

# Efficacy of a Butafosfan and Vitamin B12 Combination (Catosal®) on Biochemical and Hematological Blood Parameters in Dogs Treated with Dexamethasone

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## **ABSTRACT**

The effects of a simultaneous application of butafosfan plus vitamin B12 combination (Catosal®) on hepatic, pancreatic, and hematological parameters were evaluated in dogs injected subcutaneously with dexamethasone once daily for 7 days at a decreasing dose rate. Six dogs in group 1 were treated with dexamethasone and Catosal® and 6 dogs in group 2 with dexamethasone and NaCl 0.9%. GGT exceeded the upper reference limit in group 2 on study days 7, 9, and 11, whereas it remained inside the reference limits in group 1. An increase of RBC, HTC, and HB with respect to baseline values was observed in group 1 compared to group 2. A dexamethasone dependent decrease in amylase recovered faster in group 1 than group 2.

## **INTRODUCTION**

Corticosteroids like dexamethasone are

widely used in dogs. They are indispensable for the treatment of allergic and anaphylactic reactions, shock, skeletal disturbances, inflammations, and autoimmune diseases. However, exogenous corticosteroids, even at clinically recommended doses, have adverse effects on liver<sup>1,2,3,4,5</sup> and pancreatic<sup>6</sup> function. Furthermore, routine corticosteroid therapy causes hematological changes.<sup>1,5</sup>

In a recent study,<sup>7</sup> dexamethasone was applied to dogs with acute thoracolumbar intervertebral disk herniation. The dexamethasone treated group of dogs was 3.4 times, 11.4 times, and 3.5 times more likely to have a complication, urinary tract infection, and diarrhea respectively, when compared with other-glucocorticoid or nontreatment groups. In several controlled studies,<sup>1,2,3,4,5,6</sup> and in a retrospective study of clinical cases,<sup>8</sup> hepatopathy and increased values of serum enzymes such as gamma-glutamyl-transferase (GGT), alanine-amino-transferase (ALT), glutamat-dehydrogenase (GLDH), alkaline-phosphatase (AP), and aspartate-

**Table 1.** Mean values of AP, ALT, AST and GLDH in dexamethasone + Catosal® (group 1) and dexamethasone (group 2) treated dogs before and after treatments (AP: alkaline phosphatase, ALT: alanine aminotransaminase, AST: aspartate transaminase, GLDH: glutamate dehydrogenase, SD: study day).

Parameter	Reference limits@	Groups	SD -3	SD3	SD4	SD7	SD9	SD11	SD14	SD18
AP U/L	23-212	1	84±25.7	212±100.1*	<b>227±97.6*</b>	<b>278±143.8*</b>	<b>225±120.7*</b>	170±80.2*	128±55.3*	110±38*
		2	83±19.4	173±62.1*	193±65.2*	<b>286±62.4*</b>	<b>270±63.9*</b>	<b>221±52.4*</b>	143±33.9*	103±15.8
ALT U/L	10-100	1	43.3±14.0	72.7±12.6	84.7±18.4*	76.3±16.9*	87±32.7	68.5±23.8	54.3±17.5	42.5±9.5
		2	32.3±9.6	62.8±9.5*	79.2±24.7*	78.7±25.1*	72±14.9*	65.8±16.8*	52±11.8*	35.3±7.6
AST U/L	0-50	1	24.7±12.48	14.2±7.94	14.8±7.44	2±3.03*	8±4.73	8.8±6.88*	5.7±4.84*	4.3±5.28*
		2	22.2±7.19	13.2±5.88	17.7±9.5	5.3±4.55*	4.2±3.76*	3.2±4.49*	8.5±5.58*	6.5±4.68*
GLDH U/L	0-9.6	1	4.73±1.31	9.28±2.03	<b>28.32±13.32*</b>	<b>11.48±4.51*</b>	9.40±5.76	6.48±2.93	4.03±0.96	4.70±0.87
		2	4.28±1.12	9.33±2.15*	<b>30.47±18.82*</b>	<b>14.50±8.46*</b>	8.43±1.53*	6.83±2.70*	4.38±1.20*	4.30±0.36*

@: Reference limits were given by the manufacturer of test system (IDEXX company). \* $p < 0,05$  significantly different from baseline (SD-3) within group. Bold letters mean out of reference limits.

amino-transferase (AST), were observed in all corticosteroid-treated dogs. Hepatocellular vacuolation<sup>2,9</sup> is a major morphologic alteration that occurs with experimentally induced glucocorticoid hepatopathy and produces marked alterations in serum gamma-glutamyl-transaminase (GGT) activity.<sup>2</sup> It has been demonstrated that serum GGT is a specific indicator for alteration of hepatic functional.<sup>3,8</sup> This membrane-associated enzyme is widely used as a marker to test the integrity of liver cell membranes that may be damaged as a result of drug application or any disease related hepatic disorders.<sup>1</sup> Exposure to exogenous glucocorticoids can variably increase the serum GGT activity.<sup>2,3,4,5</sup> The extent and duration of enzyme induction is dependent on the specific drug used, dose rate, duration of treatment, and individual sensitivity of the treated animal. Significant increases of GGT activity after systemic<sup>3,8</sup> and topical<sup>1</sup> treatment with dexamethasone have been reported in the dog.

Butafosfan is a phosphonic acid compound that is available in many countries in combination with vitamin B12 as the injectable veterinary product Catosal®. Studies with butafosfan and/or Catosal® in cattle, horses, swine, broilers, and mice have shown that these products improved general health status by stimulating feed intake,<sup>10</sup> the immune system,<sup>11</sup> and digestive function,<sup>10,12</sup>

improving liver<sup>10,13,14,15,16</sup> and muscle<sup>10,15,16</sup> function and haemoastasis.<sup>17,18,19</sup> Catosal® supports red blood cell production<sup>19,20,21</sup> by improvement of concentrations of hemoglobin, hematocrit, and total plasma protein. There is also evidence that Catosal® is able to reduce stress responses in pigs<sup>22,23,24</sup> sheep<sup>22</sup>, cattle,<sup>25</sup> and mice<sup>26</sup>.

