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NEUTROPHIL/LYMPHOCYTE RATIO DYNAMICS IN DOGS WITH THE INVOLVEMENT OF CANNABIS SATIVA L. IN GRANULATED FOOD

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ABSTRACT

We obtained a reliable differential difference for the neutrophil leukocyte/lymphocyte ratio after 30 days of feeding dogs with the granulated food "REBEL PLUS" containing *Cannabis sativa* L. Although this granulated food does not claim to be a veterinary diet food, we established that after 30 days of feeding the dogs with it, it has a beneficial effect on the number and ratio of white blood cells in the peripheral blood of the examined dogs.

Key words: neutrophil/lymphocyte ratio, *Cannabis sativa* L., granulated dog food.

Introduction

The granulated food "REBEL PLUS" differs from other granular foods by the content of *Cannabis sativa* L., which defines it as unique for the Bulgarian market.

The addition of hemp seed protein to the diet of rats with polycystic kidney disease resulted in reduced pathological severity of the renal disease and amelioration of the associated cardiovascular damage. Furthermore, hemp seed enzymatic hydrolysates have proven their effectiveness during *in vitro* and *in vivo* tests as antioxidant and antihypertensive agents. Therefore, proteins and hemp seed hydrolysates have the potential to be used as ingredients in functionally active foods (Aluko 2016).

On the other hand, the neutrophil/lymphocyte ratio (Neu/Lym ratio) in many studies related to various pathologies in dogs and humans appears as an independent predictor of the outcome of the disease.

In a study of dogs and cats with pancreatitis and prolonged recovery, significantly elevated platelet/lymphocyte and neutrophil/lymphocyte ratios were established compared to the control group, which consisted of healthy animals (Neumann 2021). In another study comparing dogs with severe and mild *Babesiosis*, the authors established higher values of the NLR (neutrophil/lymphocyte ratio) in dogs with severe *Babesiosis*. This gives them reason to conclude that the NLR ratio is an easily measurable parameter that can express the severity of the disease and a decrease in this ratio can serve as a good prognostic marker (Kučer *et al.* 2008).

In a study of dogs with enteropathy sensitive to treatment with steroids or other immunosuppressants (immunosuppressant–responsive enteropathy IRE) and enteropathy not sensitive to the administration of steroids or other immunosuppressants (nonresponsive enteropathy NRE), it is established that they have significantly higher values of the neutrophil/lymphocyte ratio NLR, compared to dogs in which enteropathy can be corrected via the diet. The authors establish no difference in NLR between dogs with immunosuppressant–responsive enteropathy and those diagnosed with non–immunosuppressant–responsive enteropathy, respectively.

These findings suggest that leukogram changes (*i.e*, NLR) may be clinically useful in dogs with chronic enteropathy (CE). NLR can be easily assessed in routine haematology tests and can potentially aid the sub classification of dogs with chronic enteropathy (CE) based on the response to treatment (Becher *et al.* 2021).

These facts from the literature we reviewed gave us a reason to follow how ″REBEL PLUS″ granulated food affects the granulocyte/lymphocyte ratio in a small group of dogs (n=5).

Materials and methods

Our group of dogs consists of 5 mixed–breed dogs which live in a yard in dog houses. In some of them (n=3) various abnormalities in their electrocardiograms were established during the preliminary examinations. In one of the dogs with ECG abnormalities, a systolic peak murmur was detected via auscultation, and in another an accentuation of the first heart sound. The remaining dogs (n=2) didn't have any ECG abnormalities or deviations in the auscultation of the heart. In all dogs there was no evidence of clinically overt heart failure.

After the preliminary examinations and taking blood samples from *v. cephalica antebrachii* for hematological and biochemical examination (day 0), without pre–feeding, we started feeding the dogs with granulated food "REBEL PLUS" containing *Cannabis sativa* L.

Again, we performed clinical tests and took blood samples on day 15 and day 30 of feeding the dogs with the "REBEL PLUS" food. The complete blood count (CBC) was examined with an Auto Veterinary Haematology Analyzer Mindray BC – 30 VET (Shenzhen Mindray Animal Medical Technology Co., LTD., China).

All parameters were analyzed using a computer program Statistica, v. 6.0.10. (**Informer Technologies, Inc., USA**). To establish the reliability of the obtained differences, we used a parametric method (One–way ANOVA) and a non–parametric method (Mann–Whitney U–test), respectively. Parameter values are presented as Mean values (M) and its Mean Arithmetic Standard Deviation (SD). Differences are considered statistically significant at $P < 0.05$.

Results

Table 1: White blood count (count x10^9/L) and the ratio between the number of neutrophils and lymphocytes Neu/Lym, depending on the days, in dogs fed with granulated food ″REBEL PLUS″ (Mean ± SD).

