

Application potential of the IPFOS-Bacillus synbiotic product in military health protection

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ABSTRACT

Prebiotics and probiotics are known to play beneficial roles in human nutrition and health. In this study, the effects of IPFOS-Bacillus synbiotic product, a combined prebiotic-probiotic formulation, were evaluated for digestive support and immune modulation in an animal model. Administration of IPFOS-Bacillus at doses of 37.2 mL/kg/day and 44.6 mL/kg/day significantly reduced diarrhea incidence and restored intestinal microflora balance ($p < 0,05$) in rats. Both doses also increased total spleen protein content ($p < 0,05$) and lymphocyte count in diarrheic rats. No pathological abnormalities were observed in the heart, liver, kidneys, lungs, spleen, or digestive system. These findings suggest that IPFOS-Bacillus has strong potential for development as a health protection product to be issued to soldiers.

Keywords: Synbiotic; Immune; Digestion; IPFOS-Bacillus.

1. INTRODUCTION

In the field of military medicine, digestive disorders, psychological stress, and sleep deprivation have been recognized as critical health concerns during military operations. Under field conditions, the preservation and transportation of fresh, perishable foods such as green vegetables and fruits is challenging, leading to dietary imbalances and potential gastrointestinal disturbances [1]. Nutritional interventions, including synbiotic supplementation, have therefore gained increasing attention for enhancing the resilience and health of military personnel [2]. Some previous studies have reported that synbiotic products can yield notable benefits in this population [1, 3].

In this study, we developed a liquid jelly-based synbiotic supplement, IPFOS-Bacillus, formulated by combining probiotic spores of *B. subtilis* and *B. coagulans* with soluble dietary fibers, including inulin and inulooligosaccharide. Members of the Bacillus genus produce diverse metabolites and exhibit enzymatic activities with potential health benefits. Among the numerous Bacillus species, the most extensively studied spore-formers include *B. clausii*, *B. coagulans*, *B. cereus*, and *B. subtilis* [4]. Of these, only *B. coagulans* and *B. subtilis* var. *natto* are widely recognized as safe probiotics for human use [5].

The effects of Bacillus spores or their mixtures on gut microbiota composition and host physiology, such as oral *B. coagulans* and *B. pumilus* administration or a combination of *B. subtilis* and *B. coagulans* spores in animal models [6]. However, the synergistic impact of these probiotic spores combined with prebiotics has not been comprehensively investigated. Therefore, this study aimed to evaluate the immunomodulatory, digestive-supporting, and safety-related effects of the IPFOS-Bacillus synbiotic product in a murine model.

2. MATERIALS AND METHODS

2.1. IPFOS-Bacillus synbiotic preparation

The IPFOS-Bacillus was formulated by the Biotechnology Laboratory of the Institute of Materials, Biology, and Environment. Per 100 mL of water at pH 4.0, the formulation contained

inulin (8 g), inulo-oligosaccharide (8 g), trehalose (17 g), orange powder (3 g), sodium chloride (0.2 g), citric acid (0.1 g), sodium benzoate (0.05 g), and jelly powder (0.1 g). The solution was packaged in aluminum spout pouches and sterilized at 115 °C for 30 minutes. Following sterilization, each pouch was supplemented with 100 µL of a probiotic *Bacillus* spp. mixture (*Bacillus subtilis* VTCC51, 1×10^{10} CFU/mL, and *Bacillus coagulans* VTCC12866, 1×10^9 CFU/mL) and sealed with a cap.

2.2. Experimental animals

Wistar rats (200 ± 20 g) of both genders were used in this study, which was conducted by the National Institute of Hygiene and Epidemiology. The animals were grouped (3 to 4 rats) and housed in cages in a controlled temperature and humidity room.

2.3. Experiment design

A total of twenty-eight male Wistar rats were randomly assigned to four groups (n = 7 per group). Group A received IPFOS-Bacillus orally at a dose of 37.2 mL/kg/day (human equivalent dose). Group B was administered IPFOS-Bacillus orally at 44.6 mL/kg/day, representing 1.2 times the dose given to group A. Groups C and D served as control groups and were administered distilled water orally at 44.6 mL/kg/day. All treatments were administered once daily in the morning for 28 consecutive days. On day 29, groups A and B (treatment groups) and group D (positive control) were orally administered castor oil (5 mL/kg) one hour prior to treatment to induce diarrhea [7]. Group C served as the negative control and did not receive castor oil. The treatment regimen was continued for an additional 10 days.

