NEW NATURAL FEED SUPPLEMENT FOR DOGS
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Abstract

Dogs fed an appropriate balanced commercial diet should not require any supplements such as vitamins and micronutrients. On the other hand, everyday life stresses can lead to various violations. Consequently, a requirement appears to enrich the diet of animals with antioxidants, immunomodulating and biologically active substances.

R&D Center of Enzym Company in Lviv developed natural feed additive for dog’s everyday diet. The additive combines components to act in synergy: inactive dry yeast – with high antioxidant and prebiotic activity, organic essential microelements – with high bioavailability rate and natural phytocomponents – that provide antioxidant and immune modulating action.

Developed additive was examined in few steps trials:

- on laboratory animals – to establish dosing, exclude toxicity, and analyze basic values.
- on sport dogs – to prove efficacy of additive and its safety, and to find an optimal dosage.

Trials were provided on sport dogs, 10 animals per group, which were fed the diet without developed additive and with 0.3% and 0.6% of additive respectively.

Animals fed with the diet with 0.3% supplement dosage have shown the best performance. We confirmed the stimulation of the antioxidant system in the blood, which is especially important for us, in animals that were given 0.3% supplements in their diets.

The developed feed additive with synergically combined biologically active substances has a stimulating effect on the antioxidant and immune systems confirmed in numerous studies, in a recent study on sporting dogs.

Keywords: inactive yeast, feed supplement, immune response

1. INTRODUCTION

Pet owners nowadays are more aware of, and responsible for, their animal’s welfare. Fortunately, this trend coexists with a rapid increase in the scope of veterinary services for dietary counseling [1]. Both pet owners and practitioners know that an adequate diet is crucial for their animal’s good health [2]. Diets of dogs and cats have shifted, as a result of domestication, from hunting and scavenging to diets formulated for their specific nutritional requirements. Changes in human diets through the development of agricultural practices have fostered this shift [3]. The expansion of the natural pet food segment has led to a wide spectrum of products with different nutritional strategies applied across brands and introducing unique philosophies on what defines a natural pet food product [4]. This variability has led to confusion and disagreement as to the true definition of natural pet foods and natural pet nutrition. Additionally, the application of human food trends often is used to support functional health benefits of natural pet food products despite limited scientific evidence supporting the benefits in companion animals [5].

Today, an important niche in the proper balanced diet of dogs is occupied by natural fortified supplements. The main role of such supplements is to stimulate and maintain the functioning of the body at the proper level. Such supplements enrich the diet with biologically active substances. This allows the daily diet to increase the ability of the animal's body to respond to constant stresses around it: changes in diet, changes in housing conditions, vaccinations, infections, puberty, periods of strain of the immune
system, childbearing, etc. Thus, the main function of natural feed additives is to then maintain the animal’s health, improve quality of life, and increase the lifespan.

In this context supplementation of diets with functional ingredients such as yeast products, some special phytoextracts and amino acid chelates of essential microelements may serve as a potential approach to modulate immune and antioxidative response and increase body resistance. For example, dogs supplemented with yeast products had reduced potential pathogenic bacteria (e.g., Escherichia coli; Clostridium perfringens) and increased beneficial bacteria (e.g., Lactobacillus; Bifidobacterium) in feces [6, 7]. Yeast products may help with digestive upset during diet transition due to the bioactive compounds they possess, which may lead to improved intestinal morphology and integrity, modified gut microbiota, and modulated immune responses [8]. Study from Shen et al. (2009) reported that pigs supplemented with yeast culture had enhanced T helper-1 (Th1) responses in the gut. Increased mucosal immunity, which serves as the first barrier, may prevent pathogens from migrating into the mucosa, thereby mitigating the need to generate an immune response. In another study, the populations of AP cells, including B cells and monocytes expressing surface MHC class II were increased in SCFP-supplemented dogs. AP cells process antigens and present antigen peptides to helper T cells in an MHC class II-dependent manner. As such, MHC class II expression is needed to stimulate helper T cells. In addition, the populations of activated Th1 (IFN-γ-secreting helper T cells) were increased by SCFP. The enhanced Th1 response could be caused by β-glucan, one of the yeast cell wall components that has been shown to trigger Th1 responses [9]. Those findings suggest that SCFP may enhance the capacity of immune cells, especially Th1 responses, to react to antigens entering the body potentially requiring fewer immune cells to maintain health [10].

