Protein Profile of Buffaloes of Different Ages

Raquel Teresinha França¹, Márcio Machado Costa¹, Danieli Brolo Martins¹, Marciélen Pagnoncelli¹, Marta Lizandra Leal², Cinthia Melazzo Mazzanti¹, Heloisa Einloft Palma¹ Conrado Pfüller Kunert², Francine Chimelo Paim¹ & Sonia Terezinha dos Anjos Lopes¹

ABSTRACT

Background: In Brazil, the official buffalo herd has about three million animals, distributed over many states of the country. Many times, abnormalities found in proteinogram are not related to illness conditions, but with physiological organic and individuals conditions, that are relatively constant. The interpretation of biochemical constituents depends on the disponibility of knowledge of the variation that exists among different species of animals. Factors like age, stage of development, breed, hormones, pregnancy, nutrition, stress and loss of fluid are directly related to changes in proteinogram. Proteinogram is an important auxiliary exam, helpfull to clinical biochemistry, and represents one of the most reliable methods for identification of blood proteins. The aim of this research was to evaluate the proteinogram of buffaloes of different ages reared in extensive system in Rio Grande do Sul State, Brazil.

Materials, Methods & Results: Forty-five buffaloes were separated into three groups: group 1 (n = 15), six-months old animals; group 2 (n = 16), twelve-month old animals and group 3 (n = 14), twenty-four months old animals. The total serum protein were determined by the biuret method, using commercial reagent Labtest® and the analysis were realized in semi-automatic spectrophotometer BioPlus-Bio-2000®, according to fabricant instructions. The fractionation of serum proteins were determined by electrophoresis on cellulose acetate strips celugell Labex®. The samples were layered on the strip and, after the closing of the horizontal plane, it was applied a constant voltage of 220 volts for 15 min. Significant differences were verified among groups in the following protein fractions: total serum protein, betaglobulin, gammaglobulin and albumin:globulin ratio. In correlation tests, there was a positive correlation between total proteins and gamaglobulins, albumin:globulin ratio and albumin and negative correlation between the albumin:globulin ratio and gamaglobulins.

Discussion: With age, there is a significant increase in total serum protein, as can be seen in our work. Thus, it is possible to justify the increase in total serum proteins with the increase of gamaglobulins. In a study realized, who evaluated pregnant sheeps, observed that the total serum protein concentration diminishes with the proximity of the parturition, indicating the importance in evaluating the total serum proteins in different moments of life in different species. With age, the total serum protein tend to increase, resulting in decrease in albumin, with progressive increase in globulins. However, in this study, it was not noticed any significant statistic difference in albumin among the groups. Proteins that compose the alfaglobulin, are acute phase proteins and their concentrations increase rapidly in response to antigenic and traumatic stimuli in bovine. However, in our study, no statistic difference was observed. The proteins that compose the betaglobulin fraction are: lipoprotein, transferrin, ferritin, hemopexin, complement C3, protein C-reactive, complement C4, plasminogen and fibrinogen. Some authors observed a decrease in fraction beta at the end of gestation in sheeps, other did not observed any variation in this fraction in neonatal bovine. The proteins that compose the gamaglobulin fraction are immunoglobulins IgG, IgA, IgE and IgM. In general, due to the age, there is an increase in gamaglobulin concentration, because of a higher exposure of organism to pathogens including bacteria, viruses, fungi and parasites. Similar data were observed in this study. The results indicate that there are some variations according to the analyzed age. Then, some abnormalities found in the protein profile may be due to physiological variations.

Keywords: electrophoresis, proteinogram, influence of age, ruminant.
INTRODUCTION

The buffalo is a ruminant from Asia and, at the present time, is in Europe, Australia, Africa and America. Buffalo is a multiple purpose animal because its capacity to produce meat, milk and work; it also has high resistance to illness, and ability to adapt to different climatic conditions [5]. The Brazilian herd is significantly increasing. Because all these factors, it is extremely important to know the physiological parameters and, thus, be able to distinguish them to the pathologic conditions.

The interpretation of biochemical constituents depends on the disponibility of knowledge of the variation that exists among different species of animals [1]. Proteinogram is an important auxiliary exam, helpfull to clinical biochemistry, and represents one of the most reliable methods for identification of blood proteins [3]. The electrophoretic techniques most commonly used in veterinary medicine are made with cellulose acetate strips, agarose films and polyacrylamide gel [4,6].

Nowadays, bubalinoculture is an activity in expansion in American Continent [2]. Thus, it is necessary to know the protein profile of healthy buffaloes of different ages, for posterior comparison with pathologic processes that these animals may develop during their lives. As the studies about proteinogram of buffaloes reared in extensive systems in Brazil are scarce, this study aims to evaluate the influence of age on proteinogram of healthy buffaloes with six, twelve and twenty four months old.