2.4. Microbiological and biochemical analyses

Animals were observed daily for feed and water intake, weight gain, physical condition, locomotor activity, fecal and urinary output, and any abnormal clinical signs. Fecal samples were collected from all groups for the determination of the ratio of Gram-negative to Gram-positive bacteria using Gram staining method [8]. Biochemical assessments of hematopoietic function, including red blood cells (RBC), lymphocytes, neutrophils, platelets, and hemoglobin (HGB), were performed both before and after the experimental period. At the end of the experiment, all animals were subjected to a full gross necropsy, and 30% of rats of each group were subjected to histopathological examinations [9] of the spleen, small intestine, and large intestine.

2.5. Total spleen protein

The spleens were collected, rinsed in 0.9% NaCl solution, and blotted dry with filter paper. Each sample was weighed and homogenized in 0.9% NaCl at a ratio of 0.3 g/mL. Total protein content was then determined using the Bradford method [10].

2.6. Statistical analysis

Data were analyzed using SPSS software version 20 (SPSS Inc., Chicago, IL, USA). Comparisons between experimental and control groups were performed using Student's t-test, while comparisons among three or more groups were conducted using one-way ANOVA followed by Dunnett's post hoc test. Results are presented as mean ± standard deviation (SD), and differences were considered statistically significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. General condition and body weight changes

Before castor oil administration, all rats were active, exhibited normal food consumption, produced dry stools, and had smooth fur, with no abnormal signs in physical condition or locomotor activity. During the castor oil administration period, rats in group C (negative control) maintained normal food intake, dry stools, smooth fur, and steady weight gain. In contrast, rats in group D (positive control) showed reduced food intake; some were lethargic, exhibited rough fur, and passed

loose stools that gradually thickened but did not form distinct pellets. Rats in groups A and B (treatment groups) demonstrated better food intake compared with the positive control group.

Table 1. Monitoring body weight gain increases in rats over time during the experiment.

Group		Group A (n = 7)	Group B (n = 7)	Group C (n = 7)	Group D (n = 7)
Before castor oil administration	Body weight,g (0 day)	205,0 ± 9,9 (<i>p</i> > 0,05)	210,3 ± 10,1 (<i>p</i> > 0,05)	206,3 ± 10,8 (<i>p</i> > 0,05)	203,9 ± 9,3
	Body weight,g (28 day)	264,4 ± 25,8 (<i>p</i> > 0,05)	274,6 ± 19,2 (<i>p</i> > 0,05)	256,4 ± 15,8 (<i>p</i> > 0,05)	255,3 ± 13,2
After castor oil administration	Body weight,g (38 day)	268,6 ± 27,7 (<i>p</i> < 0,05)	278,9 ± 19,4 (<i>p</i> < 0,05)	268,6 ± 18,2 (<i>p</i> < 0,05)	241,4 ± 10,4

p : compared with the control group (D).

Following castor oil administration, all treatment-group rats initially passed loose stools, which gradually thickened. Approximately 4 hours after ingestion of the test sample (equivalent to 5 hours post-castor oil administration), 2–3 rats per treatment group exhibited soft stools. Administration of the test sample at both dose levels ameliorated castor oil-induced loose stools and mitigated associated weight loss (table 1).

The synbiotic product can reduce diarrhea incidence due to its ability to restore and maintain a healthy balance of gut bacteria [11]. Clinical trials and studies have shown that the combination of prebiotics and probiotics in synbiotic products can help improve gut health and strengthen the immune system, leading to a reduced risk of developing diarrhea. These products work by promoting the growth of beneficial bacteria in the gut, which can outcompete harmful pathogens that may cause diarrhea [2].