Some researchers are concentrating on finding the link between gut health and immunity [11]. Some yeast products are tailor-made to target certain functionality, such as immune modulation, cognitive support, gut health, etc.

“ENZYM GROUP” is a Ukrainian company with century-long traditions in baker’s yeast production. Nowadays “ENZYM” produces yeast-based biotechnological products for baking, food, and animal husbandry industries.

A unique Saccharomyces cerevisiae strain is grown using specially developed technology that provides exceptionally high natural antioxidant action: reduction the impact of toxins and pathogens:

- Contains higher amount of glutathione that has the ability to neutralize free radicals before they harm animal cells.
- A natural solution to increase feed efficiency.
- Antioxidant action and strengthened natural protection.
- Improved performance and overall health status.

R&D team developed and tested a natural feed supplement for dog’s everyday diet based on inactive yeasts as main component. The idea of developing a supplement was based on the creation of a complex of exclusively natural ingredients. The components of the feed additive have a synergistic effect. Some components trigger cellular immunity, others - stimulate the balance of the humoral link. Inactive yeast promotes improved digestion, assimilation and is a source of complete protein.

The objective of the present study was to investigate the effects of natural feed supplement based on special inactive yeasts with antioxidant properties in complex with phytoextracts and organic chelates of trace elements in adult dogs.
2. MATERIALS AND METHODS

Formulation and composition

Balanced commercial feed was used to form the dogs' rations. According to the formed groups (see below), an experimental Supplement was added to the feed in different dosages.

**Analytical constituents:** crude protein – 26 %, crude fat – 17 %, crude ash – 5,5 %, crude fiber – 1,2 %, calcium – 1,05 %, phosphorus – 0,8 %, magnesium – 0,09 %, humidity – 9 %.

**Per kg of product:** omega-3 fatty acids – 7,1 g, including EPA (Eicosapentaenoic acid) / DHA (docosohexanoic acid) – 3,8 g, omega-6 fatty acids – 36,4 g. 84,53 % of proteins of animal origin.

**Additives (per kg of product), mg/kg.** Nutritional additives: vitamin A (3a672a): 15 000 IU, vitamin D3 (3a671): 800 IU, vitamin E (3a700): 500, vitamin C (3a300): 200, vitamin B6 (3a831): 2,22, biotin (3a880): 0,14, taurine (3a370): 1 400, zinc (3b603):104,96, copper (3b405): 11,24, manganese (3b502): 2,57, iodine (3b201): 1,44, selenium (3b801): 0,08. Technological additives: natural antioxidants: 330, tocopherol-rich extracts of natural origin: 600.

**Metabolizable energy per 100 g of petfood:** 1681,09 kJ (401,8 kcal). The manufacturing and best before date, lot number are on the package. Store in a cool, dry place, avoiding direct sunlight. In addition to the control diet, two test diets containing the experimental supplements at two different levels of inclusion were created for this testing purpose. (Table 1).

**Table 1. Diets produced for the research.**

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Experimental supplement %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control or Diet 1</td>
<td>Standard commercial balanced diet for dogs</td>
<td>0</td>
</tr>
<tr>
<td>Test 1 or Diet 3</td>
<td>Standard commercial balanced diet for dogs</td>
<td>0.3</td>
</tr>
<tr>
<td>Test 2 or Diet 5</td>
<td>Standard commercial balanced diet for dogs</td>
<td>0.6</td>
</tr>
</tbody>
</table>
Table 2. Declared and analyzed proximate values (provided by Nutrilab, Netherlands).