Taking into account all the effects of Catosal® found in the above studies, this present study in dogs was intended to investigate the effects of Catosal® on dexamethasone-dependent liver stress. Furthermore, the purpose of this study was to evaluate the effects on liver function and hemogram on dexamethasone treated dogs simultaneously treated with Catosal®. With this aspect, it was our aim to see whether Catosal® can eliminate any of the observed negative effects of dexamethasone on liver enzymes and blood cells.

## MATERIAL AND METHODS

An exploratory and controlled laboratory study was performed in a parallel group design. Twelve adult, clinically healthy beagle dogs between 1.5 and 2.3 years of age were randomly assigned to two groups, each with six dogs, taking into account their gender (four males and eight females) and their body weight (6.8 – 11.3 kg). Selection criteria were biochemical and hematological parameters within the normal reference limits and full health as determined by a clinical

**Table 2.** Mean values of CK, LDH, albumin, glucose, amylase, lipase, WBC, neutrophils, lymphocytes, eosinophils and PLT in dexamethasone + Catosal® (group 1) and dexamethasone (group 2) treated dogs before and after treatments (CK: creatin kinase, LDH: lactate dehydrogenase, WBC: white blood cells, PLT: platelets, SD: study day).

Parameters	Reference limits@	Groups	SD -3	SD3	SD4	SD7	SD11	SD18
CK U/L	10-200	1	137±51.6	156±75.7	118±43.1	108±24.7	141±77.5	118±45.7
		2	196±104.6	89±34.8	104±40.7	142±48.5	96±41.3	111±21.1
LDH U/L	40-400	1	273±123.5	208±48.6	162±46.7	166±43.7	210±68.1	192±91.9
		2	292±237.3	240±79.8	210±57.2	297±114.2	158±26.5	195±46
Albumin g/L	23-40	1	27.7±2.4	33.5±2.4*	34.3±2.8*	32.2±2.6*	29.8±2.5*	28.8±1.0
		2	29.5±1.6	34.2±2.1*	34.8±2.7*	32.8±2.1*	31.5±2.9	30.7±2.6
Glucose mmol/L	4.11-7.94	1	5.27±0.28	5.31±0.64	5.86±0.53*	5.82±0.46*	5.69±0.45*	5.62±0.48
		2	5.10±0.85	5.06±0.75	5.37±0.37	5.19±0.61	5.9±0.62*	5.46±0.36
Lipase U/L	200-1800	1	1433±976	842±512*	562±260*	1607±2172	725±334*	1289±590
		2	1534±941	509±222*	447±175*	548±179*	520±250*	1183±478
WBC x103/µL	5.3 - 13.9	1	5.90±1.64	<b>17.64±3.65*</b>	<b>14.74±4.44*</b>	10.68±2.8*	7.37±1.68	5.89±0.66
		2	8.64±3.03	<b>19.23±3.52*</b>	<b>16.06±2.94*</b>	13.03±3.27*	7.86±2.45	8.42±3.13
Neutrophils x103/µL	3.9 - 8.0	1	4.01±0.97	<b>15.05±3.07*</b>	<b>12.08±3.70*</b>	7.75±2.36*	5.30±1.34	3.90±0.41
		2	5.59±2.81	<b>16.10±3.15*</b>	<b>13.14±2.51*</b>	<b>9.38±2.59*</b>	5.45±2.28	5.58±2.85
Lymphocytes x103/µL	1.3 - 4.1	1	1.34±0.55	1.42±0.41	1.65±0.42	2.05±0.26*	1.30±0.29	1.46±0.22
		2	2.08±0.44	1.71±0.39	1.78±0.45	2.36±0.68	1.51±0.28*	1.98±0.40
Eosinophils x103/µL	0 - 0,6	1	0.23±0.10	0.06±0.06*	0.07±0.07*	0.05±0.03*	0.15±0.08*	0.1±0.03*
		2	0.38±0.18	0.08±0.07*	0.04±0.01*	0.07±0.03*	0.24±0.04	0.21±0.09*
PLT x103/µL	143.3 - 400	1	323±147.09	<b>442±107.11</b>	<b>413.17±138.45</b>	<b>454.83±117.53</b>	391.17±126.25	383.83±127.87
		2	397±69.26	<b>414.67±86.62</b>	<b>415.5±84.28</b>	385.83±151.04	<b>401.33±117.68</b>	<b>402±92.51</b>

@: Reference limits were given by the manufacturer of the test system (IDEXX company for biochemical parameters, ADVIA 120 for haematological parameters). \*p<0,05 significantly different from baseline (SD-3) within group. Bold letters mean out of reference limits.

examination. The dogs had been vaccinated regularly and not treated with any corticosteroid substance or butafosfan / vitamin B12 combination for at least 4 weeks prior to the study start. The animals were housed in individual boxes and fed commercial dry food once daily. Water was available at all times.

All dogs were treated with dexamethasone (100 mg dexamethasone-dihydrogenphosphate-disodium / 10 ml, Dexamethason®, Jenapharm GmbH & Co.KG, Jena; Germany) daily for 7 days. The dose was tapered from an initial rate of 2 mg / kgbw on day 0 to 0.1 mg / kgbw on day 6 to reduce the risk of induction of a Cushing's syndrome. In the first 2 days, dogs received 2 mg/kg; then in the next 2 days, 1 mg/kg and 0.5 mg/kg respectively. In the last 3 days, 0.1 mg/kg dexamethasone was applied. Simultaneously, all dogs in group 1

received 2 ml / 10 kgbw of a solution of 100 mg / ml butafosfan and 0.05 mg / ml vitamin B12 (Catosal® Injectable solution, Bayer Animal Health GmbH, Leverkusen, Germany). A dose of 2 ml per 10 kgbw of NaCl 0.9 % (B. Braun, Melsungen AG, Melsungen, Germany) was applied to the dogs in the control group (group 2) at the same time of dexamethasone treatment. All injections were subcutaneous.

