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EFFECTS OF ALETA™ IN PROMOTING THE GROWTH OF PROBIOTIC BACTERIA: *IN VITRO* STUDY

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Abstract: It is well known that beta-glucan is not digestible by animals but can be used by probiotic bacteria in the colon. Dietary supplementation of Beta-glucan can result in the release of beneficial metabolites, such as short chain fatty acids, lower gut pH, and increase the population of probiotic bacteria such as *Lactobacillus* and *Bifidobacteria*. The goal of this study was to evaluate the *in vitro* growth of mixtures of *Lactobacillus* and *Bifidobacteria* using dried algae (Aleta™) as the substrate in comparison with dextrose found in common media preparations. Aleta™ stimulated the *in vitro* growth of mixtures of *Lactobacillus* and *Bifidobacteria*.

KEYWORDS:
Beta glucan, Aleta™, probiotic bacteria, *Lactobacillus*, *Bifidobacteria*

MATERIALS AND METHODS

Method:

The ability of various probiotic bacteria to utilize dried *E. gracilis* as carbon sources for growth was evaluated *in vitro*. A probiotic cocktail containing 22 bacteria and 1 yeast was used as a source of *Lactobacillus* and *Bifidobacterium* sp. Table 1 summarizes the probiotic blend of bacteria expected to grow under two different conditions. Modified thyoglycolate medium (pH 7.2) without sugar was used for culturing *Bifidobacterium* spp. and modified M9 mineral medium (pH 6.5) supplemented with Tween 80 and trace elements was used for culturing *Lactobacillus* spp. The probiotic cocktail was cultured overnight at 37ºC in brain heart infusion (BHI) broth, washed using chilled thyoglycolate or modified M9 medium under anaerobic condition for *Bifidobacterium* spp and in a CO_2 incubator for *Lactobacillus* spp, and plated on BHI agar plates overnight to determine colony count (CFU). Broth cultures were then diluted in appropriate media to obtain about 100 CFU/mL final count in each culture tube. Culture tubes were filled with additional medium alone, or medium containing 0.05% dried algae (Aleta™). A minimum amount of glucose (0.05%) was added to one set of culture tubes before initiating the culture and the other set was cultured in the absence of added glucose. The standard glucose concentration used for most bacterial cultures is 2% and, therefore, 0.05% is considered a minimum amount of glucose. Bacterial culture tubes were incubated at 37°C for up to 48 hours in anaerobic condition (to ensure *Bifidobacterium* growth) and in a CO_2 incubator (to ensure *Lactobacillus* growth). Sets of triplicate cultures were terminated at different time-points (0, 8, 16, 24, 32, 40, and 48 hours), concentrated by centrifugation or diluted as needed and plated onto complete thyoglycolate, blood or BHI agar plates to determine bacterial count (CFU/mL). The same procedure was repeated for 32-hours for single-species cultures of *L. acidophilus, L. fermentum* and *L. reuteri* along with *B. longun, B. bifidum and B. animalis.*

Table 1. Probiotic blend bacteria expected to grow under different growth conditions

Primary anaerobes	Facultative anaerobes
Bifidobacterium bifidum	*Lactobacillus delbrueckii (bulgaricus)*
Bifidobacterium breve	*Lactobacillus rhamnosus LB3*
Bifidobacterium animalis lactis	*Lactobacillus plantarum LM*
Bifidobacterium infantis	*Lactobacillus acidophilus*
Bifidobacterium longum	*Lactobacillus casei*
	Lactobacillus helveticus
	Lactobacillus plantarum
	Lactobacillus rhamnosus
	Lactobacillus salivarius
	Lactobacillus lactis
	Lactobacillus paracasie
	Lactobacillus brevis
	Lactobacillus gasseri
	Bacilus coagulans

RESULTS AND DISCUSION

Results of the *in vitro* growth study are shown in Figures 1 and 2**.** As shown in Figure 1 and Figure 2, the incorporation of Aleta™, which is approximately 50% paramylon and 50% non-glucan biomaterial (sugars, proteins, nucleic acid, lipid, etc.) in the culture medium that is devoid of any added sugar, promoted a considerable growth of both *Lactobacillus* spp. (Figures 1) and *Bifidobacterium* spp. (Figure 2). Aleta™ also accelerated sugar-dependent growth of both *Lactobacillus* and *Bifidobacterium* members

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Figure 1. Effect of Aleta™ with or without added sugar on the growth of individual *Bifidobacterium spp* in modified M9 medium (pH 6.5) under anaerobic conditions.

