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Original article

BLOOD FIBRINOGEN CONCENTRATIONS IN NEW ZEALAND WHITE RABBITS DURING THE FIRST YEAR OF LIFE

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Summary

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The aim of this study was to examine the reference range of blood plasma fibrinogen, which belongs to the group of moderate acute phase protein in rabbits. The experiment followed the dynamics of blood plasma fibrinogen (Fb) during the first year of life in 12 New Zealand White rabbits, 6 males and 6 females, starting at 1 month of age until the 7th month. It was shown that fibrinogen concentrations increased with the age. There was a statistically significant difference in Fb concentrations in rabbits at 3 ($P<0.05$), 6 ($P<0.01$) and 7 months of age ($P<0.001$) compared to levels obtained at 1 month of age in both genders. At ages of 1, 2 and 3 months, fibrinogen values were higher in male rabbits while in 7-month-old rabbits, plasma fibrinogen levels in females were significantly higher than those in male rabbits ($P<0.01$).

Key words: acute phase proteins, fibrinogen, rabbits

INTRODUCTION

The acute phase response (APR) refers to a nonspecific and complex reaction of an animal that occurs shortly after any tissue injury (Kushner *et al.*, 2006). During this response, there is an increased production and release of certain proteins known as acute phase proteins (APPs), which can be produced by hepatocytes and peripheral tissues (Schmidt & Eckersall, 2015). APPs production and secretion by the liver, mainly of glycoproteins, is one of mechanisms involved in the response to mediators produced by leukocytes and macrophages during episodes of infection or inflammation (Conner *et al.*, 1988). The maximum serum concentrations of APPs are typically attained within 24 to 48 h after the challenge (Jain *et al*., 2011).

One classification divides APP into ”positive” and “negative”. Plasma proteins, whose levels increase in response to challenge are in the group of positive,

while those exhibiting a decrease – in the group of negative APPs. The positive APPs are glycoproteins synthesised into the bloodstream and include haptoglobin, C-reactive protein, serum amyloid A, ceruloplasmin, fibrinogen and alpha 1-acid glycoprotein (Murata *et al*., 2004). The negative APPs comprise albumin – the most abundant constitutive plasma protein, and transferrin.

Kushner *et al*. (2006) classified positive APPs into 3 groups: 1) APPs whose concentration increase by 50% (ceruloplasmin and C_3 complement); 2) APP exhibiting 2- to 3-fold increase (haptoglobin, fibrinogen and α-albumins with antiprotease activity); and 3) proteins, increasing extremely rapidly up to 1000 times (C-reactive protein and serum amyloid A). The changes in plasma APPs concentrations are often used for diagnostic purposes (Marshall, 1994; Andonova, 2002).

In most species, the fibrinogen (Fb) belongs to the group of positive and moderate APP. It is involved in haemostasis, providing a substrate for fibrin formation, and tissue repair providing a matrix for the migration of inflammatory related cells (Thomas, 2000). Fb specifically binds to CD 11/CD 18 integrins on the cell surface of migrated phagocytes, thereby triggering a cascade of intracellular signals that lead to enhancement of degranulation, phagocytosis, antibody-dependent cellular cytotoxicity and delay of apoptosis (Rubel *et al*., 2001).

In order to assess the usefulness of Fb as a member of the group of moderate APPs and as a measure of the acute phase response in rabbits, the reference concentration range of this marker should be determined in male and female New Zealand White rabbits during the first year of their life. In previous experiments of ours we have established that fibrinogen increased 2 or 3 times after experimental infection with *E. coli* and *St. aureus.* (Mircheva *et al*., 2009; Dishlyanova *et al*., 2013). Therefore, we belelive that Fb could serve as a good indicator for the incidence of infection or inflammation. Moreover, the methods used for its determination are very inexpensive (the nephelometric method of Podmore; heat precipitation method), as compared to the determination of the major APPs SAA and CRP for rabbits, which are species-specific and very costly.

To the best of our knowledge there is no available information about the concentration of blood plasma fibrinogen in healthy male and female New Zealand White rabbits at different ages, except the data of Dishlyanova *et al*. (2014) in male and female rabbits at age of 1, 2 and 3 months. According to some directives of the Society of Clinical Pathology for scientific purposes the normal ranges of fibrinogen in rabbits should be preferably known for comparison with the values accompanying disease states with inflammation, including infection.

Therefore the aim of this study was to study the age-related and gender-related changes in plasma concentration of fibrinogen in rabbits during the first year of their life.

MATERIALS AND METHODS

The experimental procedure was approved by the Ethic Committee at the Faculty of Veterinary Medicine. The experiments were carried out on 12 New Zealand white rabbits divided in 2 groups of 6 rabbits in each: I^{st} group – 6 male and II^{nd} group – 6 females, at the age of 1 month. All animals were born from healthy doe rabbits and kept in the rabbitry. They were fed

pelleted feed according to their age and had free access to tap water.

Blood samples for analysis of Fb were drawn with heparinised sterile tubes from *v. auricularis caudalis* from all rabbits at the age of 1, 2, 3, 6 and 7 months and immediately centrifuged (1,500 min^{-1}, 15 min, 4 °C) to obtain plasma.

Plasma Fb was determined immediately by the nephelometric method of Podmore with 10% Na_2SO_4 at a wavelength of 570 nm (Todorov, 1972).

The statistical analysis of the data was performed using SPSS 16.0 for Windows (SPSS Inc.). The concentration of Fb in male and female rabbits at different ages were compared with the concentrations at 1 month of age were assessed by paired t-test. The differences between genders were evaluated by the Student t-test. All data were expressed as mean ± standard deviation (SD) and the differences were considered significant when $P<0.05$.