Blood samples were collected from all dogs 3 days before starting the study (study day -3) and on days 3, 4, 7, 9, 11, 14, and 18 from V. jugularis. The first day of treatment started was determined as study day 0. For hematological analyses, EDTA blood samples, were stored on a shaker and analysed directly within 6 hours after sampling. The hematological parameters, red blood cells (erythrocytes, RBC), hematocrit (HCT),

hemoglobin (HB), white blood cells (WBC), lymphocytes (Lymp), neutrophils (Neut), eosinophils (Eos), and thrombocytes (PLT) were analyzed in whole blood samples using the ADVIA 120 System (Bayer HealthCare Diagnostics). All reference limits (normal physiological range) for RBC, HCT, HB, WBC, Neut, Eos, Lymp, and PLT were given for dogs by the manufacturer of the test system (ADVIA 120 System). For serum separation, the blood samples were centrifuged at approx. 4° C and 1,800 x g for 10 minutes. The supernatants were transferred to pre-labelled micro test tubes and analyzed directly. The biochemical parameters such as gamma-glutamyl-transaminase (GGT), alanine aminotransaminase (ALT), glutamate dehydrogenase (GLDH), alkaline-phosphatase (AP), aspartate transaminase (AST), creatine kinase (CK), lactate-dehydrogenase (LDH), albumin, glucose, amylase, and lipase were analyzed using dry-slide technology (Vet-Test 8008, IDEXX company). The remaining serum samples were stored frozen at -18° C or below until their delivery to an external analytical laboratory ``Synlab.vet Augsburg`` (Gesellschaft für Laboranalytik mbH&Co KG, Germany) for the analysis of GLDH concentration. All reference limits (normal physiological range) for AP, ALT, GGT, AST, CK, LDH, albumin, glucose, amylase, lipase, and GLDH were given by the manufacturer of the test systems (IDEXX company and Synlab.vet Augsburg). During the whole study the dogs were monitored for potential signs of intolerance or adverse effects of the drugs administered.

### STATISTICAL ANALYSIS

Standard descriptive summary statistics were carried out for all parameters such as number of non-missing values, arithmetic mean, and standard deviation. To judge the within-group changes from baseline (SD-3) during the whole study days, each group was analyzed with the sign-test, the real symmetry test (Bowker test), and the test of marginal homogeneity (Stuard-Maxwell test).

•Sign-test: The null hypothesis is that there is no difference. The numbers of

upward differences (pluses) and downward differences (minuses) are determined. The sample size is reduced by the number of ties. The number of positive and negative signs are then tested for equality using the binomial test ( $H_0: p_1=p_2=0.5$ ).

•Bowker test (real symmetry test): The cells symmetric to the diagonal are always tested simultaneously for equality.  $H_0: p_{ij}=p_{ji}$  for all  $i<j$ .

•Stuard-Maxwell test (marginal homogeneity): The lower and the right marginal sums are tested for equality.  $H_0: p_{i.}=p_{.i}$  for all  $i$ . The between-group comparisons were analyzed for the change-from-baseline (SD-3)-values for each time point throughout the study with the non-parametric Wilcoxon-Mann-Whitney U-test. The level for statistical significance was  $p<0.05$  for all parameters.

### RESULTS

A dexamethasone dependent increase of mean AP, ALT, and GLDH serum activities were observed in both groups (Table 1). Whereas ALT did not exceed the reference range (100 U/l) at any time and did not change significantly between the groups in terms of absolute mean values, AP and GLDH increased above the reference limits. AP exceeded the upper reference limit (212 U/l) on study days 4–9 and 7–11 in groups 1 and 2 respectively. GLDH was above the upper reference limit (9.6 U/l) on study days 4-7 in both groups. Baseline change of GLDH was only significant in group 1 on study days 4 and 7, while in group 2 significant changes were seen on study days 3, 4, 7, 9, 11, 14, and 18. There was no significant difference in AP, ALT, and GLDH baseline changes between study groups. ALT changed significantly from baseline in group 1 on study day 4 and 7 only. An increasing significant trend was seen on study days 3, 4, 7, 9, 11, and 14 in group 2 (Table 1).

The mean of AST showed a continuously decreasing trend (Table 1) from baseline, which became significant from study day 7 on ( $p<0.05$ ). However, it remained within

the normal reference range. No significant difference between the two groups was observed with regard to baseline changes of AST. GGT rose significantly ( $p < 0.05$ ) in both groups after treatments (Figures 1 and 2), and a significant difference was seen between group 1 and group 2. The mean GGT concentration did not exceed the upper reference limit (7 U/l) in group 1, while in group 2, it exceeded the normal reference limit from study day 7 to 11 (Figure 1). Changes from baseline were significant on study days 7, 9, 11, 14, and 18 and 4, 7, 9, and 11 in groups 1 and group 2 respectively (Figure 2). All those changes from baseline had an increasing trend. Differences between group 1 and 2, in the baseline change from study day -3, were significant ( $p < 0.05$ ) on study days 9 and 11 as illustrated in Figure 2.

Concentrations of glucose, CK, and LDH did not change in a clinically relevant manner during the study based and remained within the normal reference range (Table 2). Positive changes in the mean concentration of glucose from baseline (SD -3) in group 1 were significant on study days 4, 7, and 11, while there was only a slight and significant increase in group 2 on study day 11. On study day 7, the difference in glucose concentration from baseline in group 1 was statistically higher than group 2 ( $p < 0.05$ ).