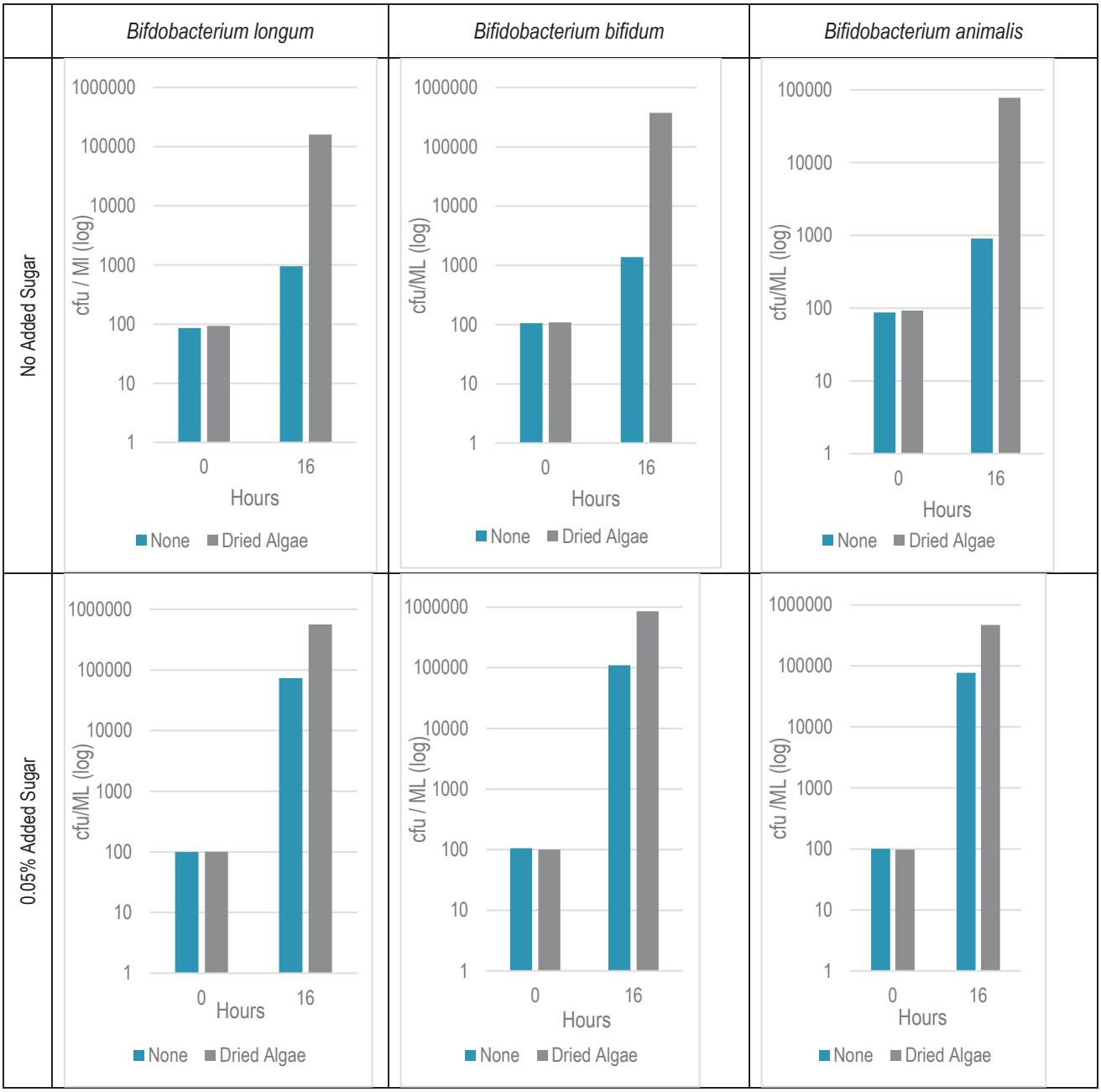
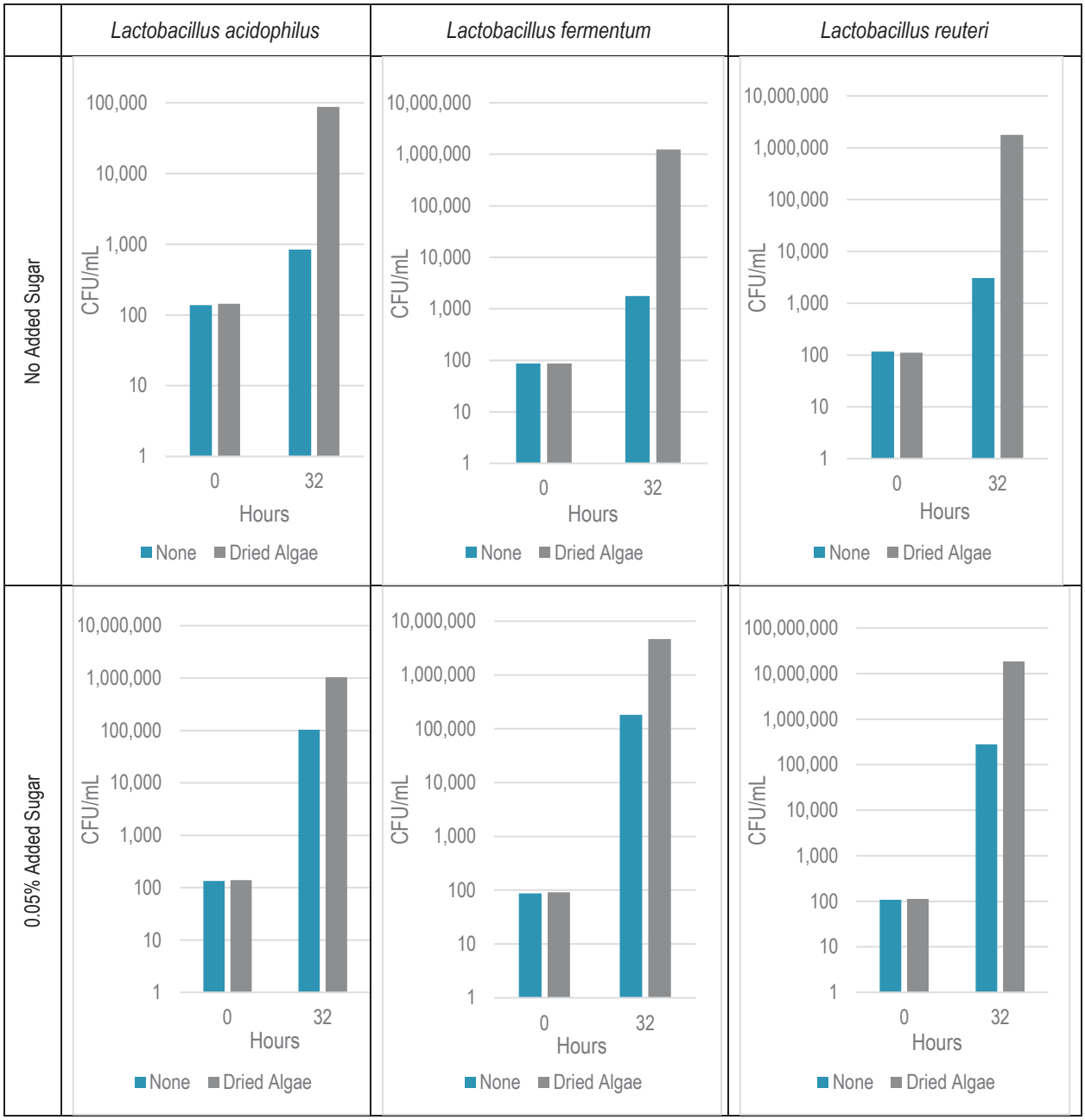