MATERIALS AND METHODS

We used forty-five undefined breed buffaloes, reared in an extensive system in the central area of Rio Grande do Sul State, Brazil. Clinical exams were performed previously the blood collection to evidence the healthy of the animals. The buffaloes were divided into three groups, according to age, with animals of both sexes: group 1 (G1) animals with six-months old (n = 15); group 2 (G2) animals with twelve-months (n = 16) and group 3 (G3) animals with twenty-four-months old (n = 14).

We collected 10mL of blood from the jugular vein of each animal, with vacuum tube without anticoagulant to determinate the total serum protein and electrophoresis. The samples were stored in a thermic box and sent immediately to laboratory. To promote the separation of the serum, the blood was centrifuged at 1509 g for 10 min, and then, stored in plastic microtubes and frozen at -20°C for posterior analysis.

The total serum protein were determined by the biuret method, using commercial reagent Labtest® and the analysis were realized in semi automatic spectrophotometer BioPlus-Bio-2000®, according to fabricant instructions. The fractionation of serum proteins were determined by electrophoresis on cellulose acetate strips celugell Labex®. The samples were layered on the strip and, after the closing of the horizontal plane, it was applied a constant voltage of 220 volts for 15 min.

After the electrophoretic running, the strips were colored with Panceau colorant for 15 min. The discoloration of the strips was done with an acetic acid 5% discolorant, until the samples became completely clear. After discoloration, the strips were divided into methanol for 30 s, for the fixation of the material. At last, the strips were divided into the destain solution for 1 min Strips were dried at 60°C for 15 min [9]. The reading of the bands were made by computer program Denscan® by scanning the strip.

Because its normal distribution (Kolmogorov Smirnov test), data were submitted to analysis of avariance (ANOVA), followed by media comparison by Tukey test. Also, in some variables, the Pearson’s correlation test was done, to check the linear relation among them. Data were considered significativelly different, with a probability (P) minor than 5%.

RESULTS

In our study, the serum proteins electrophoresis run on cellulose acetate strips resulted in the fraction of four distinct bands: albumin, alfaglobulin, betaglobulin and gamaglobulin (Figure 1). The results found in
serum proteinogram of buffaloes from different ages are presented on Table 1.

A statistic difference \( (P < 0.05) \) was observed in total serum proteins, betaglobulin, gamaglobulin and albumin:globulin ration. Highest values of total serum protein were observed in animals from G3, in relation to G1 and G2. The betaglobulin fraction presented higher values in G1 than G2 and G3. Gamaglobulin showed lower values in G1 than G2 and G3. Albumin:globulin ration showed highest values in G1 than G2 and G3. In correlation tests, there was a positive correlation \( (P < 0.01) \) between total proteins and gamaglobulins \( (r = 0.45) \) (Figure 2A), followed by positive correlation \( (P < 0.01) \) between the albumin:globulin ratio and albumin \( (r = 0.83) \) (Figure 2B) and negative correlation \( (P < 0.01) \) between the albumin:globulin ratio and gamaglobulins \( (r = -0.86) \) (Figure 2C).

### Table 1. Influence of age in total serum protein, albumin, alfaglobulin, betaglobulin, gamaglobulin and albumin:globulin ratio.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>G1 (n=15)</th>
<th>G2 (n=16)</th>
<th>G3 (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>2.76±0.36( ^a )</td>
<td>2.51±0.25( ^a )</td>
<td>2.65±0.32( ^a )</td>
</tr>
<tr>
<td>Alfaglobulin</td>
<td>1.21±0.11( ^a )</td>
<td>1.29±0.14( ^a )</td>
<td>1.21±0.12( ^a )</td>
</tr>
<tr>
<td>Betaglobulin</td>
<td>0.71±0.11( ^a )</td>
<td>0.56±0.09( ^a )</td>
<td>0.66±0.10( ^a )</td>
</tr>
<tr>
<td>Gamaglobulin</td>
<td>1.27±0.29( ^a )</td>
<td>1.79±0.33( ^b )</td>
<td>1.86±0.28( ^b )</td>
</tr>
<tr>
<td>Total serum protein</td>
<td>5.93±0.41( ^a )</td>
<td>5.99±0.51( ^a )</td>
<td>6.34±0.27( ^b )</td>
</tr>
<tr>
<td>Albumin:globulin ratio</td>
<td>0.87±0.16( ^a )</td>
<td>0.69±0.12( ^b )</td>
<td>0.71±0.13( ^b )</td>
</tr>
</tbody>
</table>

Different letters in the same line indicate statistic difference among the groups, with probability minor than 5% (ANOVA, Tukey test). SD= Standard deviations.