Treatments significantly changed the amylase (Figures 3 and 4) and lipase concentrations (Table 2) negatively compared to mean baseline values in both groups. Amylase activities in both groups were below the lower reference limit on study days 3, 4, and 7 (Figure 3). Lipase in both groups did not exceed the reference limits (Table 2). Changes of amylase from baseline in group 1 were significant on study days 3 and 4 and in group 2 on study days 3, 4, and 7 (Figure 4). Those changes were negative. There was not a significant difference of the mean amylase and lipase concentrations between treatment groups. But, a clear and significant recovery of amylase activity ( $p < 0.05$ ) in group 1 was observed on study day 11 in the baseline change compared to group 2

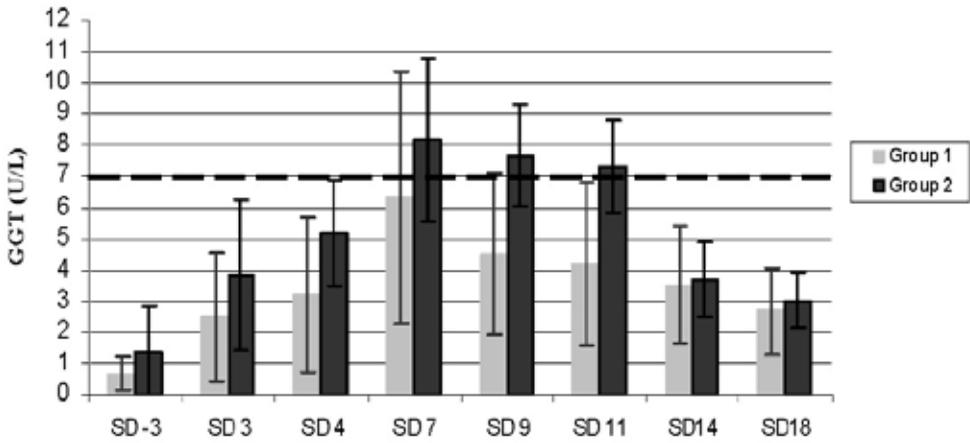
(Figure 4).

Baseline values of albumin concentration increased significantly after dexamethasone and dexamethasone+Catosal® treatments (Table 2). There was no significant difference in baseline change between the groups.

Changes in the RBC, HCT, and HB are presented in Figures 5–10. The mean values of the groups did not exceed the reference limits on any study day (Figures 5, 7, and 9). No statistical difference between the groups was observed in the absolute values. A significant rise in the baseline change of RBC and HCT ( $p < 0.05$ ) was observed in group 1 compared to group 2 on study days 3, 4, and 7 (Figures 6 and 8). RBC and HCT changed significantly from baseline, with an increasing tendency in group 1 on study days 4 and 7, while a significant decrease of RBC from baseline was observed on study day 18 in group 2. HB changed significantly from baseline in group 1 on study day 4, and in group 2 on study day 3 (Figure 10). Changes from baseline were positive in group 1 while it was negative in group 2. As illustrated in HCT and RBC values, a significant difference in baseline change ( $p < 0.05$ ) of HB was seen between groups on study days 3, 4, and 7 (Figure 10).

There was no significant difference in the number of WBC, Neut, Eos, Lymp, and PLT between groups in absolute mean values or baseline changes (Table 2). On study days 3 and 4, the mean values of WBC and Neut were above the upper reference limit in both groups. Neut exceeded the upper reference limit in group 2 on study day 7 as well. The number of WBC and Neut significantly changed from baseline on study days 3, 4, and 7 in both groups positively. A negative change from baseline was seen in the number of Eos in both groups (Table 2). Change from baseline in Lymp was positive in group 1 on study day 7, but a negative change from baseline was observed in group 2 on study day 11. These baseline changes were significant ( $p < 0.05$ ). There were no clinically relevant observations during any

**Figure 1.** Mean serum gamma-glutamyltransferase (GGT) activities in dexamethasone+ Catosal® (group 1) and dexamethasone (group 2) treated dogs. *n* = 6 dogs per group. Normal upper reference limit (given by the manufacturer of the test system: IDEXX company). SD = Study day.

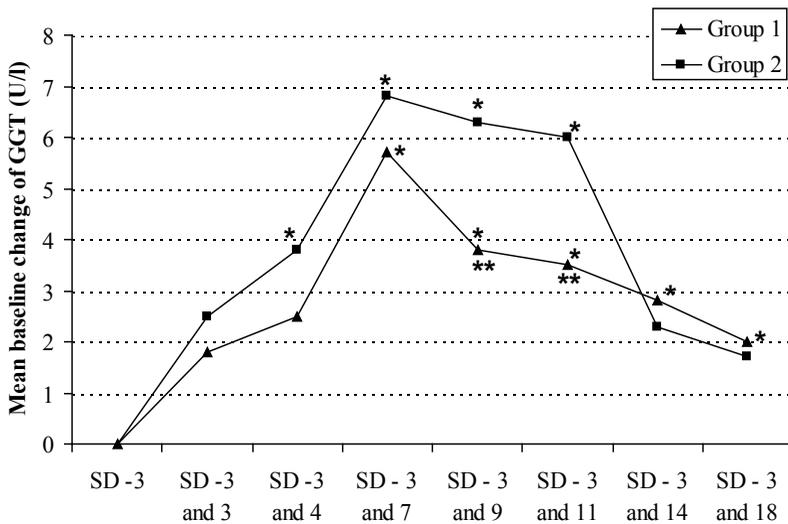


**Figure 2.** Mean baseline changes of gamma-glutamyltransferase (GGT) activities in the dexamethasone + Catosal® (group 1) and dexamethasone (group 2) treated dogs comparing with study day - 3.