Parameter	Day 0 (n=5)	15th day (n=5)	30 th day (n=5)	P-value		
				0–15th day (n=5)	15–30th day (n=5).	0–30th day (n=5)
WBC (10^9/L)	10.634±1.549	9.518±1.937	9.388 ± 1.739	0.3438 0.3472	0.9138 0.9168	0.2660 0.1745
Neu (10^9/L)	6.936 ± 1.083	5.976±1.541	5.578 ± 1.342	0.2874 0.3472	0.6747 0.9168	0.1162 0.1745
Lym (10^9/L)	2.014 ± 0.383	2.124±0.468	2.448±0.551	0.6948 0.7540	0.3455 0.2962	0.1862 0.1745
Mo (10^9/L)	0.868 ± 0.182	0.796±0.164	0.76 ± 0.181	0.5293 0.4034	0.7500 0.6761	0.3743 0.2506
Eos (10^9/L)	0.816 ± 0.357	0.622±0.206	0.602±0.098	0.3229 0.2963	0.8494 0.6015	0.2320 0.2506
Neu/Lym ratio	3.488 ± 0.492	2.864±0.676	2.343±0.617	0.1333 0.1745	0.2383 0.2506	0.0117* 0.0163⬩

*Statistically significant difference using One-Way ANOVA: * p - < 0.05; ** - p < 0.01; *** - p < .001.*
Statistically significant difference using Mann-Whitney U-test: ⬩ p - < 0.05; ⬩⬩ - p < 0.01.

The results of the comparison of white blood cell (WBC) count and neutrophil/lymphocyte ratio (Neu/Lym ratio) in dogs fed at 0, 15, and 30 days with "REBEL PLUS" granulated food, containing *Cannabis sativa* L. are presented in Table 1. The table shows that the mean value of the total number of leukocytes (WBC) of the dogs on day 0 was the highest, followed by day 15, and the lowest on day 30 of the conducted study. This trend is also observed with respect to neutrophils

(Neu), monocytes (Mo) and eosinophils (Eos). Only in lymphocytes (Lym) we established a reversed trend. For them, their lowest Mean value is on day 0, followed by day 15, and on day 30 their number is the highest.

Regarding the Neu/Lym ratio, we established that depending on the duration of feeding the dogs with "REBEL PLUS" granulated food, it gradually decreases. Its mean value is the highest on day 0, followed by day 15, and on day 30 the mean value of the Neu/Lym ratio is the lowest. For this ratio, we also established a statistically significant difference between its values on day 0 and day 30 by both methods for calculating statistical significance.

Discussion

In all articles known to us, a decrease in the Neu/Lym ratio is considered an independent predictor of recovery in various pathological states in humans and animals. In this regard, after the conducted examinations, we established a dynamic change of this ratio in dogs which were fed with granulated food "REBEL PLUS" containing *Cannabis sativa* L. for 30 days. The results obtained by us related not only to the change in the total number of leukocytes and the number of individual leukocyte classes but also to the change in the neutrophils/lymphocytes ratio towards a more favourable white blood count, which confirmed what we expected – that this food has a favourable effect on the general health of the dogs fed with it.

A similar trend was observed when examining leukocytes in healthy dogs immediately after transportation and 24 hours after transportation and placement in a new environment. The authors additionally examined several biochemical blood parameters that change during stress (Radisavljević *et al.* 2017). In contrast to their studies in which they established a decrease in total leukocyte count, neutrophil count, lymphocyte count, and Neu/Lym ratio dependent on the attenuation of the stress reaction, in our study, the lymphocyte count dynamically increases depending on the number of the days on which the dogs were fed the tested food. This, in our opinion, is related to the fact that the impact of the granulated food "REBEL PLUS" containing Cannabis sativa L. is not based solely on a beneficial effect on the stress response, which is modulated by the high–quality nutrients contained in cannabis, and above all on Ω6 and Ω3 fatty acids in the very favourable ratio of 3:1 (LA 52–62% – LNA 12–23%), but there are probably other mechanisms that lead to a more favourable white blood count modulated towards a lower tendency to inflammatory reaction and accordingly show a better health condition of the dogs fed with this food (Leizer *et al.* 2000). In our study, dogs started with lower Neu/Lym ratio values compared to the stated–above authors, however, during the course of the study, the Neu/Lym ratio in dogs fed with granulated food ″REBEL PLUS″ gradually decreased even more. We hypothesize that this is further related to the good balance of high–quality nutrients, vitamins, minerals, and probiotics contained in the tested food, which naturally stimulate the organism of the dogs and provide them with better health. The results of our study can be explained by the authors' experimental study investigating the effect of *Cannabis sativa* L. oil (CSO) and nano–emulsion based on the same plant (NCS) on snake venom–induced inflammation in rats. Their results showed that treatment with CSO and NCS was able to significantly reduce ($p<0.001$) the formation of eodema and granulation tissue. The groups additionally treated with cannabis oil or cannabis oil nano–emulsion showed a significant reduction ($p<0.001$) in the number of leukocytes in the peritoneal fluid in the *peritonitis* tests.

The histopathological analysis of the *gastrocnemius* muscle showed a reduction in tissue damage caused by the snake venom (de Oliveira Carvalho *et al.* 2021).

Thus, the authors conclusively prove the anti–inflammatory effect of *Cannabis sativa* L. oil. We assume that this anti–inflammatory effect also affected the dogs fed with granulated food "REBEL PLUS" containing *Cannabis sativa* L. that we tested for 30 days and that the same anti–inflammatory effect could explain the results obtained in our study.

Conclusion

1. The tested food "REBEL PLUS" containing *Cannabis sativa* L. has a favorable effect on the number and ratio of white blood cells of the dogs fed with it.
2. The neutrophils/lymphocytes ratio can also be used in healthy animals to determine the usefulness of the additives used in the food of the dogs.
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