## WHITE PAPER





NOVEL PROTEIN AND OIL
INGREDIENTS TO SUPPORT ORAL HEALTH



#### **KEY POINTS**

1

Bioactive peptides and lauric acid in black soldier fly larvae (BSFL) protein and oil favorably affected the growth of microbial species commonly associated with the development of oral biofilm in dogs.

2

BSFL protein and oil were able to modulate biofilm development in an ex vivo canine oral model using canine saliva inoculum.

3

BSFL ingredients are novel options for developing innovative pet products that offer high-quality nutrition while supporting the maintenance of oral health.

#### INTRODUCTION

Daily oral care is essential for the health and well-being of pets. By the age of three years, most dogs and cats are starting to show signs of poor oral health. The lack of a regular oral hygiene routine can lead to halitosis, periodontitis and possible tooth loss. While we should be brushing our pets' teeth daily, this is not always possible. Preventing the formation of dental plaque is vital for maintaining oral health in pets as dental plaque calcifies to form tartar. Dental plaque builds up on the teeth as a sticky biofilm due to bacteria. Slowing down plaque formation may also reduce tartar buildup, which often needs veterinarian intervention to remove.

# THE POWER OF BIOACTIVE PEPTIDES AND MEDIUM CHAIN TRIGLYCERIDES IN ORAL HEALTH

The larvae of *Hermetia illucens*, commonly known as the black soldier fly, are powerful up-cyclers that efficiently turn low-quality feedstock into premium ingredients. Black soldier fly larvae (BSFL) protein and oil can help meet consumer demand for nutritious, high-protein, and palatable pet foods, treats and supplements.

Black soldier fly larvae ingredients provide unique dietary compounds, such as lauric acid (a medium chain triglyceride) and bioactive peptides. These compounds are known for favorably affecting the growth of microbes. Pet foods containing BSFL have been shown to affect markers of oral health in dogs. After 50 days, dogs fed a BSFL food maintained favorable breath odor compared to a control food containing poultry byproduct meal. In addition, the presence of Moraxella, a positive indicator of oral health, was maintained in dog's saliva with the BSFL food.¹ Based on this finding, the objective of the present study was to evaluate the effect of BSFL protein and oil on indicators of canine oral health.



### SCIENCE-BASED INNOVATION FOR ORAL HEALTH

Two studies were conducted to determine the effect of BSFL protein and oil on the growth of microorganisms found in the oral cavity of dogs. Study 1 was an *in vitro* study using two reference microorganisms associated with the production of dental biofilms, *Neisseria weaveri* and *Neisseria zoodegmatis*. Study 2 included *ex vivo* testing using saliva from dogs in a canine oral biofilm model.

Prior to testing in both studies, the BSFL protein and oil were autoclaved to sterilize the samples. Since most pet foods undergo heat and/or pressure processing, autoclaving also serves the added purpose of simulating pet food production. This initial treatment ensures the active components in the BSFL protein and oil remain intact and are not deactivated by heat. A control (C+) was included for each test that consisted of culture media and a bacterial inoculant (either saliva or a reference microorganism).

Dental and oral care were the top category for treats in 2024<sup>2</sup>

Health claims & functional ingredients are key to communicating targeted health benefits.<sup>3</sup>

18% of pet treat and 8% of pet food launches in 2023 had an oral health claim.<sup>4</sup>

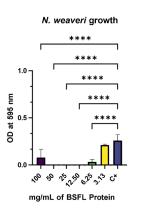
The *in vitro* assays using the biofilm reference microorganisms tested serial concentrations of BSFL protein and oil on the growth of *N. weaveri* and *N. zoodegmatis*. The autoclaved solutions containing the protein or oil were serially diluted and added to the biofilm microbial cultures (24h at 37 $^{\circ}$ C) to assess their effect on microbial growth. The minimum concentrations of protein and oil required to affect the growth of the biofilm microorganisms were 1.56 mg/mL and 1.76 µg/mL for *N. zoodegmatis* and 6.25 mg/mL and 225 µg/mL for *N. weaveri*, respectively (Figure 1).