3.2. Effect on intestinal microbiota

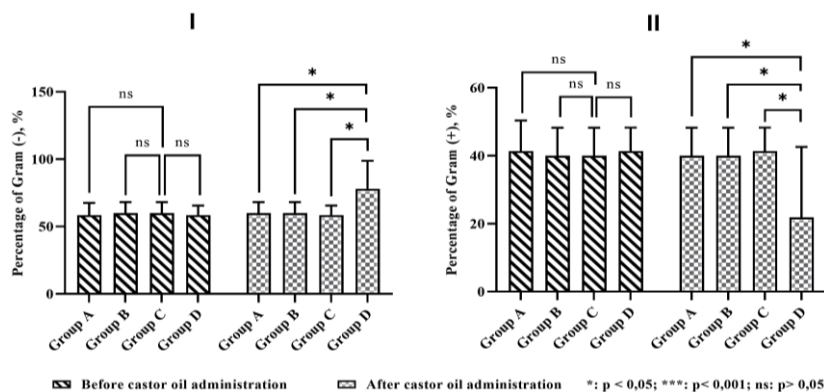


Figure 1. Percentage changes of Gram-negative (I) and Gram-positive (II) bacteria in fecal animals. (Group A and B) Rats dosed respectively with IPFOS-Bacillus and challenged with castor oil. (Group C) Rats not dosed (negative control). (Group D) Rats were challenged with castor oil alone (positive control).

As shown in figure 1, prior to castor oil administration, the percentages of Gram-negative and Gram-positive bacteria in rat feces did not differ significantly among the groups (*p* > 0,05). During the castor oil administration period, the proportions of both Gram-negative and Gram-positive bacteria changed markedly, with significant differences observed between the positive control group and all other groups (*p* < 0,05). The gut microflora of the groups receiving the IPFOS-

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Bacillus product remained relatively stable even under diarrheal conditions, whereas the positive control (group D) exhibited marked microbial imbalance during diarrhea (figure 1). These results suggest that the probiotic and prebiotic components of the IPFOS-Bacillus formulation may contribute to maintaining gut microbial balance.

Previous studies have reported that probiotics can restore the balance of beneficial bacteria disrupted during diarrhea, thereby alleviating symptoms and promoting recovery [7]. Prebiotics, including inulin and oligosaccharides, selectively stimulate the growth of these beneficial bacteria. When combined in a synbiotic formulation, prebiotics and probiotics can exert synergistic effects on the gut microbiota, enhancing their capacity to suppress pathogenic microorganisms and maintain a stable digestive environment [2, 3].

3.3. Effect on hematological examination

Table 2. Effect of IPFOS-Bacillus on hematopoietic function.

Parameters	Group (n = 7)	Before castor oil administration	<i>p</i>	After castor oil administration	<i>p</i>
RBC ($\times 10^{12}/L$)	A	8,1 ± 0,9)	> 0,05	7,6 ± 0,9	> 0,05
	B	8,3 ± 1,2	> 0,05	7,3 ± 0,4	> 0,05
	C	8,0 ± 0,5	> 0,05	7,6 ± 0,8	> 0,05
	D	8,0 ± 1,1		7,3 ± 0,9	
Lymphocytes ($\times 10^9/L$)	A	9,1 ± 2,0	> 0,05	11,4 ± 3,7	> 0,05
	B	9,0 ± 2,2	> 0,05	11,5 ± 2,4	> 0,05
	C	8,3 ± 1,2	> 0,05	8,9 ± 3,2	> 0,05
	D	8,9 ± 1,4		8,2 ± 2,3	
Neutrophils ($\times 10^9/L$)	A	1,4 ± 0,5	> 0,05	3,0 ± 1,3	> 0,05
	B	1,1 ± 0,4	> 0,05	2,2 ± 0,7	> 0,05
	C	1,6 ± 0,6	> 0,05	2,6 ± 1,0	> 0,05
	D	1,5 ± 0,4		3,0 ± 1,1	
HGB (g/dL)	A	12,5 ± 4,5	> 0,05	12,5 ± 1,2	> 0,05
	B	14,7 ± 2,8	> 0,05	12,3 ± 0,6	> 0,05
	C	14,2 ± 1,4	> 0,05	12,6 ± 0,2	> 0,05
	D	14,1 ± 1,0		12,6 ± 1,0	
Plateles ($\times 10^9/L$)	A	632,0 ± 236,2	> 0,05	778,7 ± 98,2	> 0,05
	B	631,6 ± 149,4	> 0,05	782,0 ± 86,9	> 0,05
	C	695,1 ± 148,6	> 0,05	739,9 ± 111,6	> 0,05
	D	660,9 ± 125,5		773,7 ± 59,4	

p: compared with the control group (D).

