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EFFECT OF NIGELLA SATIVA (BLACK SEEDS) ON THE AMINO ACID PATTERNS AND SOME METABOLIC CONSTITUENTS IN THE SERUM OF PEKIN DUCKLINGS

(With 3 Tables)

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تأثير الحبة السوداء على أنماط الأحماض الأمينية وبعض المكونات الأيضية في مصل البط البكيني الصغير

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أجريت هذه الدراسة لتوضيح تأثير التغذية على ٢,٥ ٪ من الحبة السوداء وكذلك سحبها على الأحماض الأمينية ووظائف الكبد والكلى في مصل البط الصغير ومدى إستمرار هذا التأثير بعد توقف إضافة الحبة السوداء بحوالي ١٢ يوماً. وقد أوضحت الدراسة أن التغذية على الحبو السوداء أدى إلى نقصان جوهري في تركيزات الأحماض الأمينية ماعدا الهستيدين والذي زادت قيمته زيادة معنوية. بينما أدى توقف إضافة الحبة السوداء من العليقة المغذى عليها البط إلى عودة قيم حامض الجلوتاميك – جلايسين – الألنين – لايسين و الأرجينين لمثيلاتها في مجموعة المقارنة وعلى العكس من ذلك إرتفعت قيم السيستين – تيروسين والفالين الميثولين وأيزوليسين والهستيدين ، أما بقية الأحماض الأمينية فظلت كما هي . بالنسبة لوظائف الكبد والكلى فلم تتأثر كثيراً بالتغذية على الحبة السوداء ماعدا حمض البوليك والذي إنخفض مستواه معنوياً نتيجة التغذية على الحبة السوداء.

SUMMARY

This study was conducted to determine the effect of black seed (B.S.) feeding (2.5%) and its residual effect on chemistry of ducklings serum. The obtained results reveal a significant decrease in the concentrations of all amino acids except histidine which showed a significant increase in ducklings

supplemented with (B.S.). On the other hand, stopping of B.S. supplemetation resulted in re-elevation of some amino acids such as glutamic acid, glycine, alanine, phenylalanine, lysine and arginine to the normal values after 12 days, while it significantly increased the values of cystine, tyrosine, valine, methionine, isoleucine, and histidine. The remainder amino acids still decreased. The activities of serum amino transferases {Aspartate amino transferase (ASAT) and Alanine amino transferase (ALAT)}. Alkaline and acid phosphatases, and creatinine values were not greatly affected, while the serum uric acid was significantly decreased in ducklings fed black seed.

Key words: *Nigella sativa - Effect on serum - Pekin ducklings*

INTRODUCTION

Black seed (Nigella sativa) is a herbaceous plant which is a member of Ranunculocea family. It has been shown to contain more than 30% fixed oils and 0.4-0.45% w/w of a volatile oils (Hashim and El-Kiey, 1962, El-Alfy et al., 1975 and Abd El-Aal and Attia, 1993).

The black seed has antibacterial and antifungal properties, (Rathee et al., 1982), anthelmintic activity against tape worms (Agarwal,et al., 1979) and chloretic action, (Mahfouz et al., 1962).

Recently, Hedaya, (1995) found that the injection of black extract to male rats decreases the serum glucose and cholesterol levels, but it increases the total serum protein and globulins levels meanwhile the values of serum albumin and urea are not significantly altered. Nearly similar results were observed by Mandouret al., (1995) and El-Gazaar (1997) for serum proteins.

Many reports and articles have been written indicating the significant role of black seed in increasing immunity and maintaining good health, while information are still deficient about the effect of black seed on blood chemistry, therefore this study was planned to clarify the effects of black seed feeding as well as its stopping supplementation on amino acid patterns, and also liver and kidney functions of Pekin ducklings.

MATERIALS and METHODS

Materials:

Fifty one week-old Pekin ducklings purchased from local commercial hatchery. Duckling were leg banded, individually weighed and allowed to their experimental floor per unit.

Twenty five birds were used as control group and kept on basal diet, while the remainder 25 ducklings used as experimental group and kept on basal diet in addition to 2.5% crushed black seed/Kg basla diet. The experimental diet was offered from the day 14 of age and lasts for 12 day after which the black seed was stopping supplementation and the birds received only basal diet.

All birds were kept under continuos light program and feed and water were offered ad libitum.

