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THE EFFECTIVENESS OF NATURAL AND SYNTHETIC IMMUNOMODULATORS IN THE TREATMENT OF INFLAMMATORY BOWEL DISEASE IN DOGS

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The aim of the study was to evaluate the usefulness of immunomodulators in the treatment of inflammatory bowel disease (IBD) in dogs. Twenty-eight dogs diagnosed with IBD took part in the study. The animals received a food containing extruded immunomodulators: β-1,3/1,6-D-glucan, β-hydroxy-β-methyl-butyrate (HMB) and levamisole for 42 days. Whole blood samples were analysed before and after therapy assessing changes in phagocyte activity (respiratory burst activity, RBA and potential killing activity, PKA), evaluation of proliferation response of mitogen-stimulated lymphocytes and serum gamma globulin levels, lysozyme activity, ceruloplasmin levels and interleukin activity (IL-6 and IL-10). In this experiment, β-1,3/1,6-D-glucan delivered the highest level of treatment efficacy by producing the quickest therapeutic effect, lowering Canine Inflammatory Bowel Disease Activity Index (CIBDAI) values to below 3, improving histopathological parameters, decreasing IL-6 levels, increasing IL-10 concentrations, and producing remission periods longer than six months. HMB and levamisole were also effective in lowering CIBDAI scores, but the abatement of clinical symptoms was slower and less pronounced in comparison with β-1,3/1,6-D-glucan. The results indicate that β-1,3/1,6-D-glucan can be useful in the treatment of canine IBD.

Key words: Immunomodulators, inflammatory bowel disease, interleukin-6, interleukin-10, dog

The aetiopathogenesis of inflammatory bowel disease (IBD) has not been fully elucidated to date. According to numerous research sources, the oversensitivity of intestinal lymphatic tissue to intestinal antigens is a key contributor to the disease. Inflammations of the intestinal tract result from the activation of T helper lymphocytes, B lymphocytes and the production of proinflammatory cytokines (Mancho et al., 2010; Simpson and Jergens, 2011; Jergens and Simpson, 2012). The immune system plays a significant role in the development of IBD, which is why recent years have witnessed a growing number of investigations

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into the use of immunomodulators in the treatment of inflammatory bowel disease in humans and animals. Immunotherapy not only boosts immunity, but it also aims to elicit the correct immune response. Immunomodulators are a group of drugs and xenobiotics that affect the immune system. They enhance humoral and cellular activity by specific and nonspecific mechanisms of action (Markowitz et al., 2002). Natural immunomodulators, in particular beta-glucans, as well as synthetic immunomodulators such as levamisole are becoming increasingly popular in the treatment of immune disorders (Ramberg et al., 2010). β-1,3/1,6-D-glucan, β-hydroxy-β-methyl-butyrate (HMB) is also a promising substance due to its ability to stimulate muscle tissue growth and protect muscle proteins (Kornasio et al., 2009). The above three compounds are common drugs of choice in human and veterinary medicine, but they have never been used in the treatment of canine IBD. The discussed immunostimulants could boost resistance to bacteria and modify cellular activity and humoral immune responses in the gastrointestinal tract of animals with mild to moderate IBD. There is a lack of research investigating the effectiveness of immunomodulators in the treatment of IBD. Therefore, the objective of this study was to compare the efficacy of selected natural and synthetic immunomodulators in the treatment of inflammatory bowel disease in dogs.

Materials and methods

The study was performed on 28 dogs of both sexes and various breeds, aged from two to six years, with a body weight from 5 to 30 kg, admitted to the Veterinary Clinic of the University of Warmia and Mazury in Olsztyn. The study obtained the approval of the local research ethics committee. The animals selected for the experiment displayed symptoms characteristic of inflammatory bowel disease, including chronic diarrhoea specific of the small intestine, and vomiting of varied intensity and frequency. Complete blood count (CBC), serum biochemistry including canine pancreas-specific lipase (spec cPL) and trypsin-like immunoreactivity (TLI), ultrasound (with Doppler) and radiographic examinations were performed to exclude systemic disease. Faecal exams ruled out parasitic and bacterial infections. Furthermore, the animals received oxytetracycline (20 mg/kg, every 8 hours). A positive response suggests antibiotic-responsive diarrhoea (ARD). After the antibiotic trial, all animals received a hypoallergenic diet (Royal Canine Hypoallergenic). Dogs, which showed a positive response and were rechallenged with the original diet, were not qualified for the study (Simpson, 2010; Simpson and Jergens, 2011). The animals were finally classified for the experiment based on the results of endoscopic and histopathological analyses of intestinal mucosa sections. The final group of 28 dogs comprised animals with mild to moderate IBD and Canine Inflammatory Bowel Disease Activity (CIBDAI) scores in the range of 4 to 8. Histopathological evaluations of

