

## Serum Adenosine Deaminase Activity in Domestic Animals: Reference Values

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**Abstract:** This study was aimed to determine normal serum adenosine deaminase (ADA) activity in domestic animals in both sexes and that were considered healthy on the basis of clinical and laboratory examinations. In this study, 262 non-pregnant animals of both sexes, belonging to 5 species [cattle (n = 50), sheep (n = 65), goat (n = 52), dog (n = 55), and cat (n = 40)] were used. Serum total ADA and ADA1 isoenzyme activities were found to be  $6.34 \pm 0.31$  IU/l and  $6.34 \pm 0.31$  IU/l in cattle,  $7.57 \pm 0.30$  IU/l and  $7.31 \pm 0.47$  IU/l in sheep,  $7.52 \pm 0.36$  IU/l and  $6.44 \pm 0.72$  IU/l in goat,  $2.56 \pm 0.13$  IU/l and  $2.03 \pm 0.30$  IU/l in dogs,  $23.36 \pm 1.59$  IU/l and  $21.71 \pm 2.13$  IU/l in cats, respectively. The serum levels of ADA2 were low in sheep, goats, dogs, and cats, whereas in these species ADA1 was high. Furthermore, there was no serum ADA2 activity in cattle. Additionally, there were no significant differences between genders in ADA activities in all species. As a result, determination of serum normal ADA and its isoenzyme activities in domestic animals will be useful for scientists who want to investigate ADA activity in future in different diseases.

**Key Words:** Adenosine deaminase, reference value, domestic animals

### Evcil Hayvanlarda Serum Adenozin Deaminaz Enzim Aktiviteleri: Referans Değerler

**Özet:** Bu çalışmada klinik ve laboratuvar muayeneleri sonucunda sağlıklı olduğu tespit edilen evcil hayvanlarda serum adenozin deaminaz (ADA) aktivitelerinin normal değerlerinin her iki cinsiyette saptanması amaçlandı. Çalışmada 5 türe ait [sığır (n = 50), koyun (n = 65), keçi (n = 52), köpek (n = 55) ve kedi (n = 40)] her iki cinsiyetten ve gebe olmayan 262 hayvan kullanıldı. Serum total ADA ve ADA1 izoenzim aktiviteleri sırasıyla; sığırlarda  $6,34 \pm 0,31$  IU/l ve  $6,34 \pm 0,31$  IU/l, koyunlarda  $7,57 \pm 0,30$  IU/l ve  $7,31 \pm 0,47$  IU/l, keçilerde  $7,52 \pm 0,36$  IU/l ve  $6,44 \pm 0,72$  IU/l, köpeklerde  $2,56 \pm 0,13$  IU/l ve  $2,03 \pm 0,30$  IU/l, kedilerde  $23,36 \pm 1,59$  IU/l and  $21,71 \pm 2,13$  IU/l olarak belirlendi. Koyun, keçi, köpek ve kedilerde serum ADA2 seviyeleri düşüktü. Halbuki bu türlerde ADA1 yüksekti. Bununla birlikte, sığırlarda serumda ADA2 aktivitesi yoktu. Üstelik bütün türlerde ADA aktivitelerinde cinsiyetle önemli bir farklılık yoktu. Sonuç olarak, evcil hayvanlarda serum ADA ve izoenzim aktivitelerinin normal değerlerinin tespiti, ileride farklı hastalıklarda ADA aktivitesini araştırmak isteyen araştırmacılar için yararlı olabilir.

**Anahtar Sözcükler:** Adenozin deaminaz, referans değer, evcil hayvanlar

### Introduction

Adenosine deaminase (ADA, EC 3.5.4.4) is an enzyme that catalyses hydrolytic deamination of either adenosine or deoxyadenosine to produce inosine and deoxyinosine, respectively (1). Firstly, the presence of enzyme was detected in whole blood and serum by Conway and Cooke

(2). In domestic animals, ADA is present in all organs, although the highest activity has been found in lymphoid tissues (3). In animals, ADA and its isoenzyme activities in the spleen, lymph nodes, and thymus have been found in high levels, and in the brain, adrenal gland, muscles, kidney, and liver in low level (4). It has been detected in

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the cell cytoplasm and nucleus (5). Furthermore, its enzymatic activity has also been reported in blood cells, serum, and plasma (6).