Rats between groups were not significant different ($p > 0,05$) in hematopoietic parameters before oral administrated with castor oil. As shown in table 2, lymphocyte, neutrophil, and platelet counts in all groups exhibited an increasing trend following castor oil administration. Among these parameters, only lymphocyte counts in the treatment groups (A and B) were higher than those in the control groups (C and D); however, the differences were not statistically significant ($p > 0.05$). The remaining hematological parameters (RBC and HGB) did not differ between the treatment and control groups. These findings indicate that long-term administration of the product does not adversely affect hematological indices. In diarrheic rats, certain immune-related hematological parameters, including lymphocytes, neutrophils, and platelets, exhibited an increasing trend. The higher lymphocyte counts observed in the treatment groups suggest a potential immune-supporting effect of IPFOS-Bacillus, although the differences were not statistically significant compared with the control groups.

Similar observations were reported by Flore et al. (2010), who noted increased lymphocyte counts in diarrheic rats supplemented with probiotics [7]. These results are noteworthy and align with previous reports that synbiotic formulations can modulate immune cell populations through gut microbiota regulation.

3.4. Effect on total protein in the spleen

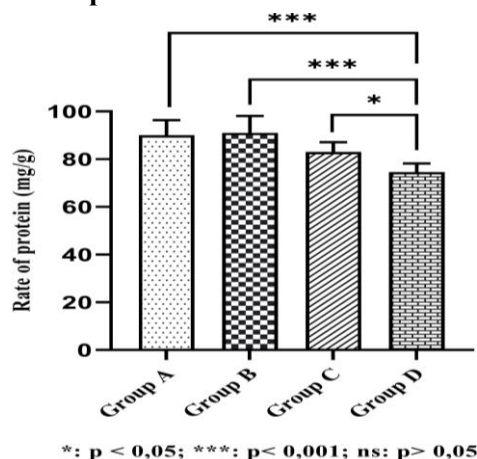


Figure 2. The level of total proteins in the spleen of animals. (Group A and B) Rats dosed respectively with IPFOS-Bacillus and challenged with castor oil. (Group C) Rats not dosed (negative control). (Group D) Rats were challenged with castor oil alone (positive control).

The total protein content in the spleen is presented in figure 2. In the positive control group, a significant reduction in splenic total protein was observed compared with the negative control group ($p < 0.05$). In contrast, both treatment groups exhibited significantly higher splenic total protein levels compared with the positive control ($p < 0.001$) and the negative control ($p < 0.05$). These results indicate that administration of the IPFOS-Bacillus product in both treatment groups effectively increased splenic total protein content in rats with castor oil-induced diarrhea.

The observed increase in splenic protein content in groups A and B, accompanied by elevated lymphocyte counts, suggests stimulation of the immune system, as the spleen is a key lymphoid organ involved in immune function [7]. It is plausible that this increase in splenic protein reflects enhanced antibody production. Consequently, the probiotic strains present in the IPFOS-Bacillus product may contribute to diarrhea prevention by modulating and strengthening host immune responses, a mechanism consistent with findings reported by previous studies [12].

3.5. Histopathological examination

Gross pathological examination revealed no visible lesions or alterations in the size or morphology of the heart, liver, lungs, kidneys, or abdominal organs in any experimental group. Histopathological evaluation of the spleen, small intestine, and large intestine demonstrated no treatment-related abnormalities, with tissue architecture preserved and no evidence of inflammation, necrosis, or structural disruption. No significant differences were observed between IPFOS-Bacillus treated groups (A, B) and control groups (C, D) following 38 days of administration (figure 3).

Moreover, the product may confer a protective effect at the cellular level, as suggested by the absence of tissue damage in treated animals. This observation aligns with findings by Chen et al. (2021), who reported that dietary supplementation with a synbiotic formulation attenuated liver cell damage in rats [13]. Collectively, these results support the safety of the IPFOS-Bacillus synbiotic and suggest its potential for organ-protective benefits alongside its gastrointestinal and immunomodulatory effects.

