SERUM PROTEIN CONCENTRATION IN THE AFRICAN FREE-RANGING CHICKENS OF DIFFERENT AGES AND SEXES.

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SUMMARY

Serum total protein, albumin and globulin concentrations were determined in 63 free-ranging chickens aged 1.5 to 10 months old and of different sexes using refractometric (nontemperature-compensated) and spectrophotometric methods. Serum albumin concentrations were determined spectrophotometrically and globulin calculated. The purpose was to determine the trend of serum proteins with age and sex, and at the same time compare the refractometric and spectrophotometric serum total protein values. Significant differences in serum total protein ($P<0.001$), albumin ($P<0.01$) and globulin ($P<0.05$) concentrations were observed between age groups but not between male and female birds. Spectrophotometric values were significantly ($P<0.001$) greater than refractometric values but highly correlated ($r=0.85$).

Keywords: African chicken, free-ranging, serum

INTRODUCTION

The free-ranging African chicken has recently been recognized as of major socioeconomic importance in rural human communities (Cooper, 1995). Many families keep such chickens providing only a shelter at night and no further investment. It is the most frequent source of animal protein for the family, guests and a source of traditional medicine (wizard detecting). It is therefore a target for improvement in health, breeding and production. Little routine clinical laboratory testing has been done in avian species, compared with that in mammalian species (Spano *et al.*, 1988). Studies focused particularly on the free-ranging chicken of Africa, for which there are numerous breeds and types, are missing.

Serum proteins are affected by various health and nutritional problems. Total serum proteins, albumin and globulin levels increase in dehydration resulting

from for example severe vomiting or diarrhoea, or in compensatory hyperglobulinemia due to hypoalbuminemia of chronic blood loss resulting from internal helminthiasis (Saad *et al.*, 1984a, b). Hypoproteinemia occur in nephrotic syndrome causing loss of albumin through the damaged kidneys, in intestinal malabsorption or in malnutrition. Determination of these values makes a valuable aid to diagnosis. Many free-ranging chickens are said to resist a number of clinical diseases including avian leukosis-Marek's disease complex, fowl pox and even newcastle disease (Minga *et al.*, 1989). However, they habour diseases that are subclinically manifested, transmitting them to the well cared and nourished commercial stocks (Minga and Nkini, 1992; Mellau and Cooper, 1994). To recognize subclinical infections serum protein analysis may assist, particularly when the baseline values in healthy animals are known. Unfortunately this information does not exist so far in Africa.

This study was undertaken with a view of determining the level of proteins in free-range chickens, particularly to examine the magnitude of the influence of age and sex as a baseline information from which alterations due to health and nutrition may be detected.

MATERIALS AND METHODS

Experimental design

A total of 63 free-ranging chickens in good physical health were collected from Morogoro market, eastern Tanzania. Blood samples were collected from the brachial vein into clot activated tubes (vacutainers, Becton-Dickinson, England), the clot removed 24 hours later and supernatant centrifuged to obtain serum. Total proteins were determined using both refractometer (SPR T.2, Atago, Japan) a direct reading and spectrophotometer (1201 S Beckman) for the purpose of comparison of these methods. The analysis was done under the same laboratory at approximately the same environmental temperature. In the spectrophotometer serum proteins were determined by the biuret method with a standard protein solution of 60 g/l bovine serum albumin or 60 g/l egg albumen. Serum albumin was determined by bromocresol green (BCG) method under the principle that, serum albumin reacts specifically with BCG to form a stable yellowish - green colour complex with an absorption maximum at 628 nm, whose colour intensity is proportional to the albumin concentration in the sample.

The absorbance of this complex was measured in spectronic 21

Table 1: Mean ± standard deviations of total serum protein by refractometer and spectrophotometer, albumin and globulin concentration in chickens aged 1.5 (n=7), 3 (n=9), 4 (n=9), 5 (n=16), 6 (n=18) and 10 (n=4) months.

Age	Refractometric	Spectrophotometric	Albumin	Globulin
1.5	20.3±3.4	30.2±7.5	16.9±3.1	13.3±6.8
3	36.2±6.9	54.5±20.9	26.9±4.7	27.7±19.6
4	40.1±8.3	57.5±11.8	31.5±8.6	26.0±17.3
5	37.3±9.2	50.1±7.8	28.5±8.5	21.7±10.7
6	39.6±9.1	52.9±18.3	24.8±8.0	28.1±13.8
10	45.3±13.1	63.6±19.3	30.7±10.9	32.0±21.3

Table 2: The range and mean ± standard deviation of total serum protein (by refractometer and spectrophotometer), albumin and globulin concentration in female and male chickens

Protein (g/l)	Female (n = 40)		Male (n = 23)	
Refractometer	15-60.0	36.6±11.0	20.0-58.0	37.2±9.2
Spectrophotometer	22-117.6	52.9±19.0	26.4-82.3	48.4±10.8
Albumin	10-46.7	26.25±9.0	12.0-48.7	26.9±7.5
Globulin	2.5-75.6	26.6±16.5	-1.7-56.3	21.6±11.1

spectrophotometer at 628 nm wavelength together with an albumin standard of 80 g/l and the albumin concentration calculated. The serum globulin concentrations were calculated as the difference of total serum protein and albumin concentration.