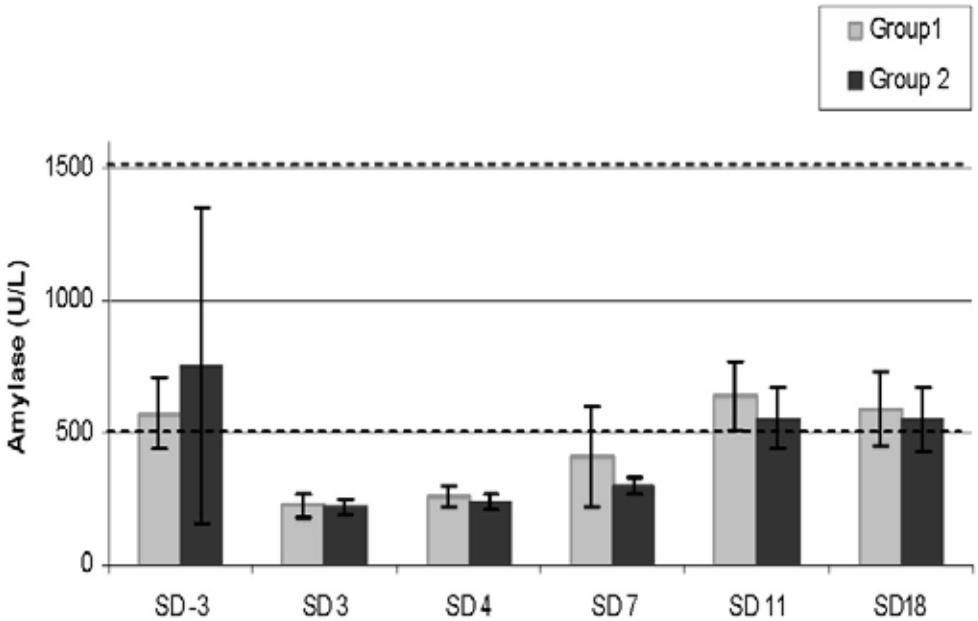
*n* = 6 dogs per group.

\**p* < 0.05 significant difference from baseline within group; \*\**p* < 0.05 significant difference between groups.

SD = Study day.



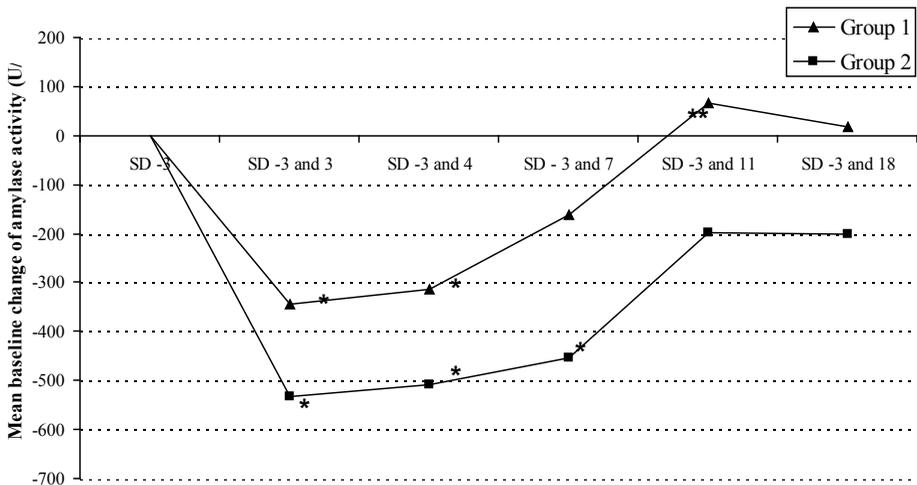
**Figure 3.** Mean amylase activities in dexamethasone + Catosal® (group 1) and dexamethasone (group 2) treated dogs. *n* = 6 dogs per group. *n* = 6 dogs per group. Normal reference limits (given by the manufacturer of the test system: IDEXX company). SD = Study day.



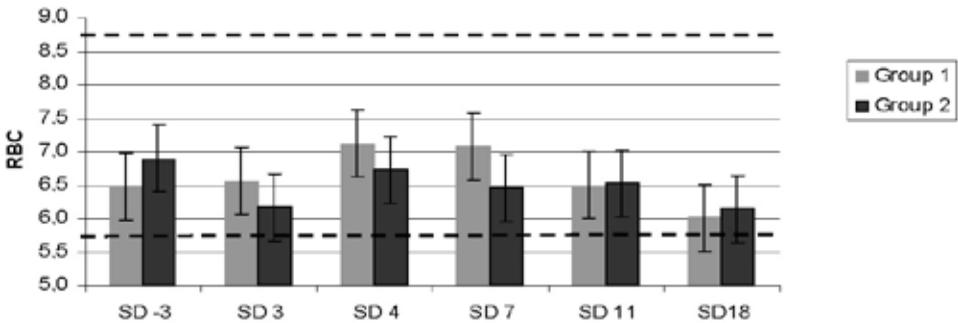
**Figure 4.** Mean baseline changes of amylase activities in dexamethasone + Catosal® (group 1) dexamethasone (group 2) treated dogs. *n* = 6 dogs per group.

\**p* < 0.05 significant difference from baseline within group; \*\**p* < 0.05 significant difference between groups.

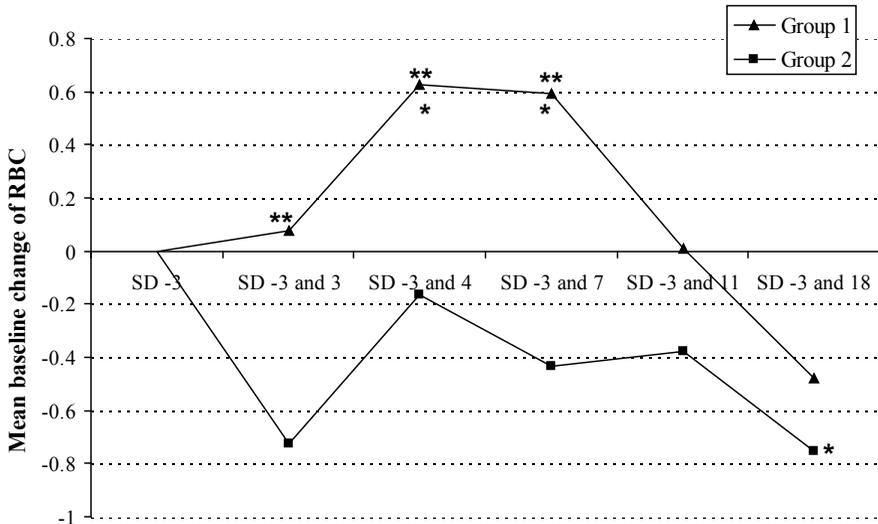
SD = Study day.



