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Evaluating the efficacy of Bactase pet PB in improving oral health in canines: A randomized, placebo controlled, double blinded and interventional clinical study

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Abstract

Background: Maintaining optimal oral health in canines remains a persistent challenge, as poor oral hygiene can lead to dental pathologies and systemic complications.

Aim: The present study aimed to evaluate the clinical efficacy and safety of Bactase Pet PB in promoting oral hygiene in dogs.

Methodology: A total of 27 healthy dogs were enrolled in a 30-day randomized, placebo-controlled, double blinded, interventional trial. The animals were allocated into two groups: Control (G1, $n = 14$), receiving a Placebo and Treatment (G2, $n = 13$), receiving Bactase Pet PB, both administered twice daily at standardized doses. Efficacy was assessed based on plaque accumulation scores, gingivitis index, oral malodor (organoleptic scale), and bacterial counts, while safety was monitored through owner-completed questionnaires.

Results: Daily administration of Bactase Pet PB in drinking water resulted in significant reductions in plaque accumulation (65.71%; $p = 0.0018$), gingival inflammation (66.67%; $p < 0.0025$) and oral malodour (61.11%; $p < 0.0089$) after the 30-day intervention period in dogs. In addition, there was a considerable reduction in oral bacterial counts post-intervention with Bactase Pet PB. In contrast, the control group exhibited no improvement or a slight increase in these parameters. Inter-group comparison revealed statistically significant reduction in these parameters after intervention with Bactase Pet PB as compared to the control treatment ($p < 0.001$). No adverse effects were reported during the study, confirming the safety and tolerability of the investigational product.

Conclusion: These findings demonstrate that the Bactase Pet PB improves key oral health parameters in canines, suggesting its potential utility as a safe and beneficial adjunct in routine dental care for companion animals. Further, large-scale and long-term clinical trials are needed to warrant these outcomes and to elucidate the underlying mechanisms contributing to its therapeutic efficacy.

Keywords: Bactase pet PB, dental plaque, gingival inflammation, oral malodor, oral bacterial counts, safety and tolerability

1. Introduction

Oral health has the greatest overall impact on canine well-being. This is due to its widespread prevalence, prolonged duration, and the severity of symptoms observed in affected dogs [1]. The World Small Animal Veterinary Association (WSAVA) has classified oral diseases as major welfare concerns due to their chronic progression and their potential to cause pain and distress in companion animals [2]. Epidemiological studies indicate that oral diseases affect a substantial proportion of the canine population, with gingivitis alone reported in 44 - 100% of dogs, depending on factors such as breed and age [3]. This broad prevalence range reflects inconsistencies in diagnosis during routine veterinary examinations and variations in disease progression among individual dogs.

Although most oral health issues in dogs are reversible with appropriate care, persistent conditions frequently result in discomfort, halitosis, and a reduced quality of life [4]. Effective

oral hygiene management is therefore essential to maintain periodontal health and prevent development of advanced disease [5]. Nevertheless, adherence to routine oral care remains challenging for many pet owners, primarily due to factors such as limited training, time constraints, and behavioural resistance from their pets. To overcome these limitations, recent focus has shifted towards the development of innovative dental care formulations that are both easy to administer and compatible with daily management practices. Among these, functional supplements containing antimicrobial agents, systemic enzymes, and probiotic strains have shown promise in enhancing oral immunity and maintaining microbial homeostasis [6].

The current study was conducted to evaluate the effectiveness of Bactase Pet PB in improving oral health in dogs, with particular emphasis on four clinically relevant endpoints: dental plaque accumulation, gingival inflammation, oral malodor, and bacterial count. Dental plaque serves as a primary reservoir for pathogenic bacteria and precedes calculus formation, making its control a key preventive measure against periodontal disease progression [7]. Gingival inflammation, characterized by redness, swelling, and bleeding, reflects the host's inflammatory response to microbial infection and serves as a reliable indicator of oral health improvement when mitigated [8, 9]. Oral malodor, often the earliest and most perceptible sign of oral disease, results from volatile sulphur compounds produced by anaerobic bacteria. Its reduction not only improves owner perceived oral freshness but also reflects restored microbial balance within the oral cavity [10]. By evaluating these endpoints, the study aims to determine the clinical efficacy, ease of administration, and overall welfare benefits of Bactase Pet PB as an adjunct to conventional oral hygiene practices in dogs. The formulation's dual-action mechanism, targeting both microbial load and host immune modulation, positions it as a promising supplement for the long-term maintenance of canine oral health.

