Detection of serum protein fractions and their concentrations in laying and non-laying ostriches (Struthio camelus) fed with different dietary protein levels

U. POLAT*, M. CETIN1, I. AK2 and F. BALCI3

1 Department of Biochemistry, Faculty of Veterinary Medicine, University of Uludag, 16059-Gorukle, Bursa, TURKEY.
2 Department of Animal Science, Faculty of Agriculture, University of Uludag, 16059-Gorukle, Bursa, TURKEY.
3 Department of Zootechnics, Faculty of Veterinary Medicine, University of Uludag, 16059-Gorukle, Bursa, TURKEY.

* Corresponding author: U. POLAT (e-mail: upolat@uludag.edu.tr)

SUMMARY

The aim of this study was to measure serum protein fractions and their concentrations in laying and non-laying ostriches (Struthio camelus) fed with different dietary protein levels. Twenty females, eight years old ostriches were used. The ostriches were randomly divided into two groups to be fed with different protein levels (20% in group I and 23% in group II) during 5 months. There were 2 sections by group according to the laying status. The sections A (in group I) and C (in group II) were constituted by laying ostriches, whereas in the sections B (group I) and D (group II), there were non laying females. At this end of the experimental feeding, serum albumin, α1-, α2-, β- and γ-globulins were detected by agarose gel electrophoresis and serum total protein, albumin and globulin concentrations were measured by using Auto Technicon DAX 72 autoanalyzer and it’s commercial kits. The laying status induced no significant modification of serum protein concentrations (total protein and protein fractions) except for α2-globulins whose concentrations were significantly increased (p<0.05). When birds were fed with a high-protein diet, significant increases in protein (p<0.01) and in α1-(p<0.01) and in α2- (p<0.05) and α2- (p<0.01) globulin concentrations occurred whereas γ-globulin concentrations tended to decrease. Moreover, this variation was more evident in non-laying females (interaction between diet and laying status : p<0.05). Since these changes in protein concentrations did not bring a real benefit for the health of the ostriches, and since feeding costs greatly contribute to the total cost of ostrich production, it was concluded that crude protein required in diet did not need to exceed 20% of the food intake.


RÉSUMÉ

L’objectif de cette étude est de déterminer l’influence d’un régime alimentaire de teneur protéinique variable sur les différentes fractions protéiniques et sur leurs concentrations sériques chez des Autruches (Struthio camelus) en période de ponte ou non en fonction de la teneur protéinique du régime alimentaire. Par U. POLAT, M. CETIN, I. AK et F. BALCI.

Détération des fractions des protéines sériques et de leurs concentrations chez des Autruches (Struthio camelus) en période de ponte ou non en fonction de la teneur protéinique du régime alimentaire. Par U. POLAT, M. CETIN, I. AK et F. BALCI.

SUMMARY

L’objectif de cette étude est de déterminer l’influence d’un régime alimentaire de teneur protéinique variable sur les différentes fractions protéiniques et sur leurs concentrations sériques chez des Autruches (Struthio camelus). Vingt autruches femelles de 8 ans ont été réparties au hasard en 2 groupes et ont été alimentées pendant 5 mois avec un régime alimentaire contenant 20% de protéines dans le groupe I ou 23% dans le groupe II. Selon l’activité de ponte, 2 sections ont été créées dans chaque groupe, les autruches pondeuses étant regroupées dans les sections A (groupe I) et C (groupe II) et les non pondeuses dans les sections B (groupe I) et D (groupe II). A la fin de ce régime alimentaire, l’albumine sérique et les globulines (α1-, α2-, β- et γ-) ont été mises en évidence par électrophorèse en gel d’agarose et la protéinémie ainsi que les concentrations sériques en albumine et en globulines ont été mesurées par colorimétrie. L’activité de ponte n’a induit aucune modification significative des concentrations des protéines sériques (protéinémie ou concentrations des différentes fractions) à l’exception des α2-globulines dont les concentrations ont augmenté significativement (p<0.05). Lorsque les oiseaux ont été soumis à un régime riche en protéines, la protéinémie (p<0.01), l’albuminémie (p<0.05) et les concentrations en α1-globulines (α1- : p<0.05 et α2- : p<0.01) ont significativement augmenté alors que les concentrations γ-globulines tendaient à diminuer, cette variation étant plus marquée chez les femelles non pondeuses (interaction entre régime et ponte : p<0.05). Vu que les modifications observées des concentrations des protéines sériques ne constituent pas un réel bénéfice pour la santé des autruches et vu que les coûts alimentaires interviennent grandement dans le coût total de production, on peut en conclure que la teneur en protéine de l’aliment n’a pas à dépasser 20%.