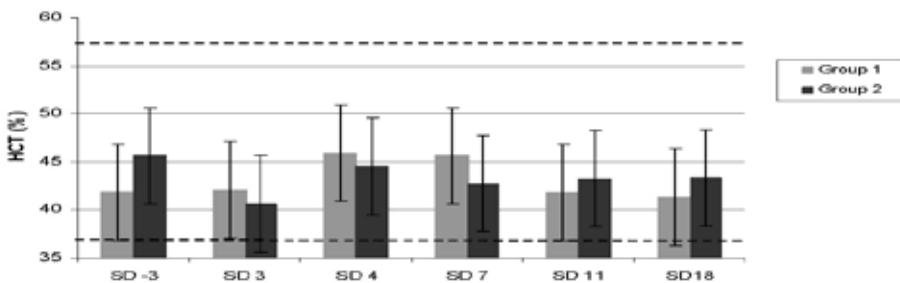
**Figure 5.** Mean erythrocytes counts (RBC,  $\times 10^6/\mu\text{l}$ ) in dexamethasone + Catosal® (group 1) and dexamethasone (group 2) treated dogs.  $n = 6$  dogs per group. Normal reference limits (given by the manufacturer of the test system: ADVIA 120).  $SD =$  Study day.



**Figure 6.** Mean baseline changes of erythrocytes (RBC,  $\times 10^6/\mu\text{l}$ ) in dexamethasone + Catosal® (group 1) and dexamethasone (group 2) treated dogs.  $n = 6$  dogs per group. \* $p < 0.05$  significant difference from baseline within group; \*\* $p < 0.05$  significant difference between groups.  $SD =$  Study day.



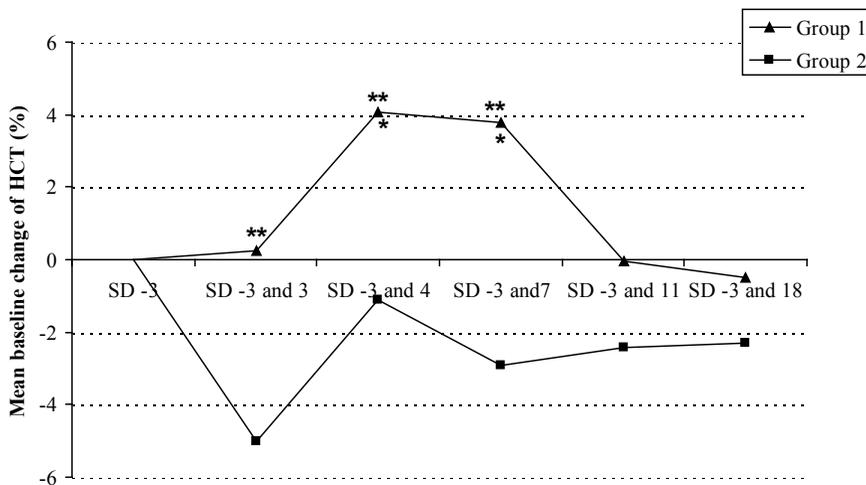
**Figure 7.** Mean hematocrit (HCT) in dexamethasone + Catosal® (group 1) and dexamethasone (group 2) treated dogs.  $n = 6$  dogs per group. Normal reference limits (given by the manufacturer of the test system: ADVIA 120).  $SD =$  Study day.



**Figure 8.** Mean baseline changes of hematocrit (HCT) in dexamethasone + Catosal® (group 1) and dexamethasone (group 2) treated dogs. n = 6 dogs per group.

\*p < 0.05 significant difference from baseline within group; \*\*p < 0.05 significant difference between groups.

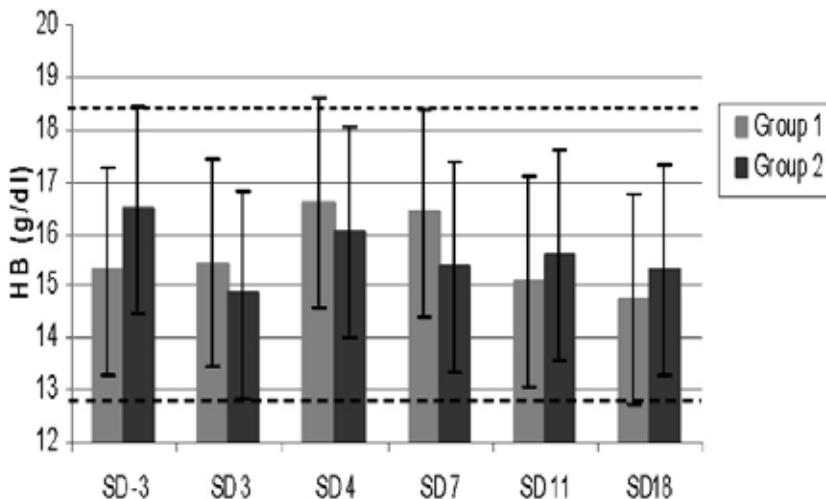
SD = Study day



**Figure 9.** Mean hemoglobine (HB) concentration in dexamethasone + Catosal® (group 1) and dexamethasone (group 2) treated dogs. n = 6 dogs per group.