<table>
<thead>
<tr>
<th>Name</th>
<th>Declared</th>
<th>Diet 1, 3 and 5</th>
<th>Diet 1</th>
<th>Diet 3</th>
<th>Diet 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>9</td>
<td>8</td>
<td>7,5</td>
<td>7,5</td>
<td></td>
</tr>
<tr>
<td>Crude protein</td>
<td>26</td>
<td>28,4</td>
<td>28,4</td>
<td>27,5</td>
<td></td>
</tr>
<tr>
<td>Crude fat</td>
<td>17</td>
<td>15,2</td>
<td>15,3</td>
<td>16,7*</td>
<td></td>
</tr>
<tr>
<td>Crude fibre</td>
<td>1,5</td>
<td>1,5</td>
<td>1,3</td>
<td>1,2</td>
<td></td>
</tr>
<tr>
<td>Crude ash</td>
<td>5,5</td>
<td>6,2</td>
<td>6,2</td>
<td>5,6</td>
<td></td>
</tr>
<tr>
<td>NFE, Nitrogen-free extract</td>
<td>40,7</td>
<td></td>
<td>41,3</td>
<td>41,5</td>
<td></td>
</tr>
<tr>
<td>ME (metabolic energy), kcal pr kg</td>
<td>401,8</td>
<td>371,05</td>
<td>374</td>
<td>383,45</td>
<td></td>
</tr>
</tbody>
</table>

Animals in the study

Thirty healthy pet dogs were engaged in the study. All dogs belonged to training dogs of medium activity (dogs participating in sports activities). Tests were conducted on active sports dogs aged 1 to 11 years. Most of the dogs were between 1 and 8 years old, only two dogs were 11 years old.

All dogs were placed on Diet 1 (control) for three weeks. The owners received instructions on feeding strategy and were allowed to give only limited amount of the pure meat treats (only if necessary). This period in the graphical results is referred to as «Before». After three weeks, the dogs were randomized in the three groups by using the MINITAB 21. The responsible veterinarian examined the dog’s general condition and collected blood. Before starting the test diets, the project leader collected the stool samples for each dog and placed them at -20°C. The food was distributed to owners for a further four weeks feeding period. After four weeks, dogs were examined for general health and blood and stool samples have been collected again.

The trials have been reported to Norwegian Food Authorities and approved under approval number: FOTS id 28938.

Trials scheme (Table 3).

Table 3. Experimental design

- Control diet 1 (Diet 1): Three weeks adaptation on a control diet
  - Four weeks standard diet - trials

- Experimental diet 3 (Diet 3): Three weeks adaptation on a control diet
  - 4 weeks experimental diet (with supplement 1) - trials

- Experimental diet 5 (Diet 5): Three weeks adaptation on a control diet
  - 4 weeks experimental diet (with supplement 2) - trials
Blood parameters analyzed were as follows:

Hematology and Antioxidant package: GPx (Glutathione peroxide), SOD (superoxide dismutase), TAS (Total antioxidant status).

Statistical analyses and presentation methods

Statistical analyses were performed with MINITAB 21 program and some graphical presentations. ANOM and Fisher grouping tests were used to present statistical differences.

The results were presented in the form of comparative graphs depending on the period of research - before feeding experimental diets and after including supplements in diets.

3. RESULTS AND DISCUSSION

We decided to concentrate on the hematological indexes, the indicators that characterize the reaction of defense systems to the new feed component, protein fractions and total protein contents in the blood of animals among the large number of investigated parameters. During the entire time of the trial, the dogs have shown good overall health status.

The potential effects of different diets, especially raw food diets, on blood parameters have been poorly investigate. Most studies investigate the effects of age, breed, and gender on blood parameters [12, 13]. Since blood parameters are an important health assessment tool in disease diagnostics [14], it is important to know what can influence the values.

We included complex consisting of inactive yeast, organic chelates of microelements and phytoextracts to the diet. Therefore, first, we wanted to investigate the effect of these components on basic blood parameters.

The values obtained from blood analyses were mainly in the physiological range of healthy animals. There were no significant differences between the groups regarding the red blood count analyses (Figure 1).
Figure 1. Red blood cell count before and after four weeks of consumption of diets containing Supplement.

Adaptation – adaptation period (3 weeks), Trials – experimental period (4 next weeks).