Study 2 used saliva from eight healthy dogs in an impedance-based model to monitor biofilm formation. This method involves adding canine oral microbiota to a cell analyzer, which measures the cell index. The cell index increases as the biofilm forms, reaching a maximum when the microelectrode surface is fully covered by the biofilm and impedance reaches a constant maximum value. This allows for the determination of the rate of biofilm development. The effect of BSFL ingredients on biofilm development was tested separately using 30 mg/mL of BSFL protein and 200 ug/mL of BSFL oil. These concentrations were chosen based on the effective dosages in the initial *in vitro* assays (Study 1). The average cell index curves and the area under the cell index curves (AUC) are shown in Figure 2. Both the protein and the oil demonstrated the ability to modulate biofilm formation from canine saliva inoculum.

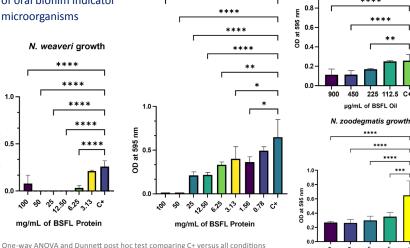




Figure 1. Effect of BSFL protein and oil on growth of oral biofilm indicator microorganisms

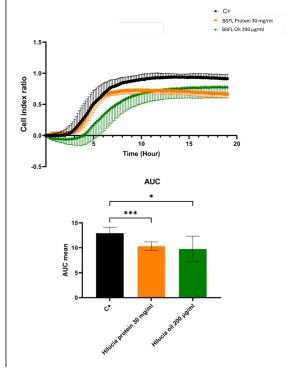


\*p-value 0.01 - 0.05; \*\*p-value 0.001 - 0.01; \*\*\*\*p-value <0.0001



N. zoodegmatis growth

Figure 2. Effect of BSFL protein and oil on cell index and area under the curve (AUC) in an impedance model of oral biofilm formation with inoculum from canine saliva (n=8 dogs)



One-way ANOVA and Dunnett post hoc test comparing C+ versus all conditions \*p-value 0.01 - 0.05; \*\*\*p-value 0.0001 - 0.001

In summary, these results suggest that bioactive peptides and lauric acid in BSFL protein and oil favorably affected growth of the microbial species, N. zoodegmatis and N. weaveri, that are commonly associated with the development of oral biofilm in dogs. Additionally, these unique components appeared to modulate biofilm formation in an ex vivo canine oral model using canine saliva inoculum. BSFL protein and oil present a novel option for developing innovative pet products that offer high-quality nutrition while supporting the maintenance of oral health.

### **ABOUT ADM**

ADM has an expansive pantry of ingredients that includes botanical extracts, vitamins, minerals, premixes and more. With ADM as your partner, you can deliver innovative, science-driven formulations to satisfy the functional health and well-being needs of pets and meet the expectations of today's pet parents. Our vertically integrated supply chain ensures the reliability and availability of high-quality products coupled with our dependable customer service, which ensures you get industry-leading, quality solutions to drive your success.

N. weaveri growth

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- 1. Santos Neto EL, Cardoso RKN, Santos LATA, et al. Black soldier fly (Hermetia illucens) larvae meal based extruded diets: Potential to improve canine oral health. Journal of Insects as Food and Feed. 2023:1-13.
- 2. Euromonitor / MCI / MKT
- 3. Euromonitor International Pet Care 25ed; Euromonitor Product Claims and Positioning; Euromonitor Voice of the Consumer: Lifestyles Survey 2024 (n=19,160)
- 4. Mintel GNPD, A year of innovation in petfood and products, January 2023-December 2023



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Dr. Jennifer Adolphe is responsible for providing technical support, nutrition training, formulation services and new product development for ADM's customers in the pet food industry. She graduated with her PhD in companion animal nutrition from the Western College of Veterinary Medicine at the University of Saskatchewan. She has a Master of Science degree in human nutrition and previously worked as a registered dietitian. Dr. Adolphe has more than 20 years of experience in both human and companion animal nutrition and previously held positions at two leading pet food companies in Canada. In addition to her role at ADM, she is an adjunct professor at the University of Saskatchewan and is pursuing a Master of Business Administration through Penn State University.