Introduction

Blood plasma contains a complex mixture of proteins with widely different structures and properties. Relative and total amounts of serum protein fractions are affected by nutritional, physiological status, infections and inflammation, and are therefore important health indicators. The occurrence of considerable species differences to the overall interpretation of avian electrophoretic data is well established and constitutes a continued challenge to the avian specialist and to the providing laboratories to obtain species-specific, even age- and gender-specific, reference ranges. The albumin and globulin components of avian serum protein are composed of separate albumin, alpha 1-2, beta, and gamma fractions. Usually, decreased albumin precedes the development of generalized hypoproteinemia in dietary protein deficiencies [4, 16, 26, 27].

Ostriches are prevalently vegetarians capable of digesting plant fiber. For health and optimal performance they need balanced rations just like other intensively kept livestock (as turkey, hen). As for many researchers ostriches are too expensive to be used as experimental animals, some of the nutritional information are still extrapolated from other species, mainly from fowl and turkey. For establishment of the nutritional requirements of the other ratite species, these extrapolations are more frequently used than in ostrich spe-
cies. CILLIERS and ANCEL [10] have formulated diets on the basis of poultry requirements, however some nutritionists suggested that turkey was a better model to extrapolate nutritional requirements to the ostriches [12]. There are many gaps in the information on ostrich-nutrient requirements as well as in the utilization of nutrients in the different feedstuffs. Nutritionists do not still agree about the nutritional standards which have to be used for ostriches [6, 14, 22].

The aim of this study was to measure serum protein fractions and their concentrations in laying and non-laying ostriches (Struthio camelus) fed with different dietary protein levels.

Material and methods

ANIMALS

The experimental birds used in the present study were obtained from « The Ostrich Raising Unit », Faculty of Agriculture, Uludag University at Bursa region in the western part of Turkey. Twenty females, eight years old ostriches (Struthio camelus) were used. The extensive breeding ostriches were randomly divided into two groups to be fed with different protein levels, 20% (group I) or 23% (group II) of crude protein. There were ten females ostriches which were fed with 20% or 23% crude protein levels at first laying and then non-laying periods in each group. Each group was divided in 2 sections according to the laying status of the birds: in sections A (group I) and C (group II), there were laying ostriches, and in sections B (group I) and D (group II) there were non-laying animals.

DIETS

During 5 months (from May to September) ostriches were fed with alfalfa ad libitum and with the layer feed (2900 kcal/kg Metabolic Energy (ME), 20% (group I) or 23% (group II) crude protein). Afterwards, alfalfa hay, corn silage (ad libitum) and 700 g/day per animal of mixed grain were given to ostriches in the winter period for seven months. Drinking water was available ad libitum.

BIOCHEMICAL ANALYSIS

The composition of the experimental diets of ostriches used in the present study was analyzed by using the Weende method [1]. Phosphorus analysis of feed mineral matters was estimated with Vanodomolibdo Phosphoric Yellow Color method and, calcium, sodium, potassium and manganese were determined with Dry Burning method [15] and shown in Table I. There is no difference in the food composition except for protein levels.