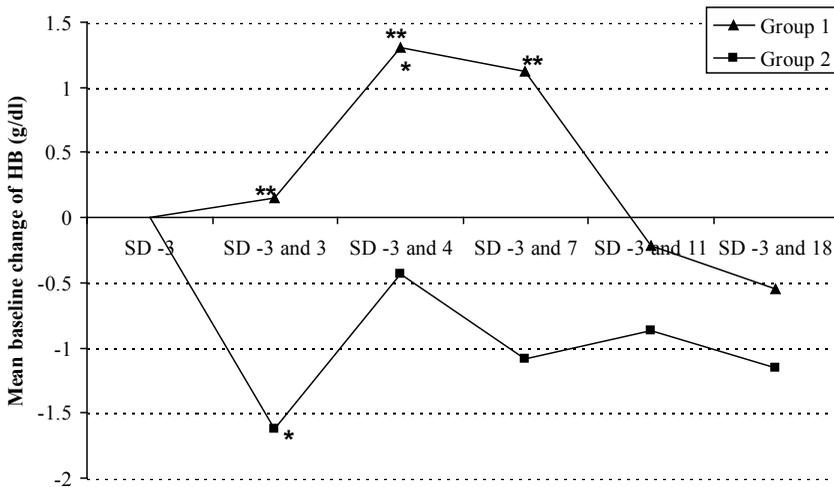
Normal reference limits (given by the manufacturer of the test system: ADVIA 120).

SD = Study day.



**Figure 10.** Mean baseline changes of hemoglobine (HB) concentration in dexamethasone + Catosal® (group 1) dexamethasone (group 2) treated dogs. n = 6 dogs per group.

\*p < 0.05 significant difference from baseline within group; \*\*p < 0.05 significant difference between groups. SD = Study day.



of the clinical examinations. No adverse events occurred throughout the study.

**DISCUSSION**

Previous findings,<sup>1-7</sup> that exogenous corticosteroids have adverse effects on liver enzymes in dogs, were supported by the results of the present study in which treatment with dexamethasone resulted in a pronounced increase of serum GGT, AP, ALT, and GLDH. Some parameters were out of upper reference limit on at least on 2 (GLDH) or 3 (GGT, AP) study days. AST and ALT serum activities were not out of the reference range during the study days in either groups. However, there was a clear significant increasing tendency for ALT and decreasing tendency for AST during treatment with dexamethasone and/or dexamethasone+Catosal® (Table 1). Although there was no significant difference between changes of ALT and GLDH from baseline in both groups, additional treatment with Catosal® in dogs treated with dexamethasone appeared to limit the increasing trend of both enzymes within 3 days after treatment and study day 7 onwards. Dexamethasone-associated increases in serum liver enzymes were already reported by oth-

ers<sup>3,4,6</sup>. Data from Dillon et al<sup>5</sup> did not support a significant change in AST activity in prednisolone treated dogs, while Abraham et al<sup>1</sup> found a significant increase in dogs after the treatment with an ear ointment containing dexamethasone, clotrimizol, and neomycine. A significant effect of additional treatment with Catosal® on GGT was observed in group 1, keeping GGT within the reference limits during the whole study days. As illustrated in figures 1 and 2, the increase in GGT activity is more rapid in the dogs treated with dexamethasone only. The dexamethasone-induced elevation of GGT appeared to be controlled by concomitant Catosal® treatment. In studies conducted in cattle, treatment with Catosal® before surgery for abomasal displacement or as a metaphylactic treatment during the pre-partum transition period controlled the increase of AST,<sup>10,13,14</sup> bilirubin,<sup>10</sup> and free fatty acid<sup>10</sup> compared to untreated control groups. In two experimental studies in broilers,<sup>15,16</sup> continuous oral application of butafosfan was able to control the rise of AP in heat- or cold-stressed broilers with for 14 days. Treatment of racing horses with Catosal® controlled the elevation in the activities

of AP and AST.<sup>21</sup> However, GGT was not evaluated in any of these studies. GGT is a specific enzyme for obstructive and cholestatic liver diseases in dogs.<sup>3,9,27</sup>

Induction of serum GGT activity by dexamethasone has been clinically reported in dogs.<sup>1,3</sup> The highest median activity of GGT has been developed in dogs with steroid hepatopathy, cholestasis, and hepatic necrosis.<sup>3</sup> In man, an increase of GGT is often seen after alcohol consumption.<sup>28,29</sup> Corticosteroid associated lesions in hepatocytes can be detected at various intervals after treatment with small as well as large doses. In one dog, hepatic lesions were detected 1 week after a single dose of dexamethasone (0.3 mg/kg). In a second dog, hepatomegaly and ballooning of hepatocytes were detected within 24 hours of oral application of 1 mg/kg prednisolone acetate.<sup>30</sup> The corticosteroid administration associated hepatopathy was characterized by centrilobular vacuolization, perivacuolar glycogen accumulation within hepatocytes, and focal centrilobular necrosis.<sup>4,8,30</sup> Following corticosteroid administration, it is apparent that severe histologic changes, even after only 3 days of therapy, may occur in hepatocytes before significant serum enzyme elevation.<sup>5</sup> Hepatic morphologic changes attributed to dexamethasone have been reported, indicating that the histopathologic lesions preceded increased serum enzyme activity.<sup>4</sup>

Treatment with topical corticosteroids may be of benefit to minimize systemic side effects. Experimental findings have shown that even ototopical dexamethasone treatment caused significant increases in serum GGT and other liver enzymes.<sup>1</sup> In the present study, the simultaneous application of Catosal® to dogs treated parenterally with dexamethasone significantly reduced the dexamethasone dependent increase of serum GGT. Taking into account that Catosal® also limited ALT and GLDH elevation in dexamethasone treated dogs to 2 days compared to 6 or 7 days in only dexamethasone treated dogs, these results corroborate earlier findings that Catosal® prevents the

increase of indicators for liver disturbances in blood and improves the liver cell function of cattle,<sup>10,13,14</sup> broilers,<sup>15,16</sup> and horses.<sup>21</sup>

