins A, D, E and K. According to US Dept. of agriculture RJ has B1 (thiamin) 1.5-7.4 mcg/gm, B2 (riboflavin) 5.3-10 mcg/gm, B3 (niacin) 60-150 mcg/gm, B5 (pantothenic acid) 65-200 mcg/gm, B6 (pyridoxine) 2.2-10.2 mcg/gm, B7 (biotin) 0.9-0.5 mcg/gm, B9 (folic acid) 0.16-0.5 mcg/gm, B12 (cobalamin) 0.015 mcg/gm. Royal jelly is the richest natural source known for Vit B5 (2). Other components of Royal Jelly Gamma Globulin- mostly immunoglobulins which powerfully strengthen the immune system, 10-HydroxyDecanoic Acid- 20-60mcg/gm. Powerful anti-bacterial and anti-fungal (3). It keeps RJ sterile, Gelatin- Precursor of collagen for skin, tendon, ligaments, etc., Acetylcholine- up to 1 mg/gram of RJ- the richest natural source. Important in nerve transmission and production and release of glandular secretions (4,5). 52 Royal Jelly Proteins Identified, Other than the major RJ protein family and some other previously

identified proteins, 42 novel proteins were identified which are about 3% of the protein in RJ but most of their functions are still unknown (6). RJ has a stimulating effect on the metabolic activity of proteins, glucose and lipids in part due to its high vitamin B5 content (7). Vitamin B5 (Pantothenic acid) catalyses the synthesis of co-enzyme A which help synthesis and oxidation of fatty acid and helps other enzymes work. White mice have significantly increased life spans when a sufficient amount of Vit B5 is added to their food (8). RJ has a vasodilating action due to its rich acetylcholine content (9). RJ increases the oxygen consumption at tissue level. The cardioprotective action is due to its capacity to stimulate an increased secretion of adrenaline via acetylcholine. It stimulates liver to secrete glycogen raising blood sugar levels for more than 24 hrs. RJ is stimulant, Hypothalamic-pituitary functioning generally declines with aging. Reportedly RJ has favorable effects on the compensation process (10). It act as Anti-oxidant and Anti-aging through decreasing intra-cellular oxidation by acting as a scavenger of reactive oxygen species. It also affects protein expression (11). In ration supplementation to turkey feed at the doses of 10, 15 and 20 ppm from 1 to 150 d of age, RJ has improved the weight gain (respectively + 10.50%, +12.30 percent and +16.50%) and the feed utilization (respectively + 9.50%, +12.00% and +22.00%). At the doses of 15 and 20 ppm, RJ improved also the carcass (respectively + 6.00% and + 9.50%) and the meat yield (12).

Material and Methods

30 broiler egg from Rose breed were used in the experiment. The eggs weight was measured at sensitive digital balance (DIAMOND model 500), the egg were incubated in homemade hatchery and 12 days after, the eggs divided into 4 groups (A, B, C1, C2). Solution of royal jelly diluted with a phosphate buffer solution 1:1. Sterilized tool insightful to eggshell, sterile solution to clear eggshell, paraffin wax to close the holes in the egg shell, and 1ml medical sterilized disposable syringes (needle gauge 21) have been used. In order to insure the viability of chicken embryo, a special candle have been used. Group A: were injected with diluted royal jelly (with a phosphate buffer solution at 1:1) in amount of 0,05ml in to the allantoic fluid. Group B: were injected into the yolk sac with 0.05ml of diluted royal jelly, while the third group left as a control group which were divided into two groups: control 1 (C1) injected with phosphate buffer solution in allantois and control 2 (C2) injected with phosphate buffer solution in the yolk sac (13). Eggs were weighed before injection, after injection the egg were weighed every two days and the chick hatched were also weighed. Statistically analyzed by one-way analysis of variance by using SPSS program (14).

Results

Table 1 shows the weights of eggs before injecting. Table 2 shows the weights of eggs after injection observe that in group (a) there is a decrease in weights of eggs but less than Group (b) which were injected in to the yolk and two control groups (C1, C2). In Table 3 reveal that there is a decrease in weights of eggs, but the group (a) are also reveal a minimum weight loss in compare to remainder groups and at hatching directly found that a group (a) is the highest weight in compare to other groups.

Table (1) Eggs weight (g) prior to being placed in the hatchery

Weight	Grams									
No. Group	1	2	3	4	5	6	7	8	9	10
A	50	49	49	50	49	49	49	50	50	49
B	49	48	49	50	49	49	50	49	50	49
C1	50	49	50	49	48					
C2	50	49	49	50	49					

Table (2) Weight of eggs (g) after injection of the four groups

ROUT OF INJECTION	Group	No. OF EGG Day After Injection	1	2	3	4	5	6	7	8	9	10
			Allantoic injection	A	2 days after injection	49	48	48	49	48	48	48
4 days after injection	48	47			47	48	46.5	47	46.5	48	48	47
6 days after injection	47	46			46	47	46	46	45.5	47	47	46
Yolk sac injection	B	2 days after injection	47	46	47	48	47	47	48	46	48	47
		4 days after injection	45	44	45	46	45	45	46	44	46	45.5
		6 days after injection	43	42	43	44	43	43.5	44.5	42	44	43
PBS injection in the allantoic	C1	2 days after injection	47	47	48	47	46					
		4 days after injection	45	45	46	45	44.5					
		6 days after injection	43	43	44	43.5	42.5					
PBS injection in the yolk sac	C2	2 days after injection	48	46	47	48	47					
		4 days after injection	46	44	45	46	45					
		6 days after injection	44	42.5	43	44	43					

Table (3) The weight of chicks (g) immediately after hatching of the four groups

No. Group	1	2	3	4	5	6	7	8	9	10	Mean ±SD
A	46	45	45	46	45	45	45	46	46	45	45.45 A 0.16±
B	42	41	42	42	41.5	42	43	42	43	41.5	42.0 B 0.19±
C1	41.5	42	42.5	42	41						41.8 B 0.25±
C2	42	41	41	42	41						41.4 B 0.24±

Different Capital letters to indicate the presence of significant differences between groups at (P<0.05).