2. Materials and Methods

2.1. Materials

Investigational product

The investigational product (IP), Bactase Pet PB, was formulated as a powder, and packaged in 1 g sachets to ensure uniform strength, compliance, and product quality. The placebo formulation consisted solely of maltodextrin. Both Bactase Pet PB and placebo were supplied by Specialty Enzymes and Probiotics, Chino, USA.

2.2. Study design

The study was approved by The Institutional Animal Ethics Committee (IAEC), Animal Research Centre, AMTZ, Visakhapatnam, India. The experimental procedure received prior approval from the Committee for Control and Supervision of Experiments on Animals (CCSEA), Ministry of Fisheries, Animal Husbandry and Dairying, Government of India, with registration number V-11011(13)/10/2025-CPCSEA-DADF (Date of approval: September 10, 2025).

The study was designed to evaluate the efficacy of Bactase Pet PB in supporting oral health management in dogs. A total of 27 healthy, client-owned dogs of various breeds, sexes, and body sizes were enrolled. The study population comprised six distinct breeds, including Belgian Malinois ($n = 15$), German shepherd ($n = 8$), Dogo Argentino ($n = 1$), Beagle ($n = 1$), Husky ($n = 1$) and Golden Retriever ($n = 1$) (Table 1). Dogs were randomly allocated into two treatment groups: Group 1

(Placebo), consisting of 14 subjects, and Group 2 (Bactase Pet PB), consisting of 13 subjects.

Table 1: Details of the dogs recruited into the study

Sr. No.	Breed	Gender	Age (Years)	Weight (kg)
1	German Shepherd	Female	3	24
2	Dogo Argentino	Female	2	25
3	Beagle	Female	3	16
4	Husky	Male	2	28
5	German Shepherd	Female	3	24
6	German Shepherd	Female	1.5	23
7	German Shepherd	Female	1.5	22
8	German Shepherd	Female	1.5	23
9	German Shepherd	Male	1.5	18
10	German Shepherd	Female	1.5	23
11	German Shepherd	Male	3	28
12	Belgian Malinois	Male	3	28
13	Belgian Malinois	Male	5	32
14	Belgian Malinois	Female	6	27
15	Belgian Malinois	Female	7	30
16	Belgian Malinois	Male	3	32
17	Belgian Malinois	Female	5	32
18	Belgian Malinois	Female	5	33
19	Belgian Malinois	Female	4	25
20	Golden Retriever	Female	9	29
21	Belgian Malinois	Male	5	35
22	Belgian Malinois	Female	3.5	28
23	Belgian Malinois	Male	6	32
24	Belgian Malinois	Female	6	30
25	Belgian Malinois	Female	6	25
26	Belgian Malinois	Female	8	30
27	Belgian Malinois	Female	5	26

All dogs remained in their home environments and continued to receive routine care throughout the study period, except during scheduled clinical evaluations. Pet owners were instructed to maintain consistent feeding regimens, while all dental hygiene practices, including tooth brushing and the use of dental chews or treats, were restricted for the duration of the trial.

The study was conducted as a randomized, double-blinded, placebo-controlled interventional trial. Dogs were randomly assigned to groups based on their order of enrolment. All veterinarians, laboratory personnel, and pet owners were blinded to group allocations. Participant identification codes were securely maintained by Preclinical Research and Development Organization, Pvt. Ltd., Pune, until completion of the final report. Personnel responsible for dispensing treatments were not involved in either the treatment administration or outcome evaluation to ensure study integrity.