Changes in values of RBC, HCT, and HB were observed in the present study. Means of these parameters remained within the reference limits. Based on the absolute values, there was no significant difference between groups. However, when baseline changes between groups were compared, dexamethasone-dependent negative change was observed in the values of RBC, HCT, and HB in the group not treated with Catosal®. Negative effect of dexamethasone on RBC in dogs has not previously been reported to our knowledge; however treatment of New Zealand white rabbit bucks<sup>31</sup> with dexamethasone at doses of 1 mg/kg BW and 5 mg/kg BW weekly for 8 weeks resulted in an increase of RBC when used at the highest doses. As illustrated in figures 6, 8, and 10, positive significant baseline changes were observed in the Catosal®+dexamethasone group up to study day 11. Catosal® improved hematological red blood cell parameters in dogs treated with dexamethasone. This positive effect of Catosal treatment on hematological parameters, supporting red blood cell production and constitution, is in accordance with the finding of others,<sup>18,19,20</sup> and is most likely due to its vitamin B12 content. Anemia due to vitamin B12 selective malabsorption and consequently its deficiency and treatment with vitamin B12 supplementation in a border collie and in a beagle was reported by Battersby et al<sup>32</sup> and Fordyce et al.<sup>33</sup>

Corticosteroid induced alterations in the number of WBCs has been reported.<sup>4,34,35</sup> Eosinopenia,<sup>1,4</sup> leucocytosis,<sup>1,34</sup> monocytosis,<sup>34</sup> and lymphopenia<sup>1,34</sup> were induced by exogenous steroid administration. Dexamethasone treatment in the present study temporarily increased the number of WBCs (leucocytosis). In both groups the number of WBCs was above the upper reference limit on study days 3 and 4, which was most probably due to marked increase of Neut (Table 2). However, an increasing trend of

Lymph on study day 7 in dogs treated with dexamethasone+Catosal® (group 1) and a decreasing trend on study day 9 in group 2 was observed.

The corticosteroid associated increase of Neut was linked to the increased liberation of matured neutrophils from the bone marrow.<sup>1</sup> In accordance with other studies,<sup>1,4</sup> the number of Eos showed a decrease associated with dexamethasone treatment without a significant difference between the groups. This could relate to the immunosuppressive effect of corticosteroids. However, this effect was not seen in Lymph in dogs treated with dexamethasone+Catosal® and only a slight decrease was observed on study day 11 in group 2. The number of PLT was slightly above the upper reference limit on study days 3, 4, and 7, and 3, 4, 11, and 18 in group 1 and group 2 respectively. A significant difference within and between groups based on absolute values or baseline changes was not observed. To our knowledge there are no reports on Catosal® dependent alteration in the number of PLT in other animals.

Amylase and lipase are enzymes used for the monitoring pancreatic function in practice.<sup>36,37,38</sup> Diagnosis of pancreatic disease in dogs can be difficult, because clinical signs and laboratory findings are not specific.<sup>6,36</sup> Glucocorticoids are known to produce alterations in the pancreas associated enzymes in dog<sup>6,38</sup> and in humans.<sup>39</sup> In the present study, the baseline values of both amylase and lipase decreased significantly after dexamethasone treatment. Amylase activity was below the lower reference range on study days 3, 4, and 7, while lipase was never out of the reference range. A similar decrease in amylase in dogs associated with dexamethasone treatment was also reported by Lucena et al<sup>6</sup> and Parent.<sup>38</sup> In contrast to Parent,<sup>38</sup> who showed an increase of lipase associated with dexamethasone treatment in dogs without evidence of pancreatitis on necropsy, our data suggested a dexamethasone associated decrease of lipase. These results, as well as Parent's<sup>38</sup> suggest that the analysis of lipase associated with

dexamethasone treatment is not reliable to conclude pancreatitis. Furthermore, changes in amylase and lipase after dexamethasone treatment need to be evaluated to determine its specificity to pancreas tissue.

A decrease of amylase from baseline associated with dexamethasone treatment was abolished on study days 7 and 11 in dogs supported with Catosal®. This difference from baseline was significant on study day 11, and amylase activity recovered quicker in dogs supported with Catosal® compared to dogs without support of Catosal®. The reason for this effect is unknown. To our knowledge, no published study is available about the efficacy of Catosal® on amylase activity. More studies need to be conducted on the pharmacological effect of Catosal® and dexamethasone on pancreatic parameters.

There was no significant treatment associated changes in LDH and CK in the present study. Similar results were reported by Dillon et al<sup>5</sup> for CK and by Abraham et al<sup>1</sup> for LDH and CK in dogs treated with ototopical glucocorticoid formulations. However the beneficial effect of Catosal on LDH<sup>16</sup> in broilers and on CK<sup>10,15</sup> in cattle and broilers has been reported before.

Change in the dexamethasone treatment associated albumin concentration was not affected significantly by Catosal® support. Albumin concentration did not change outside of the reference range. However, a significant increase in albumin concentration compared to the baseline value was observed in both groups (Table 2). Other studies on dogs treated with dexamethasone<sup>1,7</sup> or prednisolone<sup>5</sup> did not suggest a significant effect on serum albumin concentration. The reason for this may be a dose-dependent effect of corticosteroids on serum albumin concentration.

To conclude, this is the first study to show a mitigating effect of Catosal® treatment on dogs showing dexamethasone induced biochemical and hematological changes. It can be suggested that dogs treated with dexamethasone can be support-

ed by concomitant Catosal® application to lessen the side effects on liver and pancreatic enzymes and hemogram. Treatments were clinically safe for the dogs along the study days.

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