![Figure 2. Correlation test from buffaloes of different ages. (A) Total serum protein and gamaglobulin, (B) albumin:globulin ratio and albumin and (C) albumin:globulin ratio and gamaglobulin.](image)

### DISCUSSION

Age is an important factor when we analyze serum protein. Most of the values observed in young animals are different from adult, once these parameters change with age advance [10]. The serum proteinogram of buffaloes using cellulose acetate strips and
observed the same fractionament composed by four types of proteins, albumin, alfaglobulin, betaglobulin and gamaglobulin [11]. Some species, like humans and guinea pigs, albumina predominates over the other globulins. In equine and bovine, the albumin:globulin ratio is almost the same, or globulins tend to predominate [1]. However, in our study was possible to observe that, in buffaloes, globulins predominate over albumin. This fact was confirmed by the high negative correlation between A:G ratio and globulins. The lowest the A:G ratio values, the highest the serum levels of globulins ($r = 0.82$).

With age, there is a significant increase in total serum protein, as can be seen in our work [6]. In the animals from G3, it could be seen an increase in total serum protein concentration, with statistic difference when compared to animals from G1 and G2. In relation to globulins, buffaloes with 2 years old showed higher values of gamaglobulin when compared to animals with six months old, and similar behavior among animals from G3 when compared to G2 ($P < 0.01$).

Thus, it is possible to justify the increase in total serum proteins with the increase of gamaglobulin ($r = 0.45$). In neonatal calves, the variation observed in some fractions were not enough to modify the concentration of proteins [10]. However, in a study, who evaluated pregnant sheeps, observed that the total serum protein concentration diminishes with the proximity of the parturition, indicating the importance in evaluating the total serum proteins in different moments of life in different species [1].

With age, the total serum protein tend to increase, resulting in decrease in albumin, with progressive increase in globulins [6]. However, in this study, it was not noticed any significant statistic difference in albumin among the groups. Even so, in the animals from this study were observed a tendency in decreasing the concentration of albumin in G2. However, there was a positive correlation between albumin:globulin ratio and albumin, demonstrating that the decrease in albumin is also related to decrease in albumin:globulin ratio ($r = 0.83$).

The proteins that compose the alfaglobulin fraction are: alfa 1-glycoprotein acid, serum amyloid A, haptoglobin, ceruloplasmin and alfa 2-macroglobulin [6]. These are acute phase proteins and their concentrations increase rapidly in response to antigenic stimuli in bovine [8]. There was an increase of this fraction in response to antigenic stimulation in buffaloes, similar to that observed in bovine [11]. However, in our study, no statistic difference was observed among the groups evaluated, indicating no influence of age on healthy buffaloes.

The proteins that compose the betaglobulin fraction are: lipoprotein, transferrin, ferritin, hemopexin, complement C3, protein C-reative, complement C4, plasminogen and fibrinogen [6]. Small amounts of immunoglobulin can also be present in this fraction [7]. Decrease in fraction beta at the end of gestation in sheeps [1]. Not observed any variation in this fraction in neonatal bovine [10]. Was observed a variation in betaglobulin fraction in holstein calves evaluated from birth until 30 days of life [7]. In our research, values a little bit higher of betaglobulin were detected in animals with six months old and one with 2 years old. This fact can be attributed to an increase in antibodies production, because of passive immunity, that begins after 3 months old and an increase of exposure to ambiental agents in older animals.

The proteins that compose the gamaglobulin fraction are immunoglobulins IgG, IgA, IgE and IgM. We noticed an increase of gamaglobulin in animals from G2 and G3 when compared to G1. In foals from birth until twelve-months old there is gradual increase in immunoglobulins [6]. Similar modifications were observed in animals from our study, with an increase in gamaglobulin fraction in G2 and later stabilization of this fraction in G3. In general, due to the age, there is an increase in gamaglobulin concentration, because of a higher exposure of organism to pathogens including bacteria, viruses, fungi and parasites [6].

CONCLUSION

The results obtained in our study allowed us to affirm that, with exception of albumin fraction and alfaglobulin, all the other components of proteinogram of buffaloes are influenced according to the age of the animal.

SOURCES AND MANUFACTURERS

1 Labtest, Belo Horizonte, MG, Brazil.
2 Bioplus, Barueri, SP, Brazil.
3 Labex, Aparecida de Goiânia, GO, Brazil.

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.
REFERENCES