Abbreviations and unit of measurement: RBC, $10^{12}$/L — red blood cells; Hgb, g/L — hemoglobin concentration; HCT, ml/L — hematocrit; MCV, fL — mean corpuscular volume; MCH, pG — mean corpuscular hemoglobin; MCHC, g/L — mean corpuscular hemoglobin concentration.

The RBC (red blood cells), hemoglobin and hematocrit were slightly increased for the groups fed with Diet 3 and Diet 5 (with experimental Supplement in the dose 0.3% and 0.6%, respectively (Figure 1). Hematologic and biochemical values are important measurements when evaluating the health of the patient. When assessing blood components both physiological and pathological changes can be seen indicating disease or other abnormalities [15]. We recorded minor changes in blood parameters in animals of all groups at the last stage of the research, the effect of the Supplement.

We assume that some of the Supplement components could cause slight increase in hemopoiesis and hemoglobin synthesis. But basically, all parameters were in physiological limits and cannot indicate a certain significant effect on the indicators of red blood.

Saied et al showed [16] were studying the effect of feed supplement with Saccharomyces cerevisiae in it composition. They conclude that heterophils, basophiles, eosinophils, basophiles, and neutrophils to lymphocyte ratio were not affected by dietary treatments, but the inclusion of yeast culture of Saccharomyces cerevisiae didn’t affected monocytes counts. The hematocrit and hemoglobin concentration were not affected by the inclusion of yeast culture in the diet [16]. The white blood cell count showed linear and quadratic increases in broilers fed increasing concentrations of mixed yeast culture (MYC; Saccharomyces cerevisiae YJM1592 and Kluyveromyces maxianus TB7258 in a 1:1 ratio) [17].

WBC (white blood cells) are part of the body’s immune system. They fight infection and other diseases. increase may be due to inflammation, stress, excitement, and leukemia; decreases may be due to overwhelming inflammation and bone marrow failure. Various patterns of change in numbers of NEU (neutrophils), LYM (lymphocytes), MONO (monocytes), EOS (eosinophils), and BASO (basophils) may be seen with different types of inflammation, stress, excitement, and leukemia.

White blood cells indexes were in the physiological values for all groups. There were no major differences between groups and trials periods. There was a slight reduction trend in WBC parameters in
each group after experimental diet was included to the ration. The Diet 5 had the least drop compared to Diet 1 and Diet 3. However, the differences and drops are insignificant (Figure 2).

Figure 2. White blood cell count before and after four weeks of consumption of diets containing Supplement.

Adaptation – adaptation period (3 weeks), Trials – experimental period (4 next weeks).

Abbreviations and unit of measurement: WBC, \(10^9/L\) — white blood cells; Monocytes, Lymphocytes and Neutrophils, %.

Therefore, the components of the feed additive did not have negative effect on the content of blood components. The data of other authors regarding the effect of the Supplement components on hematology in dogs confirms these results. Although sometimes researchers still note a certain influence on the basic indicators of blood in animals.

Arabinogalactan treatments did not affect (\(P > 0.05\)) serum immunoglobulin G, M or A concentrations. Specific forms and doses of AG increased white blood cell, neutrophil and eosinophil concentrations. Arabinogalactan is a unique dietary fiber that affects the digestive physiology and immunological characteristics of dogs [18].
TP (total protein) increases may indicate dehydration or an inflammatory condition; decreases may be seen in decreased liver function, blood loss, gastrointestinal loss and kidney loss. Total protein simply signifies the sum of the next two chemicals on the list, albumin and globulin. They are the two major sub-types of protein in blood serum. ALB (albumin)— increases may indicate dehydration; decreases may be seen with decreased liver function, blood loss, gastrointestinal disease, and kidney disease. As for albumin levels running too high, the only common reason is dehydration. When there’s too little water in the blood, the absolute amount of albumin stays the same, but the concentration increases. GLOB (globulin) increases may be seen during inflammation and potential chronic infection; decreases may be seen because of blood loss, gastrointestinal loss and immune deficiencies. Globulins are a combination of proteins, larger than albumin, that are important for fighting infections. They are key components of antibodies, which are made by certain lymphocytes. Thus, globulin levels will often go up when a dog is mounting a response to an infectious condition, or even a response to inflammation.