After experimental feeding, the head of the birds was covered with a hood to facilitate handling [23]. Then blood samples were taken from the wing vein by using vacutainer blood collection tubes (10 ml) and were transported on ice to the laboratory (The Central Laboratory of Uludag University) for analyses. Blood samples were centrifuged at 3000 g for 5 min, at room temperature. Serum was separated and stored at -20°C until used. Serum albumin, α₁-globulin, α₂-globulin, β-globulin and γ-globulin fractions were determined by agarose gel electrophoresis (REP Electrophoresis, Helena-Laboratories, Texas). The compositions of the electrophoretic solution were constituted from serum protein electrophoretic kit (dye, destaining solutions and gel) (REP SP Plus Kit, Cat. Number: 3163, Helena-Laboratories). Serum total protein (Abott 7D73-20) and albumin (Abott 7D53-20) were measured by using Auto Technicon DAX 72 autoanalyzer and its commercial kits (Miles Inc., Tarrytown, NY, USA). Globulin concentrations were calculated by formulating (Total Protein Concentration = Albumin Concentration + Globulin Concentrations).

STATISTICAL ANALYSIS

The statistical analyses to determine the differences between protein levels and laying and non-laying periods were performed by the statistical software program SPSS running « Repeated Measures Analysis of Variance » [24]. The results are considered as significant when p value were less than 0.05. The production parameters were also analyzed by using Minitab Statistical Packet Program SPSS 10.0.1 by Duncan’s multiple range test and student-t test.

Results

The directly measured and calculated concentrations of total serum proteins, albumin, total globulins and α₁-, α₂-, β- and γ-globulins were given in tables II and III. No significant difference was found between the concentrations of serum proteins, albumin and globulins according to the laying status except for α₁-globulins. Indeed, the α₁-globulin concentrations were significantly lower in laying ostriches (p<0.05).
When animals were fed with a high-protein diet, proteinemia, albumin concentrations and the ratio albumin to globulins were significantly increased (group I : low-protein diet vs. group II : high-protein diet : p<0.01). Moreover, α1-globulin (p<0.05) and particularly α2-globulin (p<0.01) concentrations were higher in group II. Only for γ-globulin concentrations, a significant interaction between diet and the laying period was noticed (p<0.05).

Average egg production and egg production characteristics of groups and, the effects of protein levels on some production performances of breeding ostriches are shown in Tables IV and V. Significant differences (p<0.01) were found in egg fertility ratio, hatch of fertile and hatchability of total eggs between dietary protein levels, whereas the dietary protein level had no effect on the egg production and egg weight (Table IV). These parameters were higher in birds fed with low protein diets (20% crude protein) than in birds received protein-enriched diets (23% crude protein). The food consumption per egg was approximately 0.5 kg lower in birds fed with diets containing 20% crude protein than in birds received the high protein diets (23% crude protein), although the difference was not statistically significant (Table V).
Discussion

Scientific information about the nutrient requirements and serum protein fractions of ostriches during different growth and production stages are scarce. Changes in the components of the protein fractions and changes in the relative amounts of each fraction in serum can be observed in different physiological status and in many diseases and nutrition-related problems [26].

In this study, only $\alpha_1$-globulin concentrations were significantly modified ($p<0.05$) during the laying period whereas serum proteinemia, albumin and other globulin concentrations seemed to be unaffected. The $\alpha_1$-globulin concentrations were found lower in laying periods (sections A and C) than in non-laying periods (sections B and D). Although the $\alpha_1$-globulin concentrations were generally lower in birds than $\alpha_2$-globulin concentrations and there was no functional difference between these 2 fractions [16], $\alpha_2$-globulin fraction appeared similar in laying and non-laying ostriches. For $\beta$-globulins, no notable difference was observed in laying birds. However in some psittacine species, a monoclonal spike may be present in the $\beta$-globulin fraction during periods of egg laying. The modification of the $\beta$-globulin electrophoretic pattern was due to increases of oestrogen-induced yolk protein precursors, vitellogenin and lipoproteins during the transfer of egg proteins to ovary [3]. The $\gamma$-globulin concentrations seemed to be lower in non-laying birds even if the differences between the 2 sections of the same group were not statistically significant (section A vs. section B in group I, section C vs. section D in group II : not significant), but this variation became more evident when animals were fed with a high-protein diet. Deficiencies in $\gamma$-globulins can occur with immunodeficiency states, blood loss, overwhelming infection, protein-losing gastrointestinal diseases and severe inanition. On the other hand, an important hemolysis in birds may induce a restricted spike (usually in $\gamma$-fraction) which is composed by hemoproteins [3]. But in this study, this mechanism is not responsible for the increases of $\gamma$-globulin in laying period. Besides, when birds were fed with a high-protein diet, the $\gamma$-globulin concentrations decreased particularly in non-laying ostriches. Perhaps the mobilization of proteins for egging would require preferentially other serum proteins than $\gamma$-globulins especially if the other serum protein fractions were increased.