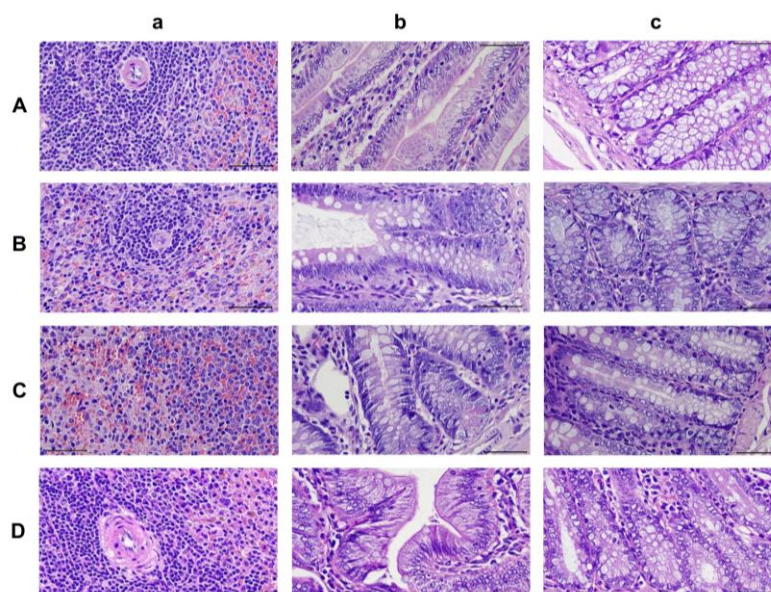


Figure 3. Histopathological images of spleen (a), small intestine (b), and large intestine (c) (HE x 400). Rats dosed respectively with IPFOS and challenged with castor oil (A, B). Rats not dosed (negative control, C). Rats challenged with castor oil only (positive control, D).

4. CONCLUSIONS

This study evaluated the effects of the synbiotic product IPFOS-Bacillus on gastrointestinal function and immune parameters in an animal model. The results demonstrated that IPFOS-Bacillus effectively alleviated loose stools, mitigated intestinal microbiota imbalance, and reduced weight loss associated with castor oil-induced diarrhea. In terms of immune modulation, the product increased spleen protein content significantly and showed a trend toward elevated lymphocyte counts, although lymphocytes did not reach statistical significance. Furthermore, no gross abnormalities or histopathological lesions were observed in the heart, liver, kidney, lung, spleen, small intestine, or large intestine, indicating that long-term administration of IPFOS-Bacillus is safe. Such results provide strong evidence supporting the product's safety for potential human use, particularly for military personnel operating under harsh environmental conditions where nutritional supplementation is essential for improving health and performance.

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TÓM TẮT

Tiềm năng ứng dụng của sản phẩm IPFOS-Bacillus synbiotic trong bảo vệ sức khỏe bộ đội

Prebiotic và probiotic được biết đến là có vai trò có lợi cho dinh dưỡng và sức khỏe con người. Trong nghiên cứu này, tác dụng của sản phẩm synbiotic IPFOS-Bacillus, một sản phẩm kết hợp của prebiotic-probiotic, đã được đánh giá về khả năng hỗ trợ tiêu hóa và điều hòa miễn dịch trên mô hình động vật. Việc sử dụng IPFOS-Bacillus với liều 37,2 mL/kg/ngày và 44,6 mL/kg/ngày đã làm giảm đáng kể tỷ lệ tiêu chảy và khôi phục sự cân bằng hệ vi sinh đường ruột ($p < 0,05$) ở chuột. Cả hai nồng độ sử dụng đều làm tăng lượng protein tổng số trong lách ($p < 0,05$) và số lượng tế bào lympho ở chuột bị gây tiêu chảy. Không có dấu hiệu bệnh lý bất thường nào được quan sát thấy ở tim, gan, thận, phổi, lách và hệ tiêu hóa. Những phát hiện này cho thấy IPFOS-Bacillus có tiềm năng to lớn trong danh sách các sản phẩm bảo vệ sức khỏe cho người lính cần được trang bị.

Từ khóa: Synbiotic; Miễn dịch; Tiêu hóa; IPFOS-Bacillus.