The globulin and albumin content increased in the blood of dogs in group D3 after the trial period. The growth rate did not go beyond the norm and did not pose any threat to the animals. The increase in albumin has been recorded in group D5 after finishing trial period (Table 3).

Table 3. Protein parameters before and after four weeks of consumption of diets containing Supplement.

<table>
<thead>
<tr>
<th></th>
<th>Total Protein g/L</th>
<th>Albumin, g/L</th>
<th>Globulin, g/L</th>
<th>Albumin: Globulin Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1 (control)</td>
<td>65.01</td>
<td>30.75</td>
<td>34.25</td>
<td>0.91</td>
</tr>
<tr>
<td>Adaptation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D1 (control)</td>
<td>65.75</td>
<td>31.63</td>
<td>34.25</td>
<td>0.94</td>
</tr>
<tr>
<td>Trials</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D3 (0.3%)</td>
<td>66.51</td>
<td>30.30</td>
<td>36.3</td>
<td>0.86</td>
</tr>
<tr>
<td>Adaptation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D3 (0.3%)</td>
<td>69.44</td>
<td>31.22</td>
<td>38.33</td>
<td>0.84</td>
</tr>
<tr>
<td>Trials</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D5 (0.6%)</td>
<td>65.02</td>
<td>30.88</td>
<td>34.25</td>
<td>0.91</td>
</tr>
<tr>
<td>Adaptation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D5 (0.6%)</td>
<td>66.25</td>
<td>33.00</td>
<td>33.375</td>
<td>0.99</td>
</tr>
<tr>
<td>Trials</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

The increase in albumin can be due to the dehydration or due to reduced liver function. All liver enzymes and bilirubin were as well increased for the dogs in the D5 group. Therefore, this should be taken in to the consideration as D5 diet may give higher load to the liver function.

Globulins help fight infection and move nutrients throughout the body. The globulin fraction includes hundreds of serum proteins including carrier proteins, enzymes, complement. Some globulins are made by the liver. Others are made by the immune system. We found slight increase in globulins content in the blood of animals that received 0.3% of Supplement in the diet. All protein fractions and total protein content were within normal limits in the blood of all animals.

When processing the obtained results, we often analyzed and compared them with the results obtained by other researchers when they added to the diet components similar in composition to our supplement.

A diet with low amounts of protein can be detected using blood analysis. A study on the effects of low-protein diets on dogs’ blood metabolites showed that biochemical values agreed with protein metabolism either increased or decreased due to protein malnutrition [19]. For example, the albumin and total protein concentrations decreased below normal ranges whereas ALP (Alkaline phosphatase) concentrations increased over the upper reference range when feeding a protein deficient diet.
Antioxidants status

Glutathione peroxidase (GPx) plays its role to protect the organism from oxidative damage. Superoxide dismutase (SOD) is an important antioxidant defence in living cells exposed to oxygen. Free are produced during normal cellular metabolism. Antioxidants are the first line of defence and are produced by the body to neutralise the harmful effects preventing cellular damage. TAS, total antioxidant status is a quantitative measurement of the state of balance of the various components. The results are not significantly different between the groups however the D3 has shown increase in both GPx and SOD values. SOD activity was higher by 39.6% in the blood of animals that received 0.3% of Supplement during trial period compared to the level of enzyme activity before Supplement was included. GPx activity was higher by 12.4% in the same groups. TAS values show a balanced antioxidant system for all groups (Figure 4). Our Previous research done on mice as well shows increased antioxidative effects in the blood when Supplement was used. The same trial showed increased phagocytic activity. At the same time in both other groups opposite effect was found.