Identical 1 g sachets containing Bactase Pet PB (intervention) and Maltodextrin (placebo) were used in the study to maintain blinding integrity. Pet owners were instructed to dissolve each sachet in water at room temperature and administer the solution orally twice daily to ensure complete consumption. Treatment compliance was monitored through owner-reported logs and verified during scheduled clinical follow-up visits.

2.3. Inclusion and Exclusion criteria

Dogs eligible for inclusion in the study were required to be at least 8 months old and weigh a minimum of 3 kg. Each subject was required to have an intact functional dentition, including the bilateral presence of maxillary and mandibular third incisors (I3), canines (C), third and fourth premolars (P3, P4), and first molars (M1). Eligible subjects exhibited mild to

moderate gingivitis symptoms (gingivitis index ≥ 1), and visible plaque accumulation with a plaque score of ≥ 1 at premolar and molar teeth. Only client-owned dogs whose owners provided written informed consent and agreed to comply with all study procedures were included in the trial. Dogs were excluded from participation if they were receiving treatment with nonsteroidal anti-inflammatory drugs (NSAIDs), antibiotics, or any other anti-inflammatory medications at the time of enrolment or assessment. Additional exclusion criteria included the presence of periodontal disease at Stage 3 or higher as defined by the American Veterinary Dental College (AVDC) classification (Table 2); evidence of systemic or infectious disease, malignant neoplasia, neurological deficits, pregnancy, current use of mechanical or chemical anti-plaque agents, or known allergies to any component of the test supplement.

Table 2: Stages of Periodontal Disease (American Veterinary Dental College, 2014)

Periodontal Disease: Stage 0	Tartar: Starting to develop
	Gingival Margin: Flat
	Gingival Color: Pink
	Crown: Clean
	No Inflammation
	Radiographic depiction: No Bone Loss
Periodontal Disease: Stage 1	Tartar: Visible buildup
	Gingival Margin: Slightly swollen
	Gingival Color: Reddish pink
	Mild Inflammation
	Radiographic depiction: No Bone Loss
Periodontal Disease: Stage 2	Tartar: Evident
	Gingival Margin: Slightly swollen
	Gingival Color: Slight red
	Mild Inflammation
	Radiographic depiction: 0-25% Bone Loss
Periodontal Disease: Stage 3	Plaque: Present under gumline
	Gingival Margin: Swollen
	Gingival Color: Red
	Moderate Inflammation
	Radiographic depiction: Moderate Bone Loss
Periodontal Disease: Stage 4	Tartar: Present
	Gingival Margin: Swollen
	Gingival Color: Dark Red or Brown
	Moderate Inflammation
	Radiographic depiction: Serious Bone Loss

2.4. Efficacy evaluation: Oral health assessment

Baseline (Day 0) and assessments of canine oral health were performed using standardized clinical observational techniques and scoring systems. Plaque accumulation on premolars and molar teeth was evaluated visually using the Plaque Coverage Score (Table 3)^[11], Gingival inflammation was graded according to the Canine Gingivitis Score Index (Table 3)^[12], based on gingival colour, swelling and bleeding tendency. Oral malodor was assessed subjectively by the pet owners using the Organoleptic Scale (Table 3), providing a practical estimation of odor intensity^[13].

In addition, oral swab samples were collected at baseline and at the end of the study for quantification of bacterial load^[14]. Samples were cultured externally under standard microbial conditions, and total colony counts were recorded to determine bacterial concentration.

Table 3: Scores for different scales used in the study (Plaque Coverage Score, Canine Gingivitis Score Index, and Organoleptic Scale)

Plaque Coverage Score	
Score	Area Cover by Plaque
0	No observed plaque
1	Less than 25%
2	25-50%
3	50-75%
4	75-100%
Canine Gingivitis Score Index	
Score	Description
0	Normal gingiva
	Gingival Color: Pale pink to pink
1	Mild inflammation
	Gingival Color: Reddish or bluish-reddish
	Gingival Margin: Presence of slight oedema
	Colorless gingival exudate may observe
	No bleeding on probing
2	Moderate inflammation
	Gingival color: Redness or reddish-blue and glazy
	Gingival Margin: Enlarged due to oedema
	Bleeding on probing
3	Severe inflammation
	Gingival color: Marked redness or reddish blue
	Gingival Margin: Enlarged due to oedema with ulceration
	Spontaneous bleeding on probing
Organoleptic Scale	
Score	Description
0	No Odour
1	Questionable Odour
2	Light Odour
3	Moderate Odour
4	Strong Odour
5	Severe Odour