Blood sampling and the analytical methods:

Individual blood samples were collected from each bird at the day 12 from starting of the experiment and after 12 day from the stopping supplementation of black seed. Clear non hemolyzed sera were separated and stored frozen at -20°C until analysis. Each group was pooled into five samples for determination of amino acids.

Aliquots of sample from blood serum were concentrated by centrifugation and were lyophilized and weighed. The dried samples were then suspended in 6 M HCl at a concentration of 5 mg/ml. These preparation were digested under argon in sealed vials at 110°C for 24 hrs (Kats and Gerhardt 1992).

The digests were evaporated to dryness under vacuum and were resuspended in sodium citrate buffer. The amino acids compositions of these samples were then determined with a calibrated Beckman 119 CL, amino acid analyzer, which separates the amino acids by ion-exchange chromatography, and then eluted amino acids to their ninhydrin derivatives for detection and quantitation. Amino acids were eluted from the column using a pH step gradient, with pH steps of 2.85, 3.90, 4.28 and 7.19 according to Banson and Patterson, (1965).

Activities of alanine and aspartate aminotransferases (ALAT, ASAT Reitman and Frankel, 1957); alkaline and acid phosphatases, ALP, ACP (Sigma Procedures No. 104, 1989); also uric acid (Caraway, (1955), creatinine (Husdan and Rapaport, 1968), were all determined in blood serum.

All birds of both groups were weighed before blood sampling. The data obtained were statistically analyzed according to Snedecor and Cochran, (1982).

RESULTS and DISCUSSION

Black seed (Nigella sativa) is becoming commonly used for many medical purposes. For instance the seeds are used in case of delayed menses,

indigestion and cough. It is also used as diuretic and to augment lactation, (Tappozada et al., 1965). The data concerning the effect of black seed feeding as well as stopping supplementation has been quite fragmentary until now, hence the purpose of this study was to throw light on the effects of black seed feeding and its stopping supplementation on serum amino acids, liver and kidney function tests, since alteration (such increases) in the amino transferases activity may be attributed to hepatocellular damage and leakage of cytoplasmic contents into serum.

The obtained results (Table 1) clearly showed that the concentrations of amino acids were significantly decreased except for histidine which was greatly increased in the sera of ducklings fed with 2.5% B.S. for 12 day.

It is well known that, the plasma amino acids are altered in case of liver diseases (Maurice et al., 1983); Eimeria infection (Koinaraski, 1988); ascorbic acid deficiency (Schonheyder, 1962) and even feeding on high dietary ascorbic acid (Mandour et al., 1995). William (1977) mentioned that the injection of insulin hormone decreases serum amino acids concentrations. Abd El-Aziz et al.,(1995) emphasized that the feeding of black seed increases the number of β-cells of islets of langerhans of pancreas. This finding was then supported by the work of Hedaya (1995) who attributed the decrease in blood glucose level of rats to the higher insulin levels resulted from injection of black seed extract. Consequently, the higher insulin levels and its anabolic effect might be the cause of lowered amino acids in birds kept on black seeds.

On the other hand, the histidine amino acid is the only amino acid which increases significantly in birds fed B.S. which may be attributed to the inhibitory effect of the active principle (Thymoquinone) of B.S. on synthesis and release of histamine (Nirmal, 1993).

Furthermore, the obtained data in (Table 2) showed a non significant changes in the activities of ALAT, ASAT, ALP and ACP. This results clearly demonstrate that the feeding of black seed (2.5% for 12 days) has a non hepato and non nephro toxic effects in which the function tests of them are not altered. In the same table the serum uric acid was significantly decreased, this may be attributed to uricosuric effect of B.S. (El-Dakhakhny 1965; El-Gazaar 1997).

Although the black seed was withdrawn from the diet for 12 days post experiment, some amino acids still altered, which may indicated that the black seed has residual effects.

More studies are needed to confirm the findings of this investigation.

REFERENCES

Abd El-Aal, L and Attia, R. (1993): Characterization of black cumin (Nigella sativa): Chemical composition and lipids. Alex. Sci. Exch. 14(4): 467-81.

Abd El-Aziz, M., El-Sayed Soliman, Zaghloul, A. and El-Nemr, M. (1995): The effect of Nigella sativa (Black cumin) on exocrine pancreatic cells: An electron-microscopic study. Alex.J.Vet.Sci.11(3):333-343.

Agarwal, R.R. Kharya, M.D. and Shrivastava, R. (1979): "Antimicrobial and anthelmintic activities of the essential oil of Nigella sativa". Indian J. Exp. Biol. 1: 1264-1265.