Discussion

The eggs reduced in their weight during incubation and this fact agreed with some studies conducted on eggs during incubation which found that there is loss about 13% of egg weight due to evaporation of liquids (15). In this research it was seen that the first group (A) which was injected in Allantoic fluid have a minimum loss in weight compared to the other groups, although they loss weight but less than other groups and this loss of weight is normal as a result of the evaporation of liquid during incubation. Second group (b) did not show any change as compared to the two sets of control on the weight of the eggs or chicks hatched and this is because that absorption of albumin is faster than the yolk. Within 2 week of embryonic age, growth of the mucosa through this period almost exclusively represents villi, and the increased appearance of enzymes that finalize digestions correspond to surface maturation (16).

To get this result is due to the royal jelly components and bee larvae that feed it turn into queens and a size exaggerated three times in compared to the workers that do not feed RJ and even a queen-life until four to six years compared to workers. It is concluded that the injection of embryos in to the allantoic cavity with RJ stimulates embryos to grow more than the group of embryos injected in to the yolk sac or other eggs which does not injected with RJ.

Reference

1. Kucharski, R. 2008. Nutritional Control of Reproductive Status in Honeybees via DNA Methylation, *Sci.*, 28; 319(5871):1827-1833.
2. Stocker, A. 2005. Trace and Mineral Elements in Royal Jelly and Homeostatic Effects. *J. Trace Elem. Med. Biol.*; 19(2-3):183-189.
3. Fujiwara, S.; Imai, J. & Fujiwara, M. 1990. A potent antibacterial protein in royal jelly. Purification and determination of the primary structure of royalisin. *J. Biol. Chem.* Jul., 5;265(19):11333-11337.
4. Wei, W.; Wei, M.; Kang, X. J.; Deng, H. H. & Lu, Z. H. 2009. A novel method developed for acetylcholine detection in royal jelly by using capillary electrophoresis coupled with electrogenerated chemiluminescence based on a simple reaction. *Electrophoresis*, 30 (11): 1949-1952.
5. Henschler, D. 1956. Identification of choline esters in biological material, especially acetylcholine in royal jelly of bee. *Hoppe. Seylers. Z. Physiol. Chem.*
6. J. Proteome Res. 2008. Comprehensive Royal Jelly (RJ) Proteomics Using One- and Two-Dimensional Proteomics Platforms Reveals Novel RJ Proteins and Potential Phosphor/Glycoproteins June 26.
7. Guo, H.; Saiga, A.; Sato, M.; Miyazawa, I.; Shibata, M.; Takahata, Y. & Morimatsu, F. 2007. Royal jelly supplementation improves lipoprotein metabolism in humans. *J. Nut. Sci. and Vitaminol.*, 53 (4): 345-348.
8. Inoue, S.; Koya-Miyata, S.; Ushio, S.; Iwaki, K.; Ikeda, M. & Kurimoto, M. 2003. Royal jelly prolongs the life span of C3H/HeJ mice: correlation with reduced DNA damage. *Exp. Gerontol.*, 38:965-969.
9. Asafova, N.; Orlov, B. & Kozin, R. 2001. Physiologically active bee products (in Russian). *Y. A. Nikolaev Nijnij Novgorod*, P. 360.
10. Narita, Y. & Ahta, S. 2009. Effects of Long term administration of Royal Jelly on Pituitary Weight and Gene Expression in Middle-Aged Female Rats, *Biosci. Biotechnol. Biochem.*, 73: 80556-1-3.
11. Honda, Y.; Fujita, Y.; Maruyama, H.; Araki, Y.; Ichihara, K.; Sato, A.; Kojima, T.; Tanaka, M.; Nozawa, Y.; Ito, M. & Honda, S. 2011. Lifespan-Extending Effects of Royal Jelly and Its Related Substances on the Nematode *Caenorhabditis elegans*. *Plos. One.*, 6 (8).
12. Bonomi, A.; Bonomi, B. M. & Quarantelli, A. 2001. (Parma Univ. (Italy). Dipartimento di Produzioni Animali, Biotecnologie Veterinarie, Qualita e Sicurezza Alimentare) *Rivista di Scienza dell'Alimentazione (Italy)* -02-01v. 30(1): 49-60.
13. Brian, W. J. M. & Hillar O. K. 1996. *Virology Methods Manual Academic Press*, London, NW, 7DX.
14. Snedecor, G. W. & Cochran, W. G. 1982. *Statistical Methods*. 7th Edition. Iowa State University Press, Ames, U.S.A.
15. Massaro, M. & Davis, L. S. 2005. Differences in egg size, shell thickness, pore density, pore diameter and water vapour conductance between first and second eggs of Snares penguins *Eudyptes robustus* and their influence on hatching asynchrony. *Ibis*, 147:251-258.
16. Uni, Z.; Ferket, P. R.; Tako, E. & Kedar, O. 2005. In ovo feeding improves energy status of late-term chicken embryos. *Poult. Sci.*, 84:764-770.