The main biological activity of ADA is to protect lymphocytes from toxic effects of 2-deoxyadenosine, deoxyadenosine triphosphate, and deoxyadenosine diphosphate, which depress immune functions (7). It has also been shown that ADA is related to normal conditions involving lymphocyte-monocyte proliferation (8,9).

Two distinct ADA isoenzymes are known as ADA1 and ADA2 in domestic animals (4). ADA1 is found in all cells, with the highest activity in lymphocytes and monocytes, whereas ADA2 is not ubiquitous, but coexist with ADA1 only in monocytes-macrophages (1,10). Moreover, ADA1 has also been detected in neutrophils with the lowest activity (1). It is suggested that increased ADA1 is derived mainly from injured tissues and cells or lymphocyte and neutrophil, while increased ADA2 may be an indicator of monocyte-macrophage activation or turnover (10,11). On the contrary, deficiency of ADA is associated with inhibition of lymphocyte proliferation and differentiation (12,13). Moreover, it is suggested that decreased ADA activity could be a reflection of cell-mediated immune dysfunction (12-14).

It has been reported that in domestic animals serum ADA activity increases during leukemia (15), infectious peritonitis (16), hepatopathy (15), liver toxicity (3), and muscular dystrophy (17,18). Furthermore, ADA activity has also been reported to increase in peritoneal and pleural effusions (16,17,19). On the contrary, studies on the ADA inhibitors and genetic deficiency of ADA have demonstrated that inhibition and/or genetic deficiency of ADA resulted in immunodeficiency (9,12). Furthermore, it is reported that ADA activity in serum and lymphocytes decreased after administration of immune suppressive therapy and immune suppressive disorders (5,20,21).

Although serum ADA and its isoenzyme activities were reported in many diseases and conditions (3,5,9,12,15-21), normal values of enzyme activity in all healthy domestic animals have not been observed yet. Especially in Akkaraman sheep, Pure Hair goats, and Van cats, which are important Turkish local breeds, no studies were cited in the literature. Therefore, in this study serum ADA and its isoenzyme activities in all healthy animals (male and female) were investigated to determine normal values in these domestic animals.

## Materials and Methods

In this study, a total of 262 non-pregnant animals, 5 different species [cattle (n = 50), sheep (n = 65), goat (n = 52), dog (n = 55) and cat (n = 40)], were used. Health conditions of the animals were determined by clinical, hematological, and biochemical examinations. Out of 262 animals, 50 were Swiss Brown cattle aged between 2 and 8 years old (22 male and 28 female), 65 were Akkaraman sheep aged between 2 and 6 years old (30 male and 35 female), 52 were Pure Hair goats aged between 2 and 6 years old (14 male and 38 female), 55 were street dogs aged between 1 and 4 years old (22 male and 33 female), and 40 were Van cats aged between 1 and 5 years old (15 male and 25 female).

Ten milliliters of blood without anticoagulant and 5 ml blood with anticoagulant were taken from jugular vein of the animals (cattle, sheep, and goat); 5 ml of blood without anticoagulant and 2 ml blood with anticoagulant were taken in cats and dogs from *v. cephalica antebrachii*. Blood without anticoagulant was used to prepare serum samples to determine ADA and its isoenzymes levels.

Serum total ADA activity was also determined spectrophotometrically and using the method described by authors (22). To distinguish between ADA1 and ADA2 forms, the ADA activity was measured using the same technique with and without erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA). EHNA is a potent inhibitor only of the ADA1 isoenzymes and a concentration of 200 mmol/l was used in the reaction solution (11,21). In the presence of EHNA, only ADA2 isoenzyme is active. The ADA1 activity is then calculated by subtracting the ADA2 isoenzyme activity from the total ADA activity as reported by authors (11,21).

For statistical analysis, normal distribution test was applied first to all variables. Mann-Whitney U test was used to determine the difference between male and female animals in the same species. All calculations were performed with MS-Excel® (Microsoft Corp. Inc.) and SPSS software.

## Results

Serum ADA and its isoenzyme activities in the animals are given in Table 1. Highest ADA and ADA1 isoenzyme activity were detected in cats, followed by sheep, goat, cattle, and dog, respectively. The serum levels of ADA2

were low in sheep, goats, dogs, and cats, whereas in this species ADA1 was high. Furthermore, there was no serum ADA2 activity in cattle.

Serum ADA activities in domestic animals by gender are presented in Table 2. Serum ADA activity in all animals were not different in terms of sex.