Banson, J.V. and Patterson, J.A. (1965): Accelerated automatic chromatographic analysis of amino acids on a chromobeads resin. Anal. Chem. 37: 1108.

Caraway, W. (1955): J. Clin. Path. 25: 840 cited in kit of uric acid, calorimetric method provided by Egyptian American Co. for Lab. services.

El-Alfy, T.S., El-Fatatry, H.M. and Toama, M.A. (1975): Isolation and structure assignment of Antimicrobial principle from the volatile oil of Nigella sativa L. seeds Pharmazie. 30: 109-111.

El-Dakhakhny, M. (1965): Studies on the Egyptian Nigella sativa, IV Some pharmacological properties of the seeds active principle in comparison to its dihydro compound and its polymer. Drug Research (Germany) Arzneim. Forsch. 15: 1227.

EL-Gazaar, U.M.B. (1997): Biochemical studies on vit. E deficiency in rats. Thesis Master D. Fac. Vet. Med. Alex.. Univ..

Hashim, F.M. and El--Kiey, M.A. (1962): Nigella sativa seeds of Egypt. J. Pharmac. Sci. United Arab Rep. 3: 121.

Hedaya, S. (1995): Effect of Nigella sativa (Black seeds) extract on some hematological and biochemical parameters in rats. Alex. J. Vet. Sci. 11 (2): October, 95-99.

Husdan, H. and Rapaport, A. (1968): Estimation of creatinine by the Jaffe reaction. A comparison of three methods. Clin.Chem.14(3):222-238.

Kats, M.L. and Gerhardt, O.K. (1992): Methylated lysine in storage body protein of sheep with hereditary ceroid lipofuscinosis. Biochem. Biophys. Acta. 1138: 97-108.

Koinaraski, V. (1988): Dynamics of free amino acids in the blood of turkeys infected with Eimeria adenoids, Veter. Sbirka. 86 (5):41-43.

Mahfouz, M., El-Dakhakhny, M., Gemel, A. and Moussa, H. (1962): Chloretic action of Nigella sativa seed oil. Egyptian Pharmac. Bull. 44, 225-229.

Mandour, A., Korshom, M. and Assa abou El-Wafa (1995): Amino acid pattern in broiler Fayoumi chickens kept on high dietary ascorbic acid. Beni-Suef. Vet. Med. Res. 5 (1): 32-40.

Mandour, A., Mahmoud, K., Assa abou El-Wafa, El-Agamy, E. and Ommyma Ragab (1995): Effect of alfatoxine and Nigella sativa seeds on serum proteins and its electrophoretic patterns in white Pekin ducklings. 1st Egyptian Hungarian Poultry Conference. 17-19 Sep. Alex. Egypt.

Maurice, D., Bodine, A. and Rehere, N. (1983): Metabolic effects of low aflatoxin B_1 level on broiler chicks. Applied and Environmental Microbiology. Mar. 45 (3): 980-9844.

Nirmal, C. (1993): Inhibition of histamine release from mast cells by Nigellone. Annals of Allerg. 70: March 237-242.

Rathee, P.S., Mishra, S.H. and Kaushal, R. (1982): Antimicrobial activity of essential oil fixed oil and unsaponifiable matter of Nigella sativa L. Indian. J. Pharmac. Sci. 44: 8-10.

Reitman, M. and Frankel, S. (1957): A colorimetric method for determination of serum glutamic oxalacetic and glutamic pyruvic transaminase. Amer. J. Clin. Path. 28: 56-63.

Schonheyder, F. (1962): Influence of partial starvation and acute scurvy on the free amino acids in blood plasma and muscle in guinea pig. Brit. J. Nutr. 16: 75.

Sigma procedure No. 104 (1989): Phosphatases, alkaline, acid, prostatic acid: Quantitative determination in serum at 400-420 nm.

Snedecor, G.W. and Cochran, W.G. (1982): Statistical methods, 6th Ed. Iowa Univ. Press, Ames. USA.

Tappozada, H., Mazloum, H. and El-Dakhakhny, M (1965): The antibacterial properties of the Nigella sativa seeds, active principle with some clinical application. J. Egypt. Med. Assoc. 48: 187.

William, M. D. (1997): Endocrine glands. Cited after Swenson, J. editor of Ducks. Physiology of domestic animals 9th ed. Cornell Univ. Press. Ithaca and London.