2.5. Safety evaluation

All dogs who received at least one dose of the investigational product were included in the safety evaluations. Safety parameters monitored throughout the study included abnormal behaviour, adverse events, and changes in feed and water intake. Routine physical examinations and vital sign assessments were conducted by attending veterinarians, and a structured questionnaire was used to collect owner-reported observations. These evaluations ensured that the treatment was well tolerated and that no adverse effects on general health were observed during the study period.

2.6. Statistical analysis

All individual data were decoded and compiled for statistical evaluation using Microsoft Excel 2016. Results were expressed as mean $\pm$ standard deviation (SD). Data were analyzed at a 95% confidence interval with a significance level of 5% ($p \leq 0.05$). Intra group (within group) differences between the start (SOT) and end (EOT) of treatment and inter group (between the groups) difference were assessed using a paired t-test (Two-Sample for Means), while inter-group (between-group) differences were analyzed using an unpaired t-test (Two-Sample Assuming Unequal Variances). A p -value ≤ 0.05 was considered statistically significant unless otherwise specified.

3. Results

A total of 27 canine subjects were enrolled in the study. Following randomization, the dogs were allocated into two groups: a control group (G1, $n = 14$) and a treatment group (G2, $n = 13$). All enrolled subjects successfully completed the study protocol. The oral health status of all dogs in both the groups (G1 and G2) was evaluated at baseline and at the end of the study period. Key clinical parameters assessed included the presence of dental plaque accumulation, gingivitis, and oral malodor. Evaluations were based on clinical examination findings and each dog's medical history. Additionally, the safety profile of the investigational product (IP) was assessed through a structured, owner-reported questionnaire designed to record any adverse events or behavioral changes throughout the trial.

3.1. Plaque scores

Plaque scores for all enrolled dogs are summarized in Figure 1. In the control group, no significant change was observed between the start of treatment (SOT) and end of treatment (EOT), with plaque scores remaining consistent throughout the study period. In contrast, dogs receiving Bactase Pet PB exhibited a significant reduction in plaque accumulation, with mean scores decreasing from 2.69 ± 0.85 at SOT to 0.92 ± 1.61 at EOT, resulting in a 65.71% reduction ($p = 0.0018$). This demonstrates a clear positive effect of the investigational product on dental plaque control. Inter-group comparison further revealed a statistically significant reduction in plaque coverage after 30 days of Bactase Pet PB supplementation compared with the control ($p < 0.0001$).

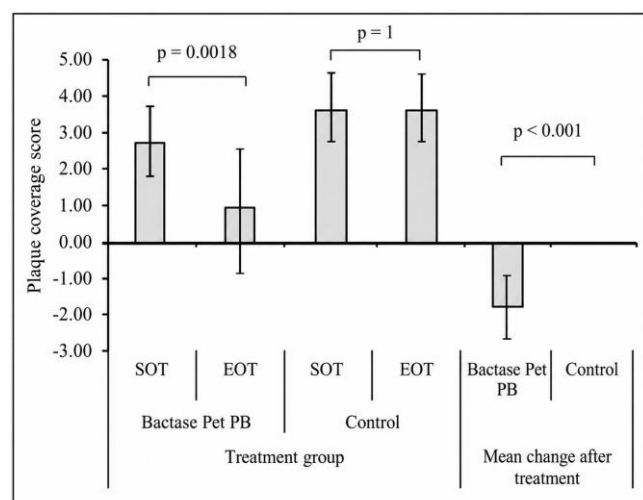


Fig 1: Effect of oral supplementation of Bactase PET PB and Placebo on presence of plaque coverage