On the contrary, total proteinemia, albumin concentrations and $\alpha$- ($\alpha_1$- and $\alpha_2$-) globulin concentrations were significantly elevated when a high-protein diet was used, whatever the laying status of ostriches. Total proteinemia in group I and II were similar to reference values previously reported [11, 18, 20, 25]. But some authors [17, 19] have obtained a lower proteinemia on a comparable ostrich population (in females older than 72 months of age). Albumin concentrations were also comparable to other data [2, 11, 20] even if the percentage of albumin to total serum proteins seemed to be more variable [5]. As far as globulins were concerned, the concentrations recorded here were compatible to values previously reported by POLAT [20] and AGAOGLU [2] in adult ostriches. Percentage of $\alpha$- ($\alpha_1$- and $\alpha_2$-) globulins found higher than the results of POLAT on eighteen months old ostriches [21].

Except for some egg characteristics, the dietary protein contents did not modify the ostrich zootechnical perfor-

**Table IV.** — Average egg production and egg production characteristics according to the groups. Group I : ostriches were fed with 20% crude protein (n = 10) ; Group II : ostriches were fed with 23% crude protein (n = 10). Results are expressed as means ± SEM. ** p < 0.01.

**Table V.** — Effect of dietary protein contents on some production performances of breeding ostriches. Group I : ostriches were fed with 20% crude protein (n = 10) ; Group II : ostriches were fed with 23% crude protein (n = 10). Results are expressed as means ± standard deviations.

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<tr>
<th>Zootechnical parameter</th>
<th>Dietary protein content</th>
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<tbody>
<tr>
<td></td>
<td>20% (group I)</td>
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<tr>
<td>Concentrate food consumption (kg / bird / day)</td>
<td>1.192 ± 0.023</td>
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<tr>
<td>Roughage food consumption (kg / bird / day)</td>
<td>1.471 ± 0.067</td>
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<tr>
<td>Total food consumption (kg / bird / day)</td>
<td>2.604 ± 0.085</td>
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<tr>
<td>Food consumption per egg (kg / bird / day)</td>
<td>4.152 ± 1.041</td>
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mannances in the present study. In previous reports [7, 9], researchers have also demonstrated that dietary high protein levels had no effect on body weight or on the growth performances of ostriches. Nevertheless, GANDINI et al. [13] showed that the minimal amount of dietary protein required for obtaining the highest mean body weight gain in ostriches was 20% otherwise birds fed with dietary low protein diets (14%, 16% and 18%) presented decreased growth performances. Although egg production and egg weight were not modified in birds fed with protein-enriched diets, decreases of fertility and hatchability were noticed. Similar findings on ostrich females were reported by BRAND et al. [8].

As proteinemia and albuminemia will increase when protein intake exceeds the requirement for maintenance and growth, use of a high-protein diet was not necessary for health and growth performances of ostriches and even lead to decrease egg fertility and hatchability. As there is not an obvious advantage to increase protein levels in feed, and because feeding costs contribute approximately 70-80% of the total cost of ostrich production, it was concluded that the crude protein ratio required in food has not to exceed 20%. Such a study will be useful for establishing ostrich nutritional requirement and for correctly interpreting variations in serum proteins in respect of the food used.

References