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Increased β-globulin Levels in Captive Oriental White Storks Fed a Diet of Sand Eels

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Abstract : The aim of the study was to identify the effects of two different diets (sand eels or saurels) on the serum protein electrophoretic patterns of Oriental White Storks held in captivity. The tests were performed on two groups of storks according to the diet (group 1 and 2). Twenty-two (group 1) or twenty-nine (group 2) storks were included. The values of complete blood count (CBC), serum biochemistry profiles, protein fractions (albumin, α-globulin, β-globulin, and γ-globulin), and lipoprotein (high density- [HDL] and low density lipoprotein [LDL]) were compared between samples obtained during two groups ($p < 0.05$). The α-globulin fraction was decreased and the β-globulin fraction was significantly increased in samples obtained from group 1 compared to those obtained from group 2. In group 1, the concentration of LDL was also significantly increased compared to that of group 2. In conclusion, we confirmed that the β-globulin fraction was significantly elevated in storks fed sand eels.

***Key words* :** Oriental White Stork, *Ciconia boyciana*, electrophoresis, β-globulin, low-density lipoprotein, sand eel.

Introduction

The Oriental White Stork (*Ciconia boyciana*) belongs to the family Ciconidae and the order Ciconiiformes. This stork is listed as an endangered species on the Red List of Threatened Species of the International Union for Conservation of Nature (IUCN) and is extinct in South Korea (19). Since 1996, the Korea Institute of Oriental White Stork Rehabilitation Research (KIOWSRR) has made an effort to reintroduce the Oriental White Stork as a breeding species in South Korea. Since 2004, the Wildlife Center of Chungbuk, Chungbuk National University has participated in the project, providing health screening programs.

For a successful reintroduction of wild animals, following rules should be preserved (7): 1) a self-sustaining captive population should be maintained, 2) adequate and protected habitat is needed, 3) the animal should be trained prior to release into the wild in order to improve survival rate, 4) after the release, adaptability of the animal should be monitored, 5) conservation education should be provided against people around the habitat. At present, the KIOWSRR is trying to secure the population and is also monitoring the body condition against different food ingestion in the breeding facility.

The goal of this current study was to identify the effects of different diets on the serum protein electrophoretic patterns of Oriental White Storks held in captivity. The results of this study will help characterize the influence of nutrition on captive storks.

Materials and Methods

Sampling and blood examinations

The study was performed in the KIOWSRR facility. Blood sampling was performed from June 2007 to January 2009. Twenty-two (group 1; 13 males and 9 females) or twenty-nine (group 2; 14 males and 15 females) storks aging 1 to 4 years old were included in each period. All storks showed visually normal behaviour and appetite, as determined by experienced keepers and a veterinarian. During the experiment, storks were housed individually in a 7 × 7 m cage with chain-link fencing. Each cage was covered with netting, and a pond and roost were established on the ground of the cage. Sand eels (for group 1) or saurels (for group 2) were provided once a day in buckets in the enclosures.

For the examination, peripheral blood was collected from the caudal tibial vein of the storks after providing each fish. The collected blood was placed into heparin-treated tube and serum separator tubes, respectively. After the collection, manual complete blood count (CBC) and serum biochemistry

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profiles were performed with a 7020 Automatic Analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan). Residual serum samples were held at −20ºC for additional analysis.

Serum protein electrophoresis

Freshly thawed serum samples were used for protein electrophoresis. The electrophoresis was performed on a cellulose polyacetate membrane in a Sepratek™ chamber (Gelman Sciences, Ann Arbor, MI, USA) as a manufacturer's protocol. After electrophoresis, the membranes were stained with Ponceau S and were analyzed by SigmaScan (SPSS Inc., Chicago, IL, USA). The protein fractions were calculated by multiplying the percentages of each fraction by the total protein concentration. The following protein fractions were determined: albumin, α-globulins, β-globulins, and γ-globulins.

Evaluation of lipoprotein fractions

Freshly thawed serum samples were used to examine lipoprotein fractions. High-density lipoprotein (HDL) and low-density lipoprotein (LDL) were examined using a 7020 Automatic Analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan).

Statistical analysis

Statistical analyses of the values between the groups were performed using SPSS (version 12.0, SPSS Inc.). Differences in the mean values between the groups were analyzed by Student's t-test. A P value of < 0.05 was considered to be statistically significant.