3.2. Gingivitis score

Oral supplementation with Bactase Pet PB resulted in a marked improvement in gingival health. The treatment group demonstrated a significant reduction in gingivitis scores, decreasing from 1.85 ± 0.69 at the start of treatment (SOT) to 0.62 ± 1.12 at the end of treatment (EOT), corresponding to a 66.67% reduction ($p = 0.0025$) (Figure 2). In contrast, dogs in the control group exhibited a worsening of gingival condition, with gingivitis scores increasing from 1.79 ± 0.89 (SOT) to 2.29 ± 0.73 (EOT), representing a 28% increase ($p = 0.1161$). Inter group comparison (EOT-SOT) revealed a statistically significant reduction in gingivitis following 30 days of Bactase Pet PB supplementation compared to the placebo group ($p < 0.0001$).

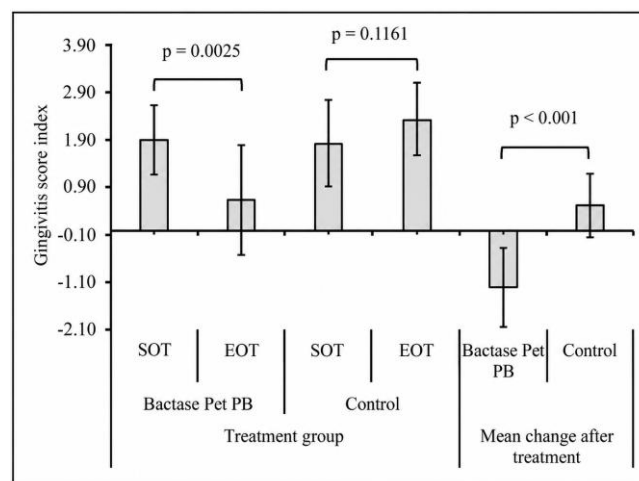


Fig 2: Effect of oral supplementation of Bactase PET PB and Placebo on canine gingivitis score index

3.3. Bad odor assessment

Bad odor assessment was performed by the pet owners using a standardized organoleptic scale, (Figure 3). Dogs supplemented with Bactase Pet PB showed a substantial reduction in bad odor, with mean scores decreasing from 2.77 ± 0.93 at the start of treatment (SOT) to 1.08 ± 1.93 at end of treatment (EOT), corresponding to a 61.11% improvement, which was statistically significant ($p = 0.0089$). On the contrary, the control group dogs demonstrated a non-significant increase in the mean scores from 4.07 ± 1.07 (SOT) to 4.36 ± 1.15 (EOT), reflecting a 7.02% rise ($p = 0.5026$). Inter-group analysis confirmed a statistically significant improvement in oral odor following Bactase Pet PB supplementation compared to the placebo group ($p < 0.001$). These findings indicate the efficacy of Bactase Pet PB in effectively reducing malodor and improving oral hygiene in canines.

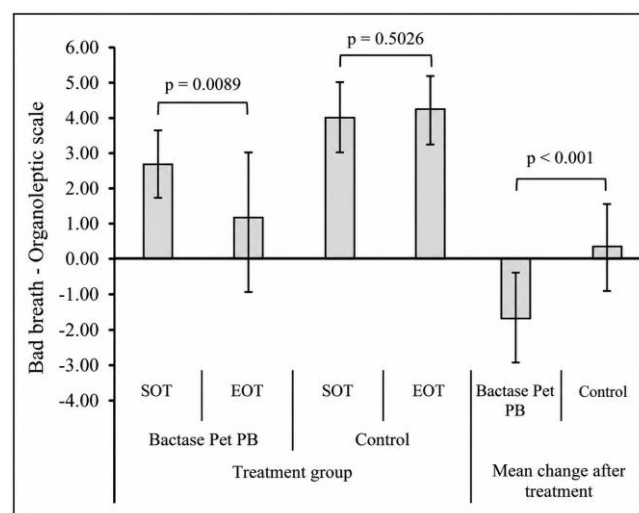


Fig 3: Effect of oral supplementation of Bactase PET PB and Placebo on bad odour scores

3.4. Bacterial count

Bactase Pet PB supplementation resulted in a modest reduction in oral bacterial counts, decreasing from 174.16 ± 71.5 at the start of treatment (SOT) to 163.19 ± 65.72 at the end of treatment (EOT), although this change was not statistically significant ($p = 0.6875$). Whereas, control group exhibited a slight deterioration in oral microbiological status, with scores increasing from 151.77 ± 65.38 to 158.79 ± 66.88 .

