COMPARISON OF HOMOLOGOUS AND HETEROLOGOUS THROMBOPLASTIN IN AVIAN PROTHROMBIN TIME IN TWO DIFFERENT TEMPERATURES


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ABSTRACT
Avian hemostasis profile represents a challenge in the laboratorial routine due to the lack of specific tests and homologous commercial reagents available to diagnose avian coagulopathies. Differences between avian and mammalian coagulation processes, especially in protein function and structure, result in analyses divergences. The aim of this study was to compare mammalian and avian thromboplastin as Prothrombin time (PT) in two different temperatures to evaluate avian hemostasis. Twenty-seven citrated whole blood samples were obtained from 28 to 33 days-old Cobb male chickens. PTs were performed at 37 and 40°C using a commercial rabbit thromboplastin (Helena Laboratories®) and an avian thromboplastin made from an extract of chicken brain as activator. Results showed that homologous thromboplastin presented a shorter TP at both temperatures (p<0.0001). Temperatures did not influence the results but homologous thromboplastin presented a tendency to a shorter PT at 40°C (p=0.06). These differences may imply in different sensitivity or specificities in test results when using prothrombin time as a diagnostic tool in avian and even other exotic or wild species. The authors highlight the importance of the choice of reagents and protocols in comparative hemostasis studies.

Introduction
Avian hemostasis is a challenge in routine laboratory currently. Direct application of diagnostic methods and commercial reagents are the most difficulties (Harr 2010). Laboratory methods that are used for mammalian diagnosis routine, can’t always be applied in avian species. Characteristics of mammal and avian coagulation are different, especially in function and structure involved in this process, establishing pattern divergences in laboratory analysis in birds. Different results found in literature suggest that more specific methodologies in laboratorial profile are needed and also more knowledge about coagulation physiology of this animals. Available tests have a limitation to understand the coagulation in vivo (Harr 2010; Oldenburg et al. 2008; Powers 2000).

Tissue factor (TF) is essential to vascular and hemostasis development. The TF in vivo is exposed in perivascular cells, active the coagulation cascade and promote fibrin formation. Factor VII (FVII) bind to tissue factor and form an enzymatic complex that activates factors X and IX. Platelets activated also participate of thrombin generation. However the level of thrombin generation is limited by inhibition of tissue factor pathway inhibitor (TFPI). (Brooks et al. 2011).

Many physiologic functions are correlated or depend on a constant regulation of appropriate temperature to animal, considered the thermoneutral temperature. The organism has mechanisms capable of temperature control, keeping inside of little variation to maintain the body homeostasis optimizing your biochemical reactions. Avian body temperature is 41.1°C and your balance is based on two variables, one related to reactions to high temperature, especially ambient temperature, and other is related to reduction of body temperature (Macari et al. 2004).

Materials and methods
Twenty seven citrated whole blood samples were obtained from 28 to 33 days-old Cobb male chickens. PTs were performed at 37 and 40°C using a commercial rabbit thromboplastin (Helena Laboratories®) and an avian thromboplastin made from an extract of chicken brain as activator.

Avian thromboplastin was extracted from chicken brain with acetone solution. A solution for work was prepared using supernatant of 0.1g of brain powder with 5ml saline 0.85% (Denson, 1976).

The results were submitted to t test as statistical analysis for paired samples. The significance level of 5% was used.

Results
Results showed that homologous thromboplastin presented a shorter TP at both temperatures (p<0.0001). Temperatures did not influence the results but homologous thromboplastin presented a tendency to a shorter PT at 40°C (p=0.06).

Discussion and Conclusions
Protein specificity is evident and according to our study and others (Kase et al. 1980; Tahira et al. 1977), activation of hemostasis by thromboplastin extracted of the same class, or homologous, result in a shorter time to clot formation.

Maintaining constant body temperature is also a key mechanism for all animals homeotermic, promoting optimal conditions for cellular properties and biochemical and enzymatic reactions. The temperature standard used to hemostatic test in laboratory routine is from 37 °C to mammals and which is also expanded to other animals. However, in the case of birds, this temperature is beyond the limit of variation of thermoneutral homeostasis which is close to 41.1 °C (Macari et al. 2004).

The thermoneutral temperature also seems to influence in clot formation, but more studies are needed.

The authors highlight the importance of the choice of reagents and protocols in comparative hemostasis studies.

Literature cited