Results

The results of CBC and serum biochemistry profiles revealed no abnormalities based on the reference intervals of items developed by the authors' previous study (unpublished data). There is no significant difference between groups or sexes.

The protein electrophoresis revealed significant differences in the pattern of α- and β-globulin fractions between group 1 and group 2 ($p < 0.05$) (Fig 1). While the α-globulin fraction of samples obtained from group 1 was decreased, the β-globulin fraction values were increased compared to those obtained from group 2 (Fig 2). Total globulin values between the two groups, however, did not differ. The test for lipoprotein fractions (i.e., HDL and LDL) revealed that the LDL value was significantly higher in the samples obtained from group 1 than in those from group 2 (Fig 3).

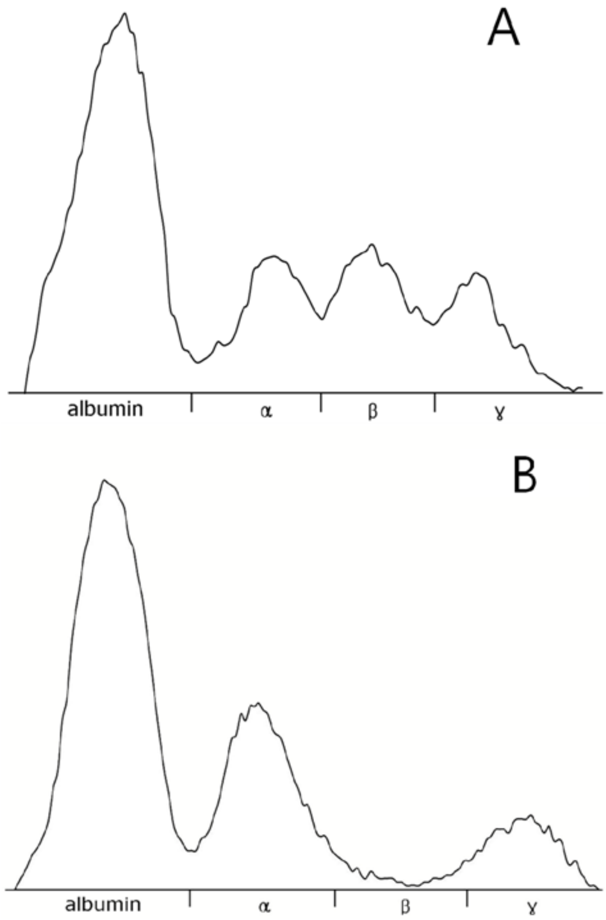

Fig 1. Representative protein electrophoresis profiles from storks obtained from group 1(A) and 2(B).

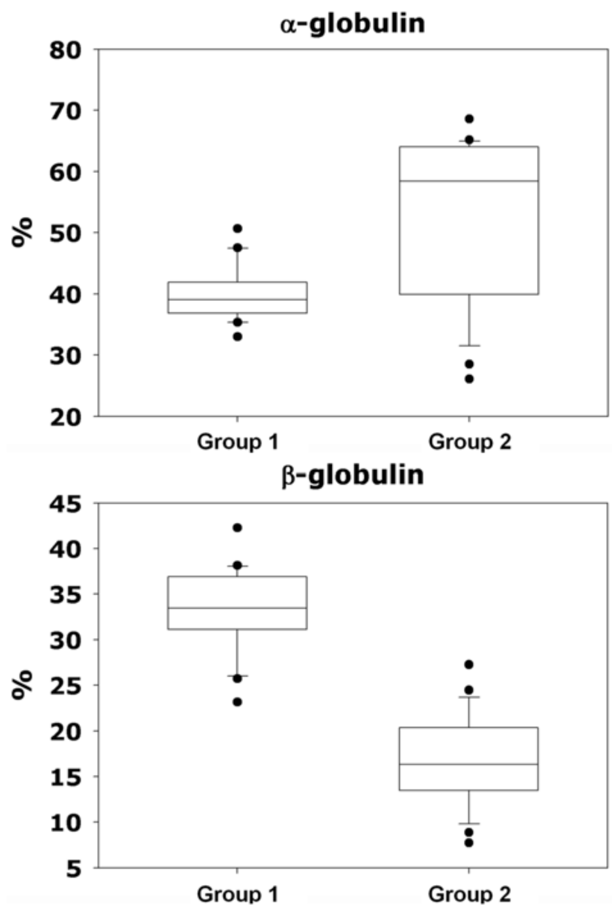

Fig 2. Mean ± SD of α- and β-globulin fractions obtained from group 1 and 2 ($p < 0.05$).