## Discussion

In the present study, ADA activities in domestic animals that were determined to be healthy after clinical and laboratory examinations were investigated.

In the present study, ADA activity obtained from animals were in agreement with results given by Tanabe (4). ADA activities determined for cats and sheep in the present study (Table 1) were in parallel with the findings reported in the literature (16,23). Furthermore, ADA activities found in dogs were in agreement with the findings of Takahashi (24), but was in disagreement with the result of Hirschberger and Koch (19), which was

quite higher than present findings for dogs. This could be due to the method used in the present study, which was different than the method used by Hirschberger and Koch (19).

Yasuda et al. (15) reported that ADA activity in cattle as  $3.9 \pm 1.9$  IU/l, which is lower than the present findings (Table 1). In contrast, Konaş and Salmanoğlu (25) reported that ADA activity in cattle as  $11.07 \pm 4.91$  IU/l, which is higher than the present findings (Table 1). However, several workers (26-28) reported that ADA activity in cattle is between  $5.4 \pm 2.6$  and  $6.63 \pm 0.11$  IU/l, which is similar to our results (Table 1).

In this study, serum ADA activity in pure hair goats was measured as  $7.52 \pm 0.36$  IU/l. However, Rodrigues et al. (29) reported that serum ADA activity for crossbred goats as  $19.5 \pm 6.0$ , which is quite higher than the present findings (Table 1). However, no other reference could be found in the literature about ADA in goats, therefore the results cannot be evaluated objectively.

Table 1. ADA and its isoenzyme activities of domestic animals.

SPECIES	ADA (U/l)		ADA1 (U/l)		ADA2 (U/l)	
	n	Mean $\pm$ SEM	n	Mean $\pm$ SEM	n	Mean $\pm$ SEM
CATTLE	50	$6.34 \pm 0.31$	10	$6.34 \pm 0.31$	10	ND*
SHEEP	65	$7.57 \pm 0.30$	20	$7.31 \pm 0.47$	20	$0.52 \pm 0.09$
GOAT	52	$7.52 \pm 0.36$	11	$6.44 \pm 0.72$	11	$0.76 \pm 0.13$
DOG	55	$2.56 \pm 0.13$	18	$2.03 \pm 0.30$	18	$0.13 \pm 0.05$
CAT	40	$23.36 \pm 1.59$	20	$21.71 \pm 2.13$	20	$1.15 \pm 0.12$

\*Not detected

Table 2. ADA activities of domestic animals by gender.

SPECIES	MALE		FEMALE	
	n	Mean $\pm$ SEM	n	Mean $\pm$ SEM
CATTLE	22	$6.13 \pm 0.47$	28	$6.52 \pm 0.40$
SHEEP	30	$7.95 \pm 0.64$	35	$7.30 \pm 0.33$
GOAT	14	$7.41 \pm 0.52$	38	$7.58 \pm 0.54$
DOG	22	$2.39 \pm 0.24$	33	$2.68 \pm 0.14$
CAT	15	$23.63 \pm 2.53$	25	$23.07 \pm 2.05$

P > 0.05

In the present study, although ADA isoenzyme activity was measured, ADA2 activity was not detected in cattle. Moreover, although ADA2 activity was detected in cats and dogs, it was very low. Therefore, the observed ADA activity could be due to the ADA1 isoenzyme. The present results were similar to Tanabe's findings (4). To the best of our knowledge, serum ADA isoenzyme activity in sheep and goats has not been observed in the literature. Therefore, results given in the present study for this animal could not be compared. Consequently, the observed ADA isoenzyme values for sheep and goats in the present study can be taken as reference values.

These enzyme activities were not statistically different in terms of sex in all the animals studied (Table 2). Similarly, Tanabe (4) reported that ADA activities in cattle, dog, cat, horse, pig, rat, and rabbit were no

different in terms of sex. On the other hand, Sywall et al. (30) reported that ADA activity was higher in male than in female cattle, which was different than the present findings. Additionally, in the present study serum ADA isoenzymes were evaluated in sheep and goats for the first time with regard to gender. Although, to the best of our knowledge, there is no study on this subject in the literature, our findings were in agreement with the findings of other studies examined other animals species (4).

As a result, determination of normal ADA enzyme activity of healthy animals will help the scientists interested in ADA activity in different situations. Determination of the changes in this enzyme activity and its clinical importance will contribute to veterinarians and scientists studying in this area.

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