intestinal mucosa sections were performed in accordance with the recommendations formulated by the World Small Animal Veterinary Association (WSAVA) Gastrointestinal Standardization Group in 2008 (Day et al., 2008). The histopathological criteria evaluate: (1) villous stunting, (2) epithelial injury, (3) crypt distension, (4) lacteal dilation, (5) mucosal fibrosis, (6) intraepithelial lymphocytes, (7) lamina propria lymphocytes and plasma cells, (8) lamina propria eosinophils, (9) lamina propria neutrophils, and (10) other. The intestinal biopsy samples were graded histopathologically (a grading scale from '0' to '+++' was used, in other publications graded with numbers) as mild lesions '+', moderate lesions '++' and severe lesions '+++' (Willard et al., 2008; Washabau et al., 2010; Simpson and Jergens, 2011). The grading scale for histological assessments did not exceed '++', suggesting the presence of mild to moderate changes in the small intestine which can be treated with immunomodulators without adverse effects on the patient's health. Every dog was diagnosed with lymphoplasmacytic enteritis (LPE) of the small intestinal mucosa. Based on the immunostimulants used in the study the animals were randomly divided into four groups:

– Group I comprised seven dogs aged from two to six years, including four males and three females. Over a period of six weeks, the patients were administered dry food supplemented with β-1,3/1,6-D-glucan, Biolex-Beta HP manufactured by Inter Yeast Poland, at a dose of 7 mg/kg body weight (bwkg);

– Group II comprised seven dogs aged from two to four years, including five females and two males. Over a period of six weeks, the patients were administered dry food supplemented with β-hydroxy-β-methyl butyrate (HMB) at 30 mg/bwkg;

– Group III comprised seven dogs aged from three to six years, including three females and four males. The animals were administered dry food supplemented with an immunostimulating dose of levamisole at 2 mg/bwkg twice every five days, and the same dry food without supplementation was administered on the remaining days;

– Group IV was the control, and it comprised seven dogs with IBD, aged from two to five years, including three females and four males, which were fed identical dry food without supplementation.

All the animals that took part in the study were fed with dry food produced by Bestfeed LTD Poland, which, depending on the group, was extruded with the aforementioned immunomodulators. The patients of Group IV received only dry food to eliminate dietary influence on clinical, histopathological and laboratory parameters. The nutritional composition of the applied feed supported healthy growth of adult dogs, and it did not contain any therapeutic additives that are commonly found in medicated feeds for the treatment of gastrointestinal disorders.

The animals were subjected to clinical evaluations, and the value of CIBDAI was determined in all dogs, as proposed by Jergens et al. (2003). CIBDAI values were determined before the experiment, after six weeks (at the

end of immunomodulation), and once a month in the course of six successive months to determine treatment efficacy and IBD recurrence rates. Haematological, biochemical and immunological examinations were performed before and after the experiment (day 42). Endoscopic examinations, including the collection of mucosal samples from the anterior section of the duodenum and the jejunum, were performed before the experiment and after six weeks. Six biopsy samples were collected from every region.

The immunological analyses of whole blood samples involved the isolation of immunocompetent cells and the determination of nonspecific cellular activity parameters. The metabolic activity of phagocytes – the levels of respiratory burst activity (RBA) stimulated by phorbol myristate acetate (PMA) were analysed by the spectrophotometric method (620 nm) described by Chung and Secombes (1988). The intracellular killing of phagocytes (PMN – polymorphonuclear and MN – mononuclear), i.e. the potential killing activity (PKA) was assessed by a spectrophotometric method (Rook et al., 1995).

The analyses of specific cellular activity parameters included: the evaluation of proliferation response of mitogen-stimulated lymphocytes (Con A – concanavalin A, LPS – lipopolysaccharides, PHA – phytohaemagglutinin) was studied by the 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide (MTT) method described by Mosmann (1983). Furthermore, the serum concentration of the gamma globulin fraction (Siwicki and Anderson, 1993), lysozyme activity by spectrophotometric assessment (Parry et al., 1965) modified by Siwicki and Anderson (1993), ceruloplasmin levels by the spectrophotometric method described by Siwicki and Studnicka (1986) and interleukin (IL-6 and IL-10) activity levels determined by enzyme-linked immunosorbent assay (ELISA) tests (R&D Systems, Canine IL-6 Quantikine ELISA Kit and Canine IL-10 Quantikine ELISA Kit) were assessed.