Table 1: Amino acids in sera of ducklings treated with 2.5% black seed (mg/dl)

Groups	After feeding of B.S.		After stopping supplementation of B.S.	
	Control	Treated	Control	Treated
Aspartic	9.53 ± 0.07^a	2.07 ± 0.04^b	13.30 ± 0.70^a	11.00 ± 0.12^b
Serine	4.47 ± 0.04^a	1.39 ± 0.05^b	6.50 ± 0.12^a	4.30 ± 0.17^b
Glutamic	24.75 ± 0.43^a	10.83 ± 0.07^b	28.30 ± 0.14^a	28.30 ± 0.14^a
Proline	9.16 ± 0.03^a	1.22 ± 0.06^b	17.30 ± 0.17^a	10.50 ± 0.26^b
Glycine	5.78 ± 0.04^a	1.91 ± 0.06^b	9.50 ± 0.06^a	10.00 ± 0.12^a
Alanine	15.65 ± 1.06^a	11.85 ± 0.08^b	18.60 ± 0.26^a	19.00 ± 0.03^a
Cystine	1.73 ± 0.08^a	1.61 ± 0.06^b	0.83 ± 0.06^b	2.39 ± 0.23^a
Tyrosine	13.10 ± 0.89^a	6.85 ± 0.06^b	8.90 ± 0.06^b	10.60 ± 0.35^a
Threonine	8.05 ± 0.03^a	2.21 ± 0.11^b	9.40 ± 0.23^a	6.30 ± 0.12^b
Valine	9.90 ± 0.06^a	6.00 ± 0.02^b	11.28 ± 0.16^b	15.00 ± 0.58^a
Methionine	5.05 ± 0.03^a	3.29 ± 0.16^b	5.10 ± 0.06^b	6.50 ± 0.15^a
Isoleucine	9.73 ± 0.12^a	3.82 ± 0.16^b	15.30 ± 0.06^b	17.60 ± 0.33^a
Leucine	15.06 ± 0.03^a	10.12 ± 0.01^b	16.50 ± 0.06^b	26.60 ± 0.41^a
Phenylalanine	9.36 ± 0.21^a	1.62 ± 0.06^b	13.30 ± 0.12^a	14.00 ± 0.02^a
Histidine	4.32 ± 0.18^b	6.36 ± 0.06^a	6.70 ± 0.15^b	8.30 ± 0.18^a
Lysine	33.45 ± 0.26^a	13.06 ± 0.03^b	30.00 ± 0.02^a	29.30 ± 0.14^a
Arginine	9.16 ± 0.41^a	5.83 ± 0.09^b	5.50 ± 0.06^a	4.90 ± 0.29^a

Means within the same column with the same superscript are not significantly different at ($P < 0.01$).

Table 2: Mean values of serum uric acid, createnine, ALAT, ASAT, ALP and ACP of ducklings kept on diet containing 2.5% B.S.

Groups	After feeding of B.S.		After stopping supplementation of B.S.	
	Control	Treated	Control	Treated
Uric acid (mg%)	24.09 ± 1.80^a	14.75 ± 0.91^b	18.14 ± 1.07^a	17.04 ± 1.42^a
Createnine (mg%)	0.21 ± 0.02^a	0.23 ± 0.02^a	0.02 ± 0.01^a	0.21 ± 0.02^a
ALAT (U/ml)	5.07 ± 1.42^a	4.33 ± 0.33^a	4.63 ± 0.33^a	5.07 ± 1.17^a
ASAT (U/ml)	16.67 ± 0.63^a	18.00 ± 3.06^a	14.0 ± 1.16^a	12.9 ± 1.15^a
ALP (.K.A.U/dl)	189.1 ± 14.4^a	207.11 ± 8.1^a	191.7 ± 18.3^a	180.7 ± 16.1^a
ACP (.K.A.U/dl)	8.53 ± 0.40^a	8.10 ± 0.80^a	7.50 ± 0.20^a	7.67 ± 0.60^a

Table 3: Body weight of ducklings after feeding and stopping supplementation of B.S.

Age Groups	5 days of age	12 days after feeding of B.S.	12 days after stopping supplementation of B.S.
Control	71.90 ± 4.00^a	350.60 ± 24.00^a	686.00 ± 29.10^a
Treated	82.80 ± 4.00^a	372.50 ± 17.00^a	732.00 ± 18.80^a