($p = 0.7809$) (Figure 4). Despite the lack of intragroup significance, intergroup comparison revealed a statistically significant difference ($p < 0.001$), indicating a relative advantage of the Bactase Pet PB in limiting bacterial proliferation within the oral cavity, indicating the antibacterial properties of the product.

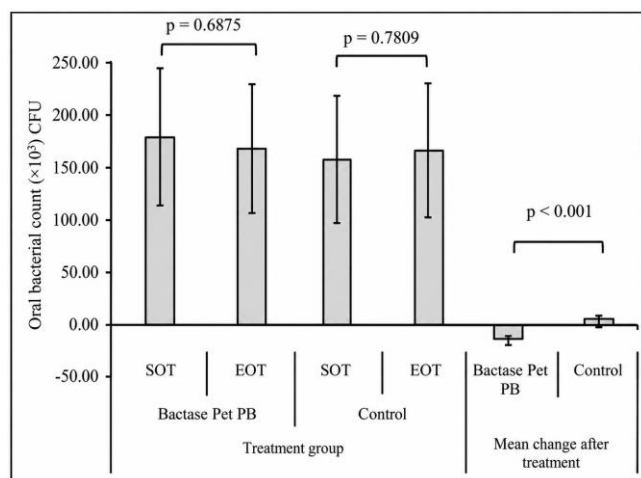


Fig 4: Effect of oral supplementation of Bactase PET PB and Placebo on oral bacterial count

Overall, the study demonstrated significant improvements across multiple parameters, including reductions in plaque accumulation, gingivitis index, and oral malodor, along with a favorable trend in bacterial counts reduction. These findings collectively support the efficacy of Bactase Pet PB in promoting oral hygiene and maintaining periodontal health in dogs.

4. Discussion

In this randomized, double-blinded, placebo-controlled interventional clinical trial, canine subjects presenting with mild to moderate gingivitis symptoms were orally supplemented with Bactase Pet PB or placebo for a period of 30 days to evaluate therapeutic efficacy. Efficacy assessments focused on key oral health parameters including plaque accumulation, gingivitis, oral malodor and bacterial load, following twice daily administration of the test or control product dissolved in water. Safety and tolerability were monitored throughout the study using structured, owner-completed questionnaires and clinical observations.

The present clinical study demonstrated that the daily administration of Bactase Pet PB via drinking water resulted in a significant reduction in key oral health parameters among dogs over a 30-day intervention period. Specifically, plaque accumulation decreased by 65.71% ($p = 0.0018$), gingival inflammation by 66.67% ($p < 0.0025$) and oral malodor by 61.11% ($p < 0.0089$). A modest reduction in total bacterial count (6.30%) was also observed. In contrast, dogs in the placebo group exhibited either unchanged or slightly elevated values for these parameters. The inter-group comparison confirmed a statistically significant improvement in oral health outcomes following intervention with Bactase Pet PB as compared to the control treatment ($p < 0.001$). The observed therapeutic effects are likely attributable to the synergistic action of the enzymatic and probiotic components of Bactase Pet PB, which contribute to the reduction of microbial load, oxidative stress, and local inflammation. The marked decrease in gingival inflammation and malodor

further supports the formulation's role in promoting a balanced oral microbiota and improved periodontal health. These components have been previously studied for their roles in regulating oxidative stress and inflammatory pathways, further supporting the therapeutic potential of such formulations in managing periodontal health. The current finding aligns with previous reports by Makinen *et al.* [15] who demonstrated significant reduction in pathogenic species such as *Porphyromonas gingivalis* and *Fusobacterium* sp. ($p = 0.005$) following probiotic supplementation in dogs and cats highlighting the potential of such formulations as adjuncts to conventional oral hygiene management.