![Graph](image)

**Figure 4.** Delta of changes of Antioxidant activity between Adaptation and Trials periods results.

Obviously, we confirmed that Supplement rich in natural antioxidants improves first main links of endogenous antioxidant defense system. But this effect was dose depended. Changes were not significant, but research of other authors using the components of our Supplement as well, confirm the data we obtained. Probably longer studies could also show more significant changes. Specially fermented inactive yeast with antioxidant properties could activate glutathione system and improve content of reduced glutathione. Glutathione (L-glutamyl-L-cysteinyl-glycine) is a tripeptide which contains three constitutive amino acids: glutamate, cysteine, and glycine. It comes from the natural metabolism of Saccharomyces cerevisiae yeasts. Glutathione is important in its reduced form – GSH, since it has the ability to scavenge toxic oxidative radicals and improve stability of biological membranes. Antioxidant enzymes serve as defense system under any kind of stress impact [20].

Interesting impact on antioxidant defense system except our work was showed in the trials of other authors as well. Zhu et al. investigated the effect of yeast *S. cerevisiae* supplementation on serum antioxidant capacity and gut microbial populations in weaned piglets. They showed an increase in serum superoxide dismutase (SOD) activity and a decrease in serum malondialdehyde concentration (P < 0.05). Zhu et al. suggested that various forms of yeast with very important antioxidant enzyme systems, such as SOD or catalase, may the body’s antioxidant capacity and boost the intestinal immunity of weaned piglets [21]. Nido et al. reported that the body weight and liver malondialdehyde level of mice fed a high-fat diet decreased significantly after treatment with selenium enriched *S. cerevisiae* [22].
In our experiments, we formed groups of sport dogs that are actively training. Sports and exercise served as a stress factor in our experiments. Because sports training as a stressor can be a good factor to study the effects of stress on its basis. That is why based on such background, we determined the effects of adding a Supplement to the diet of exactly sport dogs.

Thus, it is worth introducing functional supplements into the diet of sporting dogs, which can increase the dog’s … while minimizing the effects of exhaustion and maximizing the body’s regeneration process during rest. A well-conducted conditioning training requires supplementation to support, among other things, muscle development, joint protection and regeneration, and antioxidant activity [23]. Among other components our Supplement contained organic trace elements. Selenium, zinc, copper, and manganese contribute to the proper function of the enzymatic antioxidant system. Thus, they prevent an excessive increase in the content of reactive oxygen species caused by exercise. Selenium is important for correct function of glutathione peroxidase (GPx), which protects cells from damage under oxidative stress caused by injury, infection, or inflammation [24]. In turn, considering the role of zinc in antioxidant systems, such as those involving superoxide dismutase and catalase, maintaining optimal levels of zinc may be the key in mediating oxidative stress in skeletal and systemic muscles [25]. Sporting dogs need more zinc than non-training dogs. Therefore, raw materials rich in this element are worth thinking about.

Besides organic microelements, other components of Supplement have antioxidant properties as well. Larch arabinogalactan is considered a good source of dietary fiber and has been approved as such by the FDA [26]. It also has potential therapeutic benefits as an immune-stimulating agent and as cancer protocol adjunct. Larch bark (source of arabinogalactan) extract shows antioxidant activity comparable to ascorbic acid, which is known to be a powerful antioxidant. In depth studies are needed, but the overall results indicate that the bark of the larch is a good source of antioxidant compounds [27].

Therefore, we tried to create a synergistic product in terms of its biological activity. Suitable for the daily diet to improve the protective reserves of animals’ organism. We have conducted numerous studies on this topic, which will be published later after their comprehensive analysis.

**CONCLUSIONS**

During the seven weeks of the experimental period, dogs were in good health, although some individual dogs showed signs of non-food-related health issues. During the experimental period, no adverse effects have been recorded on the dog's general health. It is important that no significant differences have been recorded. The dogs were on the test diets for four weeks. The longer run should be considered with more animals in the group to see significant differences. Although no significant changes happened, some relevant trends appeared.

No significant effects related to the red blood cell counts and other relevant parameters have been recorded. There were no significant effects related to changes in the white blood cell parameters. Platelets parameters slightly dropped for the animals that consumed the D3 and D5 diets.

The activity of antioxidant enzymes such as GPx and SOD in the blood of dogs in group Diet 3 was much higher after trial period compared to adaptation stage. This can be of high relevance for the fortifying of the immune system and protection and regeneration of the cells, even though was not the increase was not significant a tendency was observed, and longer trials are recommended to fortify this thesis.
REFERENCES