The laboratory results are expressed in SI units and were statistically analysed with the STATISTICA 9 data analysis software. The significant differences between the dog groups at the beginning and at the end of the experiment were analysed by Friedman analysis of variance test. The examination of significant differences between the starting and ending values of certain parameters was done by Kruskal–Wallis test and Student's *t*-test for dependent variables. In each test, two levels of significance were established and marked with '*' as significant difference (P = 0.05) and '**' as highly significant difference (P = 0,01); the absence of these symbols indicates a nonsignificant difference.

Results

Before the experiment, CIBDAI values did not exceed 8 points in any of the 28 dogs classified for the experiment. The patients were diagnosed with mild

to moderate IBD. After the experiment, CIBDAI scores decreased in all groups except the control (Table 1). In Groups I and II, the difference in CIBDAI before and after immunomodulatory therapy was statistically significant. In the course of six months after the experiment, CIBDAI values were measured on a monthly basis to determine IBD recurrence rates. An absence of recurrence was observed only in Group I. Recurrence rates reached 14% in Group II and 43% in Group III (Table 1). Group IV patients had not undergone treatment.

Table 1

Changes of CIBDAI index during the study and recurrence rate – statistical comparison between dogs before and after therapy

Group	CIBDAI		n	Dogs with clinical symptom recurrence (CIBDAI ≥ 4)	Recurrence percent (%)
	Before immunomodulation (mean ± SD)	After immunomodulation (mean ± SD)			
I	6.000 ± 1.45	0.914 ± 0.45**	7	0	0
II	6.285 ± 2.12	1.286 ± 0.54*	7	1	14
III	6.143 ± 1.98	2.142 ± 0.89	7	3	43
IV	5.857 ± 1.84	7.142 ± 0.11	7	7	100

Mean ± SD: mean ± standard deviation; *significant difference at P = 0.05; **significant difference at P = 0.01; statistical comparison between dogs before and after therapy

In Groups I, II and III, immunomodulation improved the appearance and structure of the small intestinal mucosa on the basis of macroscopic and histopathological evaluations. As previously noted, no improvement was observed in Group IV (Table 2).

Immunological evaluations of nonspecific cellular activity and humoral immunity revealed statistically significant changes in all the experimental groups (Table 3).

The following differences in nonspecific humoral immune response were observed in each group between day 0 and day 42, subject to the analysed parameter, but no statistical difference was observed. The concentration of proinflammatory IL-6 showed a statistically significant decrease in Groups I and II; in Group III there was no statistically significant difference, whereas in Group IV a statistically significant increase was observed between day 0 and day 42. The highest drop in IL-6 levels was noted in Group I (91%), followed by Group II (89%) and Group III (88%). The levels of the anti-inflammatory (immunomodulant) cytokine IL-10 increased in all experimental groups with the exception of Group IV. The observed difference was highly significant in Group II and significant in Group I. No differences in IL-10 concentrations were noted in Group III and in the control group (IV) between day 0 and day 42 of the experiment.

Table 2

Macroscopic and histopathological evaluation of the duodenal mucosa according to the World Small Animal Veterinary Association (WSAVA), 2008

Group	No. of dog	Before immunomodulation		After immunomodulation	
		Macroscopic duodenal evaluation	Histopathological duodenal evaluation	Macroscopic duodenal evaluation	Histopathological duodenal evaluation
I	1	+++	++	+	+
	2	++	++	N	N
	3	++	++/+	N	N
	4	++	++	N	+
	5	+++	+	N	N
	6	++	++	N	N
	7	++	++	+	+
II	1	+++	++	+	+
	2	+++	++	+	+
	3	++	++/+	N	N
	4	++	++	N	N
	5	+++	++	++	++
	6	++	+	N	N
	7	++	++	+	+
III	1	++	+	+	+
	2	+++	++	+	+
	3	+++	++	+	N
	4	++	++/+	N	N
	5	++	+	+	N
	6	+++	++	N	+
	7	++	++	+	+
IV	1	++	+	++	++
	2	+++	++	++	++
	3	+++	++	++	+++
	4	++	++/+	++	++
	5	++	++/+	+	++
	6	++	++	++	+++
	7	++	+	+	++

