

## Short Paper

# Determination of serum haptoglobin reference value in clinically healthy Iranian fat-tailed sheep

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## Summary

Blood samples were collected from the jugular vein of 101 clinically healthy Iranian fat-tailed sheep according to their age (<1, 1-3, 3-5 and >5 years) and sex. Serum haptoglobin (Hp) was measured based upon the preservation of the peroxidase activity of haemoglobin, which is directly proportional to the amount of Hp. The reference value for serum Hp of apparently healthy Iranian fat-tailed sheep was 0.05-0.18 g/l. Age and sex had no significant effect on the serum concentration of Hp in examined animals.

**Key words:** Serum haptoglobin, Iranian fat-tailed sheep

## Introduction

Haptoglobin (Hp) is an  $\alpha_2$ -globulin synthesized in the liver (Feldman *et al.*, 2000) and is a major acute-phase protein in numerous species of production and companion animals. In ruminants, the level of circulating Hp is negligible in normal animals but increases over 100-fold with immune stimulation (Conner *et al.*, 1988, 1989). Many studies have indicated the significance of Hp as a clinically useful parameter for the evaluation of the occurrence and severity of inflammatory diseases in sheep (Pfeffer and Rogers, 1989; Skinner and Roberts, 1994), cattle (Hirvonen *et al.*, 1996, 1999; Heegaard *et al.*, 2000; Eckersall *et al.*, 2001; Ohtsuka *et al.*, 2001) and horses (Kent and Goodall, 1991; Mills *et al.*, 1998). The reference value of serum Hp has been determined for cattle, pigs, horses, ponies and camels (Kent and Goodall, 1991; Skinner *et al.*, 1991; Taira *et al.*, 1992; Eurell *et al.*, 1993; Uchida *et al.*, 1993; Salonen *et al.*, 1996; Lipperheide *et al.*, 1997; Nakagawa *et al.*, 1997;

Horadagoda *et al.*, 1999; Nazifi *et al.*, 2006). There is no published report on the reference value of serum Hp in Iranian fat-tailed sheep. The purpose of this study was therefore to determine reference values of serum Hp in clinically healthy Iranian fat-tailed sheep.

## Materials and Methods

Blood samples were collected from the jugular vein of 101 Iranian fat-tailed sheep according to their age (<1, 1-3, 3-5 and >5 years) and sex. The sheep had been reared in Fars province, especially around Kazeroun. All the animals were clinically healthy, dewormed and free from internal and external parasites. Blood samples were collected into plain vacutainers, and the serum was separated after centrifugation for 15 min at  $750 \times g$ . Any haemolysed samples were discarded. The serum samples were stored at  $-20^\circ\text{C}$  until analysed. Hp was determined according to preservation of the peroxidase activity of haemoglobin, which is directly proportional to the amount of Hp.

Serum Hp was measured using a solid phase enzyme-linked immunosorbent assay (ELISA) (Tridelta Development Plc, Co. Wicklow, Ireland). The sensitivity of this test has been determined as 0.0156 mg/ml Hp in serum.

Data were expressed in SI units and analysed by two-way ANOVA using SPSS/PC software, version 11.5 (Norusis, 1993). All values were expressed as mean and standard deviation (SD) and  $p < 0.05$  was determined as statistically significant. The reference range was determined according to mean  $\pm$  2SD.

## Results

The mean and SD of serum Hp of clinically healthy Iranian fat-tailed sheep are presented in Table 1. Age and sex had no significant effect on the serum concentration of Hp in examined animals.

**Table 1: The concentration of serum Hp (g/l) in clinically healthy Iranian fat-tailed sheep (n = 101)**

Sex	Age	Number of sheep	Mean	SD
Male	<1	15	0.113	0.069
	1-3	15	0.118	0.062
	3-5	12	0.092	0.054
	>5	8	0.133	0.100
Female	<1	15	0.113	0.013
	1-3	15	0.122	0.050
	3-5	13	0.106	0.073
	>5	8	0.111	0.108
Total	<1	30	0.113	0.058
	1-3	30	0.120	0.065
	3-5	25	0.099	0.062
	>5	16	0.122	0.104

There was no significant difference between serum Hp of male and female sheep in different age groups ( $P > 0.05$ )

## Discussion

Haptoglobin is a potent acute-phase protein in cattle, having a low constitutive level and exhibiting a high relative increase during acute-phase reaction (Hayes, 1994; Hirvonen *et al.*, 1996; Salonen *et al.*, 1996). The baseline level of Hp is often bordering on the unmeasurable (Makimura and Suzuki, 1982). The concentration of serum Hp in

this study was higher than the value reported for cattle (Makimura and Suzuki, 1982; Skinner *et al.*, 1991; Salonen *et al.*, 1996; Lipperheide *et al.*, 1997; Nakagawa *et al.*, 1997). The reference value for serum Hp of apparently healthy Iranian fat-tailed sheep was 0.05-0.18 g/l. The serum Hp concentration of healthy cows ranges from 0.022 to 0.047 g/l (Salonen *et al.*, 1996). The mean serum Hp value for horses has been reported as  $1.43 \pm 0.68$  g/l (Kent and Goodall, 1991). Eurell *et al.* (1993) reported the reference range for serum Hp concentration in ponies to be 0.25-0.6 g/l. Harvey *et al.* (1984) reported that in foals, serum Hp was generally within the adult normal range at birth. A moderate but significant decrease occurred at 1 week of age. Taira *et al.* (1992) evaluated the effects of age, parturition and inflammation on the Hp in some horses. The highest concentration of serum Hp was observed in horses under 12 months of age, and Hp concentration decreased with aging. The sheep appear to be different in these respects, as sex and age had no significant effect on serum Hp in the present study. Similarly, age and sex had no significant effect on the serum concentration of Hp in clinically healthy Iranian dromedary camels (Nazifi *et al.*, 2006). In summary, serum Hp reference value in Iranian fat-tailed sheep could be used for comparison with abnormal data in inflammatory diseases. Serum Hp is most helpful when interpreted together with herd history, clinical signs and laboratory tests.

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