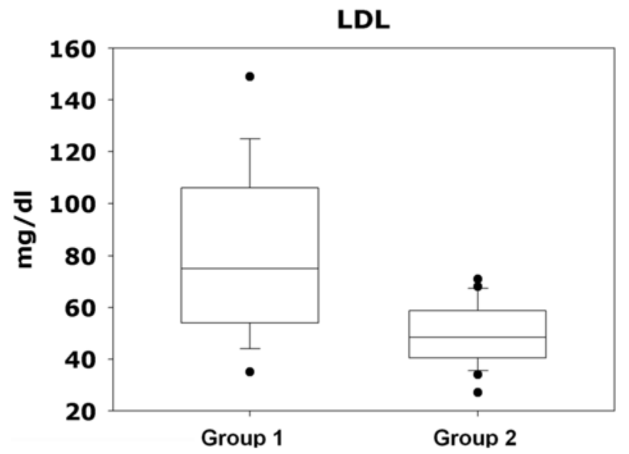

Fig 3. Mean ± SD of low-density lipoprotein fractions obtained from group 1 and 2 ($p < 0.05$).

Discussion

In this study, we demonstrated that storks fed a diet of sand eels had a significantly elevated β-globulin fraction, probably as a result of elevated levels of LDL. This difference in globulin fraction between samples obtained from group 1 and 2 was consistently observed in all storks, regardless of their sex or age. Avian β-globulin consists of acute phase protein (fibrinogen and transferrin), β-lipoprotein (LDL), and vitellogenin; thus, it can be elevated during acute inflammation, infection, malnutrition, lipemia artifact, neoplasia, and trauma (3,4,18). None of the storks exhibited any pathological changes during a physical and clinical pathological examination, and total globulin values were consistent between the two groups. Also the sample which we used was serum that does not have fibronogen. Therefore, we hypothesized that the globulin fractions were re-distributed by a non-pathogenic cause. In particular, we proposed that the differences in lipoprotein levels between the two groups induce the change in the β-globulin fraction, and that the alteration of the α-globulin fraction is a secondary event. This interpretation was further supported by additional examinations of the lipoproteins, which revealed significantly elevated LDL levels in samples obtained from group 1 compared to those from group 2.

In general, male and immature female birds are considered to have a similar lipoprotein delivery mechanism to that of mammals (9). However, mature females possess a unique lipoprotein delivery mechanism that mediates the transport of hepatically synthesized lipoproteins to the growing oocytes (2). Very low-density lipoprotein metabolism is the best-understood lipoprotein in birds (5,14,16). Recently, an avian LDL receptor that is similar in molecular structure and function to that in mammals has been described (6,18). In this study, all storks revealed a consistent change in LDL value regardless of sex. This finding suggests that the change in LDL value is caused by a mechanism present in both male and female storks.

The sand eel is a slim fish (10-20 cm) living at a depth of 10-100 m in seawater of the high latitudes of the Northern Hemisphere (1). Due to its low price and steady availability in South Korea, it was selected as a primary food source for the storks. Sand eels have a high lipid content compared to other fish and are a rich source of omega-3 polyunsaturated fatty acids (PUFAs) (1,17). Omega-3 PUFAs consist of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (11,12). In humans, EPA and DHA down-regulate the LDL receptor in hepatic cells, resulting in an increase in the circulating LDL levels in patients ingesting omega-3 PUFA (10). Although LDL metabolism is largely unknown in birds, the work presented in this study raises the possibility that EPA and DHA affect the LDL value in group 1. This is further supported by the decrease in LDL levels after the storks were fed a saurel, a reduced-lipid diet.