Macroscopic evaluation: N: no lesions; +: mild lesions; ++: folding of the mucosal membrane, mild rubor, few effusions; +++: significant folding of the surface of duodenal mucosa, significant rubor, many effusions; Histopathological evaluation defining changes in intestinal mucosa and the degree of cellular infiltration according to WSAVA (2008): N: normal structure; +: mild lesions; ++: moderate lesions; +++: significant lesions

Discussion

In the present experiment, immunomodulating agents were used to monitor changes in cellular activity and humoral immunity and to evaluate the effect of immunomodulation on the intestinal mucosa of dogs with IBD. The adminis-

Table 3

Average values and standard deviation of humoral immune response and cell activity for every group before and after immunomodulation. Statistical comparison between dogs before and after therapy

		Group I		Group II		Group III		Group IV	
		Before supplementation	After supplementation	Before supplementation	After supplementation	Before supplementation	After supplementation	Before supplementation	After supplementation
Lysozyme	mg/L	1.621 ± 0.221	1.389 ± 0.244	1.406 ± 0.208	1.419 ± 0.285	1.520 ± 0.354	1.516 ± 0.216	1.177 ± 0.178	1.286 ± 0.281
Ceruloplasmin	IU	51.800 ± 4.338	50.986 ± 4.966	50.857 ± 3.019	54.500 ± 3.634	55.271 ± 2.396	52.829 ± 3.861	51.086 ± 5.138	51.057 ± 4.417
γ-Globulins	g/L	11.929 ± 0.886	10.214 ± 1.948	13.529 ± 1.327	10.329 ± 3.844	11.543 ± 1.665	11.114 ± 1.817	13.057 ± 1.569	12.886 ± 1.574
RBA	A	0.434 ± 0.034	0.704 ± 0.024**	0.446 ± 0.021	0.603 ± 0.069**	0.444 ± 0.048	0.631 ± 0.027**	0.429 ± 0.038	0.396 ± 0.030
PKA	A	0.704 ± 0.029	0.589 ± 0.016**	0.35 ± 4 0.018	0.516 ± 0.063**	0.349 ± 0.025	0.547 ± 0.026**	0.347 ± 0.023	0.363 ± 0.021
Con A	A	0.453 ± 0.028	0.706 ± 0.019**	0.473 ± 0.010	0.646 ± 0.035**	0.447 ± 0.031	0.601 ± 0.010**	0.451 ± 0.021	0.463 ± 0.025
LPS	A	0.360 ± 0.015	0.551 ± 0.010**	0.356 ± 0.010	0.480 ± 0.042**	0.337 ± 0.024	0.480 ± 0.038**	0.349 ± 0.012	0.357 ± 0.017
IL-6	pg/mL	63.500 ± 40.901	5.557 ± 2.104*	47.72 ± 9 11.774	5.214 ± 1.456**	34.714 ± 31.917	4.114 ± 2.058	34.214 ± 5.417	37.986 ± 34.882*
IL-10	pg/mL	13.929 ± 10.307	32.85 ± 7 15.853*	11.343 ± 6.614	28.214 ± 9.310**	14.014 ± 6.475	22.471 ± 10.562	18.471 ± 20.501	17.786 ± 19.630

Values are given as mean ± standard deviation; IU: International Units; A: absorbance; RBA: Respiratory Burst Activity; PKA: Potential Killing Activity; Con A = Concanavalin A; LPS: Lipopolysaccharide; IL-6: Interleukin-6; IL-10: Interleukin-10; *significant difference at P = 0.05; **significant difference at P = 0.01; statistical comparison between dogs before and after therapy

tered immunostimulants improved cell activity and humoral immunity parameters in all groups except the control group. Immunomodulation significantly improved the cellular activity of animals in Groups I, II and III. A highly significant increase in the respiratory burst activity (RBA) of phagocytes was reported in all experimental groups (I, II and III) after 42 days of treatment (Table 3). No significant differences were observed in Group IV (control) at the end of the study. Elevated RBA values indicate that immunostimulation supported effective pathogen elimination by phagocytes. The highest increase in RBA values was induced by β-1,3/1,6-D-glucan, whereas the remaining two immunomodulating agents had a somewhat smaller impact on RBA levels. After day 42 of the experiment, a highly significant increase in the potential killing activity (PKA) of phagocytes was also observed in the experimental groups (Table 3). Higher RBA and PKA levels contribute to the elimination of pathogenic microflora which plays an important role in the pathogenesis of IBD (Mitsuyama et al., 2002).