Amylase and protease enzymes play a crucial role in maintaining oral hygiene by facilitating the breakdown of carbohydrates and protein residues adhered to the surface of teeth and gingiva. These adherent macromolecules originating from dietary sources and microbial biofilms, serve as substrates for pathogenic bacteria, thereby predisposing dogs to gingival inflammation, plaque formation, and halitosis [16-18]. Oral microorganisms ferment these residual carbohydrates and proteins to produce organic acids that can erode the tooth enamel, plaque accumulation and contribute to calculus deposition [19-20].

Amylase catalyzes the hydrolysis of starch-containing food particles into soluble sugars, aiding in their clearance from the oral cavity and thereby reducing the availability of fermentable substrates for cariogenic bacteria [21]. Moreover, amylase may facilitate the aggregation of cariogenic bacteria, enhancing their removal through salivary flow [22-23]. Proteases, on the other hand, hydrolyze the peptide linkages within proteins to release smaller peptides and amino acids, reducing the organic matrix that supports microbial adherence and biofilm development [21, 24]. Additionally, the enzymatic cascade involving glucose oxidase and lactoperoxidase plays a vital antimicrobial role. Glucose oxidase catalyzes the oxidation of carbohydrates to yield metabolites that react with salivary thiocyanate in the presence of lactoperoxidase, forming hypothiocyanite - an antimicrobial compound that disrupts bacterial metabolism and inhibits the proliferation of periodontopathic species [25-26]. This mechanism collectively contributes to reduced plaque accumulation, diminished gingival inflammation, and overall enhancement of oral health status in dogs.

Lysozyme has been well documented for its broad-spectrum antimicrobial and anti-biofilm properties. It acts by hydrolysing the β -1,4-glycosidic linkages in the peptidoglycan layer of bacterial cell walls, particularly targeting periodontopathic bacteria [27]. In addition to its bacteriolytic activity, lysozyme has been shown to inhibit glucose fermentation by *Streptococcus mutans*, thereby limiting acid production and subsequent enamel demineralization [28]. Joel *et al.* [27] further demonstrated lysozyme promotes bacterial aggregation and lysis by binding to and destabilizing the cell walls of both Gram-positive and certain Gram-negative bacteria, effectively curbing their proliferation. Naveed *et al.* [29] reported that lysozyme exhibits pronounced bactericidal activity against Gram-positive species, with the highest efficacy observed against *Staphylococcus aureus*. Using scanning electron microscopy (SEM) their study confirmed extensive structural disruption of biofilm forming bacterial colonies, supporting lysozyme's role as an antimicrobial component in maintaining oral microbial balance.

Lactoferrin, another key antimicrobial enzyme, exhibits both

anti-microbial and anti-inflammatory properties primarily by limiting iron availability, an essential nutrient for bacterial proliferation [30]. In addition to its iron binding capacity, lactoferrin interacts with specific receptors on epithelial and immune cell surfaces and binds to pathogen-associated molecular patterns (PAMPs), thereby modulating the host immune response and contributing to its anti-inflammatory effects [31]. Further, catalase, an essential antioxidant enzyme, plays a vital role in mitigating oxidative stress by catalyzing the conversion of cellular hydrogen peroxide into water and oxygen [32]. Dong *et al.* [33] demonstrated that dietary supplementation with a combination of lactoferrin and *Lactobacillus plantarum* significantly enhanced systemic antioxidant defences, increasing catalase activity by 28.7% and improving overall antioxidant status across multiple biochemical markers. The same study also reported a downregulation of pro-inflammatory cytokines: IL-2, TNF- α , and IFN- γ , cytokines known to mediate gingival inflammation and tissue degradation. Similarly, Jadhav *et al.* [34] reported a significant elevation in antioxidant capacity ($p < 0.005$) following enzyme blend supplementation. This enhancement in antioxidant status may contribute to neutralization of reactive oxygen species (ROS) generated during gingival inflammation, thereby protecting oral tissues from oxidative damage. Collectively these findings support the potential role of enzymatic supplementation in modulating oxidative and inflammatory pathways to promote better oral health outcomes in canines.