Acknowledgement

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References

1. Arnott SA, Ruxton GD. Sand eel recruitment in the North Sea: demographic climatic and trophic effects. Mar Ecol Prog Ser 2002; 238: 199-210.
2. Elkin RG. An overview of recent developments in avian lipoprotein metabolism. J Nutr 1997; 127: 793S-794S.
3. Fudge AM, Speer B. Selected controversial topics in avian diagnostic testing. Semin Avian Exot Pet 2001; 10: 96-101.
4. Harrison GJ, Lightfoot TL. Clinical Avian Medicine, 1st ed. Florida: Spix Publishing. 2006: 611-629.
5. Hermmier D. Lipoprotein metabolism and fattening in poultry. J Nutr 1997; 127: 805S-808S.
6. Hummel S, Lynn EG, Osanger A, Hirayama S, Nimpf J, Schneider WJ. Molecular characterization of the first avian LDL receptor: role in sterol metabolism of ovarian follicular cells. J Lipid Res 2003; 44: 1633-1642.
7. Kleiman DG. Reintroduction of captive mammals for conservation: guidelines for reintroducing endangered species into the wild. Bioscience 1989; 39: 152-161.
8. Lanzarot MP, Barhona MV, San Andrés MI, Fernández-García M, Rodríguez C. Hematologic, protein electrophoresis, biochemistry, and cholinesterase values of free-living black stork nestlings (Ciconia nigra). J Wildl Dis 2005; 41: 379-386.
9. Leclercq B, Whitehead CC. Leanness in domestic birds: genetic, metabolic and hormonal aspects, 1st ed. London: Butterworths. 1988: 175-188.
10. Malinowski JM, Metka K. Elevation of low-density lipoprotein cholesterol concentration with over-the-counter fish oil supplementation. Ann Pharmacother 2007; 41: 1296-1300.
11. Mattar M, Obeid O. Fish oil and the management of hypertriglyceridemia. Nutr Health 2009; 20: 41-49.

12. Mckenney JM, Sica D. Role of prescription omega-3 fatty acids in the treatment of hypertriglyceridemia. Pharmacotherapy 2007; 27: 715-728.
13. Montesinos A, Sainz A, Pablos MV, Mazzucchelli F, Tesouro MA. Hematological and plasma biochemical reference intervals in young white storks. J Wildl Dis 1997; 33: 405-412.
14. Musa HH, Cheng JH, Bao WB, Li BC, Mekki DM, Chen GH. Genetic differentiation and phylogeny relationship of functional ApoVLDL-II gene in red jungle fowl and domestic chicken populations. Pak J Biol Sci 2007; 10: 2454-2459.
15. Puerta ML, Munöz Pulido R, Huecas V, Abelenda M. Hematology and blood chemistry of chicks of white and black storks (Ciconia ciconia and Ciconia nigra). Comp Biochem Physiol A Comp Physiol 1989; 94: 201-204.
16. Salvante KG, Lin G, Walzem RL, Williams TD. Characterization of very-low density lipoprotein particle diameter dynamics in relation to egg production in a passerine bird. J Exp Biol 2007; 210: 1064-1074.
17. Sargent JR. Fish oils and human diet. Br J Nutr 1997; 78: S5-S13.
18. Schneider WJ. Low density lipoprotein receptor relatives in chicken ovarian follicle and oocyte development. Cytogenet Genome Res 2007; 117: 248-255.
19. Smirenski SM. Biology and conservation of Oriental White Stork Ciconia boyciana, 1st ed. South Carolina: Savannah River Ecology Laboratory. 1991: 165-177.

양미리를 급여한 사육 황새에서 β-글로불린 분획의 증가

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요 약 : 이 연구는 사육황새에 급여하는 먹이(양미리와 전갱이)의 차이에 따른 혈청 단백질 분획의 변화를 평가하기 위한 것이다. 사육황새를 먹이에 따라 두 그룹(그룹 1과 2)으로 나누어 연구를 진행하였다. 그룹 1에는 22마리, 그룹 2에는 29마리의 황새가 포함되었고, 각각 양미리와 전갱이를 급여하였다. 두 그룹에서 전혈구검사, 혈청화학검사, 단백질분획검사(α-, β- 및 γ-글로불린) 및 지단백질 분획검사(HDL 및 LDL)를 진행하고 결과를 비교하였다. 검사 결과 그룹 1에서 α-글로불린의 감소와 β-글로불린의 증가가 유의적으로 관찰되었다. 그룹 1에서 LDL의 농도는 그룹 2에 비해 유의적으로 증가하였다. 결과적으로 양미리를 급여한 사육황새에서 β-글로불린의 증가가 LDL의 증가에 의한 것임을 확인하였다.

주요어 : 황새, 전기영동, β-글로불린, LDL, 양미리