Immunomodulant therapy enhanced the proliferative activity of blood lymphocytes stimulated with Con A and LPS (MTT assay) in dogs, and the noted increase was significant in all experimental groups (Table 3). No significant differences were reported in the control group. The results obtained in the analysed groups varied depending on the mitogen applied. In the MTT assay, β-1,3/1,6-D-glucan proved to be the most potent immunomodulant regardless of mitogen type (Con A, LPS), whereas HMB and levamisole were somewhat less effective. The powerful immunostimulating effect of 1,3/1,6-D-glucan on cell activity has been recognised by other researchers (Volman et al., 2008). In our experiment, we used the Biolex-Beta HP supplement containing 1,3/1,6-D-glucan in almost unmodified form with 85% purity, which could have additionally contributed to the improvement in lymphocyte- and granulocyte-dependent immune responses in the dogs studied.

Our results indicate that 1,3/1,6-D-glucan, HMB and levamisole significantly influence selected parameters of lymphocyte- and granulocyte-dependent immune responses in dogs with IBD. An increase in the metabolic activity of blood phagocytes and higher RBA levels support the elimination of pathogens. This is very crucial in IBD, where microflora is recognised as an important factor inducing immunological imbalance of the alimentary tract, which may lead to the development of inflammation of the intestinal mucosa (Suchodolski et al., 2010; Jergens and Simpson, 2012). Enhanced cell-mediated immunity contributes to the effective elimination of pathogens and, consequently, the alleviation or complete subsidence of the inflammatory process. Of the immunomodulating agents studied, 1,3/1,6-D-glucan had the most expressed stimulating effect on cellular activity.

An analysis of the humoral defence parameters did not reveal significant differences in lysozyme activity, ceruloplasmin activity or gamma globulin levels in the blood serum of immunostimulated dogs, but significant variations were observed in the concentrations of the proinflammatory cytokine IL-6 and the anti-inflammatory cytokine IL-10. The involvement of IL-6 in the pathogenesis of

chronic intestinal inflammations has been widely documented in both human and veterinary medicine (Carey et al., 2008). In our study, a drop in IL-6 levels was noted in all immunostimulated groups after 42 days of the experiment. Highly significant differences were reported in Group II and significant differences were observed in Group I. In Group IV, a significant increase in IL-6 levels was noted after 42 days of the experiment, and this was confirmed by the results of macroscopic and histopathological evaluations indicating progressing inflammation of the small intestine (Table 3). High IL-6 concentrations in Group IV were also correlated with elevated CIBDAI scores (5–9 points). In Groups I, II and III, high initial IL-6 levels were correlated with an increase in CIBDAI values (4–8 points). A decrease in serum IL-6 concentrations, accompanied by a significant drop in CIBDAI scores, was observed in all immunostimulated groups. In a study by Nielsen et al. (2005), IL-6 was found to significantly contribute to inflammations of the gastrointestinal tract in patients affected by Crohn's disease and ulcerative colitis in comparison with healthy subjects. A significant correlation between high IL-6 levels (in saliva and serum) and elevated values of the clinical inflammatory activity index (equivalent to CIBDAI) was reported in animals (Nielsen et al., 2005). Similar observations were made by Hosokawa et al. (1999), who demonstrated that the concentrations of IL-6 and its soluble receptor are positively correlated with the degree of IBD activity in humans. High levels of IL-6 in dogs with mild to moderate IBD suggest that this proinflammatory cytokine plays an important role in the progression of IBD in canine patients. A similar study was carried out to investigate correlations between IL-6 gene expression and chronic bowel inflammations of unknown aetiology in cats. Nguyen Van et al. (2006) demonstrated that IL-6 gene expression is highly correlated with histopathologically revealed IBD in cats. Guarner (2011) investigated the expression of pro- and anti-inflammatory cytokine genes in German shepherds diagnosed with antibiotic-responsive enteropathy (ARE) to reveal that the immune system plays an important role in inflammations of the duodenal mucosa.