Probiotic strains such as *B. coagulans*, *A. clausii*, *B. subtilis*, *L. plantarum* and *L. casei* exhibit multiple mechanisms that contribute to maintaining and improving oral health in dogs. These mechanisms include competitive exclusion of opportunistic pathogens, inhibition of pathogen adhesion to mucosal surfaces, disruption of bacterial biofilms, and modulation of anti-inflammatory responses [35-38]. *L. plantarum*, in particular, has been shown to inhibit adhesion of *Salmonella typhimurium*, enterotoxigenic *E. coli* (ETEC), and *Staphylococcus aureus* to epithelial cells in-vitro, with the strongest inhibitory effect observed when the probiotic was introduced prior to pathogen exposure [39]. This suggests a competitive exclusion mechanism at the mucosal interface. As adhesion represents initial step in biofilm formation and inflammatory activation, *L. plantarum*'s interference with this process may reduce biofilm development and attenuate inflammatory stimuli within the oral cavity, thereby aiding in the management of gingival inflammation.

In addition, *B. coagulans* and *B. subtilis* have been reported to exert potent anti-inflammatory effects. Khosravi *et al.* [40] demonstrated that probiotic supplementation in dogs with experimentally induced allergic contact dermatitis, resulted in significant downregulation of IgE, IFN- γ , and IL-4 cytokines known to mediate hypersensitivity and chronic inflammation [41]. Notably, IFN- γ expression remained consistently lower at all evaluation points (Days 7, 28, and 35; $p < 0.001$), indicating sustained suppression of inflammatory pathways. Since IFN- γ and IL-4 are central to immune activation within gingival tissues, their downregulation may contribute to the reduced gingival inflammation observed following probiotic administration [40]. Furthermore, *B. coagulans* has been shown to enhance antioxidant enzyme activity and mitigate oxidative stress, offering an additional layer of protection to gingival tissues [42]. This antioxidant effect may help reduce tissue damage and oxidative burden associated with chronic inflammation, ultimately supporting periodontal health.

Collectively, the anti-inflammatory, anti-biofilm, anti-malodor and antimicrobial effects observed with Bactase Pet PB can be attributed to its synergistic combination of probiotic strains and enzymatic components. This multifactorial mechanism likely contributes to improved plaque control, reduction of gingival inflammation, and suppression of pathogenic microbial activity within the oral cavity. The formulation thus offers a comprehensive and practical approach for the management of common oral health disorders in companion animals. These findings are consistent with the broader literature and underscoring the therapeutic potential of probiotic-enzyme combinations as a conventional oral hygiene practices in dogs.

5. Conclusion

In the current randomized, placebo controlled and double-blinded clinical, oral supplementation with Bactase Pet PB resulted in statistically significant improvements in key indicators of canine oral health compared to the untreated control group. Dogs receiving Bactase Pet PB demonstrated marked reductions in dental plaque accumulation, gingival inflammation, and halitosis, over the 30-day intervention period, reflecting overall enhancement in oral hygiene status. The product was well tolerated and readily accepted by dogs maintained under typical home-care conditions, suggesting its practicality and suitability for routine use in companion animals. Importantly, no adverse events were reported throughout the study, confirming the formulation's safety profile. Although findings from the current clinical trial highlight the clinical relevance of Bactase Pet PB as a promising adjunctive or alternative strategy for managing oral health in dogs, further large-scale and long-term clinical trials are needed to warrant and validate these outcomes and to elucidate the underlying mechanisms contributing to its therapeutic efficacy.

6. Acknowledgments

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7. Funding

Advanced Enzyme Technologies Limited, Thane, India

8. Conflicts of Interest

None

9. Ethics Approval

The study was approved by The Institutional Animal Ethics Committee (IAEC), Animal Research Centre, AMTZ, and Visakhapatnam.

10. Institutional Review Board Statement

The experimental procedure received prior approval from the Committee for Control and Supervision of Experiments on Animals (CCSEA), Ministry of Fisheries, Animal Husbandry and Dairying, Government of India, with registration number V-11011(13)/10/2025-CPCSEA-DADF (Date of approval: September 10, 2025)

11. Data Availability

The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy restrictions.

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