RESULTS

The time course of plasma Fb concentration is presented on Fig. 1. In both genders, plasma Fb values at 1 month of age were similar. Fb value in male rabbits at month 2 was 1.23±0.26 g/L, at month 3 and 6 were 1.54±0.39; 1.62±0.58 g/L respectively and by the end of the experiment (age 7 months) – 1.66±0.39 g/L.

In female rabbits the mean Fb concentration at the first measurement was 0.46±0.13 g/L. At the age of 2 months, the Fb in female rabbits was 1.08±0.30 g/L and was further increased at month 3 (1.45±0.41 g/L). At the age of 6 and 7 months, the concentration of Fb in female rabbits were 1.68±0.23 and 2.34±0.65 g/L respectively.

Statistically significant difference was established at the age of 2 months ($P<0.05$) in male rabbits compared to 1 month of age. Substantial differences were present at 3 months of age ($P<0.01$) in both genders compared to the baseline measurement at month 1. The concentrations of Fb at the age of 6 and 7 months were statistically significantly ($P<0.001$) different compared to 1 month of age.

Statistically significant difference in Fb levels was established between genders at age of 7 month ($P<0.01$).

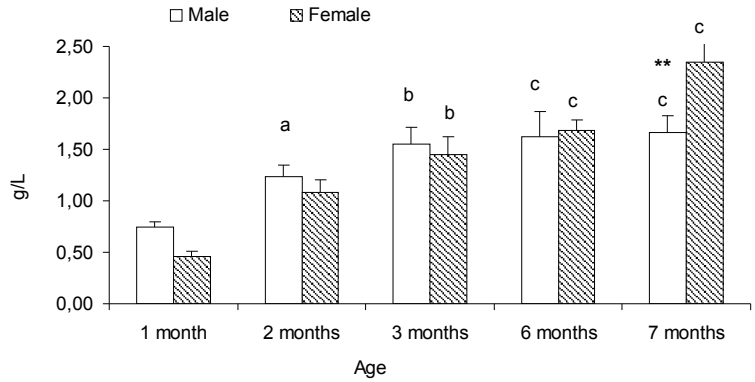

Fig. 1. Blood plasma fibrinogen concentrations in male and female New Zealand White rabbits at different ages. Data are presented as mean ± SD (n=6). [a]$P<0.05$, [b]$P<0.01$, [c]$P<0.001$ significant difference vs one month of age; ** $P<0.01$ significant difference between the genders.

DISCUSSION

The APPs are a group of blood proteins that contribute to restoring homeostasis and limiting microbial growth in an antibody-independent manner in animals with infection, inflammation, surgical trauma or stress (Murata *et al*., 2004).

Fb and haptoglobin are affirmed to be "major" and "positive" APPs in rabbits (Petersen *et al*., 2004), but the data about Fb indicating infection in rabbits are few (Kimura *et al*., 1994; Mircheva *et al*., 2009; Hana *et al*., 2011; Dishlyanova *et al*., 2013). Data about reference ranges of this parameter in the available literature are limited.

In several studies, a positive relationship was reported between the increase of the another acute phase protein: haptoglobin and aging in different animals species: pigs (Moya *et al*., 2007); mice (Ding & Kopchik, 2011); rats (Ciftci *et al*., 2012).

The study of Dishlyanova *et al*. (2014) provided proof for a positive relationship between blood Fb levels and aging in rabbits. The reported data, however, concerned only bunnies in the earliest period of their life – until 3 months of age.

The results from the present study provided the necessary and lacking information about blood plasma Fb concentrations in rabbits from both sexes according to their age until the 7th month of life.

Fb is used in cattle and sheep as a reliable indicator of the presence of inflammation, bacterial infection or surgical trauma (Pfeffer *et al*., 1993; Hirvonen *et al*., 1996; Cheryk *et al*., 1998; Hirvonen & Pyorala, 1998). In horses, Fb has been used for the diagnosis of infections (Falcon *et al*., 1985; Johnstone & Crane, 1986; Morris *et al*., 1988; Held *et al*., 1990). The increase of Fb associated with surgical castration of bulls seems to be independent of plasma cortisol concentrations (Fisher *et al*., 1997). Hyperfibrinogenaemia has been induced by intramuscular injection of Freund's complete adjuvant (Mills *et al*., 1998). The increased Fb levels observed in pregnant dogs three weeks following implantation suggest that Fb may be useful for early pregnancy diagnosis in dogs (Concannon *et al*., 1996). Zapryanova *et al*. (2013) reported that the Fb can be useful for diagnosis and follow up of a bacterial infection in dogs. Fb is used as an early marker of acute inflammation of the mammary gland in goats caused by *Staphyloccocus aureus* (Fasulkov *et al*., 2014).

CONCLUSIONS

The results showed that plasma Fb concentration increased with age. There was a statistically significant difference in Fb concentrations in rabbits at 3 ($P<0.05$), 6 ($P<0.01$) and 7 months of age ($P<0.001$) compared to levels obtained at 1 month of age in both genders. During first 6 months of their life fibrinogen concentration in male and female rabbits were similar but there was a statistically significant difference in Fb concentration between genders at 7 months of age ($P<0.01$).

The present data could be useful to the researchers using rabbits as experimental animals as reference values for comparative purposes in the interpretation of the changes of this APP in different inflammatory conditions in rabbits.

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