In immunostimulated groups, an increase in the levels of IL-10, an immunoregulatory cytokine with anti-inflammatory properties, was accompanied by a drop in IL-6 concentrations. IL-10 contributes to food tolerance and it facilitates IgA secretion from plasma cells as described by Nguyen Van et al. (2006). Under *in vitro* conditions, IL-10 inhibits the generation of proinflammatory cytokines, such as IL-1, IL-6, tumour necrosis factor (TNF) and interferon (INF), by blocking Th1 cells and macrophages (Niederau et al., 1997). In this study, the use of immunomodulating agents in the treatment of IBD in canine patients increased IL-10 levels in all animals regardless of the applied immunomodulant. Highly significant differences in IL-10 concentrations in Group I and significant differences in Group II were correlated with a marked drop in IL-6 levels after immunomodulation, implying that the administered supplements had an immunoregulatory effect on dogs with IBD. In Group III, an increase in the serum levels of

IL-10 was accompanied by a decrease in IL-6 concentrations as a result of immunomodulation, but the noted difference was statistically not significant (Table 3). In a study of cats, Nguyen Van et al. (2006) demonstrated that IL-10 gene expression levels in the duodenal mucosa of animals diagnosed with IBD were higher than those determined in other gastrointestinal disorders. In cases of mild to moderate IBD, IL-10 levels were low, suggesting that this cytokine does not play a protective function in human patients with IBD. In our study, in all study groups except the control group IL-10 concentrations rose as a result of immunomodulation. The above-mentioned changes occurred together with clinical improvement, which caused a decrease in CIBDAI scores. The changes were statistically significant in the group receiving beta-glucans (Tables 1 and 3). In the control group, IL-10 levels tended to decrease between day 0 and day 42. The changes observed in the concentrations of both interleukins were indicative of progressive inflammatory processes, and they were validated by the results of clinical and histopathological evaluations in the control group. Gasche et al. (2000) have shown that IL-10 significantly inhibits intestinal mucosal inflammations in the progression of IBD. The use of anti-inflammatory cytokines and pro-inflammatory cytokine antibodies, based on evaluations of their mucosal concentrations, combined with the intragastric administration of IL-10-producing *Lactococcus lactis*, holds promise in the treatment of inflammatory bowel disease in human medicine, as described by Steidler et al. (2000).

The limitations of this study, such as the low number of animals taking part in the study and the absence of a control group receiving a placebo, are known to the authors. All animals qualified for the study were fed the same diet (feed) with or without (control group) immunomodulant supplementation. Unfortunately, the possibility that dietary change influenced the immunological parameters cannot be excluded (dietary change can alone influence immunological parameters). Due to the idiopathic nature of IBD, a fail-safe patient qualification system does not exist. The diagnosis of IBD is a diagnosis of exclusion (based on the elimination of all chronic inflammatory intestinal diseases). Many laboratory tests and imaging exams were performed during the animal qualification stage, and in a large number of tests/exams it is easier to make a mistake. The histopathological evaluation of mucosal biopsies is subjective and depends on the skill and experience of the pathologists (Simpson, 2012). In our study, every histopathological specimen was examined by the same pathologists experienced in gastroenterological diseases.

For a given drug to achieve optimal effects in the treatment of IBD in dogs, it has to deliver an improvement in CIBDAI values, an improvement in the histological characteristics of the intestinal mucosa and an increase in the anti-inflammatory immune parameters. Drugs that meet all three requirements prolong remission, which is a very important consideration in IBD. The analysed immunomodulant agents offered an effective treatment for IBD. The best results were reported for β-1,3/1,6-D-glucan (Biolex-Beta HP), which delivered the quickest

therapeutic effects, lowered CIBDAI scores to below 3, improved histopathological parameters, decreased IL-6 levels, elevated IL-10 concentrations and induced a remission period of more than six months. HMB and levamisole were also successful in lowering CIBDAI values, but the abatement of clinical symptoms was slower, and the improvement in the histological characteristics of the intestinal mucosa was less pronounced than that achieved with β-1,3/1,6-D-glucan. As regards immune parameters, HMB and levamisole effectively stimulated both cellular activity and humoral immunity. In groups stimulated with the above immunomodulators, remission periods were shorter than six months, indicating that HMB and levamisole are less effective in preventing the recurrence of IBD in dogs. In our study, 1,3/1,6-D-glucan emerged as the most effective immunomodulant in the treatment of IBD, as it delivered remission periods longer than six months and had no side effects. The incorporation of 1,3/1,6-D-glucan into an intestinal diet is likely to prolong remission in excess of six months without harmful consequences for the patient's health.

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