Statistical procedures

Dependence of the serum protein concentrations on age was tested for significance with the linear regression analysis. The difference in protein concentrations between sex was tested with the student *t*-test. Differences in the results of the refractometric and the

spectrophotometric methods were tested for significance with the student's *t*-test for paired samples and the correlation investigated using the linear correlation coefficient. Significance was assumed at $P<0.05$.

RESULTS

There was an increase in serum total protein, albumin and globulin concentrations with age (Table 1). The mean values for serum total protein, albumin and globulin concentrations showed no significant differences between male and female chickens (Table 2).

In the comparison of spectrophotometer and refractometer measurements it was observed that the former instrument values were significantly ($P<0.001$) higher than those of the refractometer (Table 1,2). This indicates that comparisons between laboratories are subject to a very large instrument error depending on the method used.

DISCUSSION

Total serum protein concentration observed in chickens of this investigation appear to agree with values observed elsewhere. Kaneko (1989) has reported mean total serum protein concentration of 56.0 g/l, 25.0 g/l serum albumin 31.0 g/l serum globulin concentration. The mean values in this investigation were 51.2±14.9 g/l for total protein (by spectrophotometer), 26.5±8.25 g/l for albumin and 24.0±13.8 g/l for globulin. Within limitation of experimental errors these values do not differ much with those reported by Kaneko (1989). However, there is a large deviation from the mean in all cases indicating a big variation within individuals. The difference can either be attributed to age, breed, and health differences, but also as a result of the method of determination (Lumeij and Maclean, 1996).

Chickens used in this experiment were young (1.5 - 10 months). This have contributed to the low values as it is known in mammals that protein concentration is lowest at birth and increases with age (Mbassa and Poulsen, 1991).

This was observed in this study to be the trend for chickens, where age was found to have a large influence on the concentration of serum proteins. Studies by Dimopoullos and Cunningham (1956) have shown that in all animals there is a general increase in total serum protein, a decrease in serum albumin and an increase in serum globulin concentrations with age. In this study there was a general increase in total serum protein concentrations with age. A

similar trend was observed for albumin and globulin concentrations. This is in agreement with observation by Dimopoullos and Cunningham (1956). The chickens were collected from different batches. If they were of the same batch the results would have been more uniform.

The influence of sex was not apparent in these young birds. The concentration of total serum total protein, albumin and globulin were not significantly different between male and female chickens. The influence of sex is normally due to hormones such as androgens, corticosteroids, growth hormone and thyroxine (How *et al.*, 1979; Peschle, 1980).

According to Lucas and Jamoroz (1961) the breeds and conditions under which the domestic chicken live are so varied that haematological values of a single breed may not represent them all. Variations from normal in this study may have been attributed by the fact that chickens were reared in tropical environment which is different from the environment where the previous authors did their studies. The breed used in this study was adapted to Africa and reared as free-range. This system subjects the chicken to a variety of factors, including feed scarcity for which it has to fetch the whole day thus spending more energy.

The only source of proteins is from insects, little frogs, lizards, worms and sometimes little snakes. These are very scarce in dry environments. Furthermore the birds were temporarily confined to cages, during which they are provided with maize bran carbohydrates, reducing further the dietary proteins.

Specific and non-specific changes in diseases are also known to cause serum protein changes either in one or more of the serum protein fractions (Dimopoullos, 1961). Since the health of the chickens was assessed visually, some chickens might be malnourished or habour subclinical infections affecting the protein fractions differently resulting in different increasing and decreasing trends that seemed to cause fluctuations. One fact must be admitted here is that these birds were collected from the market brought for sale, thus they may represent the rejected population because some farmers only sell the poorest animal in the flock. The confinement in cages, while awaiting buyers, causes both stress and further malnutrition. However, it can be stated that the information indicates some characteristic of the original population in rural areas since due to fast sales the maximum time a hen stays in market cages was 1-2

days. Furthermore farmers who are pressed with problems that require cash are likely to sell even their prime animals like large males and female in order to obtain more money.

The use of the refractometer and spectrophotometer to measure serum proteins has revealed a very large disparity between the values of the same animal. The differences were highly significant ($P<0.001$) but highly correlated ($r=0.85$) showing that both methods are reliable for the determination of serum total protein concentration in chicken.

Spectrophotometry gave larger means (average 51.2±14.9 g/l) which were close to those established by Kaneko (1989). Refractometry resulted into lower values (average 36.8±8.5 g/l). This suggests that the former method is more accurate than the latter.

CONCLUSION

It can be concluded that there is variation in serum proteins with age. Results of avian serum proteins established by any of the reliable methods should always be compared with reference values obtained by the same method. Routine analysis of serum proteins can be a very useful tool in detection of nutritional level and monitoring of health in rural chickens.

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