Figure 2. Effect of Aleta™ with or without added sugar on the growth of individual *Lactobacillus spp* in modified M9 medium (pH 6.5) under anaerobic condition for 32 hours.



CONCLUSION

Lactobacillus spp and *Bifidobacterium* spp are two major groups of bacteria that have been used as probiotics, and reports show that they promote many health benefits. Lactobacilli and bifidobacteria are indigenous to the GIT, occupy space, and consume nutrients along the intestinal tract, limiting the colonization of pathogenic bacteria. These bacteria have been recognized for exporting bacteriocins, which can target and kill invading pathogens (Sun and O'Riordan, 2013). Since Lactobacilli and bifidobacteria are autochthonous and dominant in the GIT, they can be utilized as a control method of pathogenic bacteria by competition, for example *Clostridium perfringens* (Stephenson *et al*. 2010). Several studies have indicated that introducing a balance of beneficial microorganisms such as Lactobacilli and bifidobacteria to the poultry microbiota improves body weight gain and feed conversion ratio as well as in warding off common diseases

in poultry, such as Newcastle disease and infectious bursal disease (Talebi *et al*.2008, Salianeh *et al*. 2011, Mohan *et al* 1996). Therefore, Aleta™ 's ability to promote the growth of these beneficial bacteria *in vitro* was tested. The results suggested that incorporation of Aleta™, which is 50% paramylon and 50% non-glucan biomaterial (sugars, proteins, nucleic acid, lipid, etc), to the culture medium devoid of any added sugar promoted the growth of both *Lactobacillus* and *Bifidobacterium*. Aleta™ also enhanced the growth of these probiotic bacterial communities in cultures that were supplemented with small amount of sugar (0.05% glucose).

REFERENCES

1. Russo P1, López P, Capozzi V, de Palencia PF, Dueñas MT, Spano G, Fiocco D. (2012) Beta-Glucans Improve Growth, Viability and Colonization of Probiotic Microorganisms, International Journal of Molecular Sciences, 2012, 13(5):6026-6039
2. A Vasile , D Paraschiv , S Dima , G Bahrim (2011) Growth and cell viability improve of the probiotic strain Lactobacillus casei ssp. Paracasei in the presence of oat bran and buckwheat flour. Innovative Romanian Food Biotechnology, 2011
3. Sun Y, O'Riordan MX.(2013) Regulation of bacterial pathogenesis by intestinal short-chain fatty acids. *Adv Appl Microbiol* . **85**:93–118. doi:10.1016/B978-0-12-407672-3.00003-4
4. Stephenson DP, Moore RJ, Allison GE. (2010) *Lactobacillus* strain ecology and persistence within broiler chickens fed different diets: identification of persistent strains. *Appl Environ Microbiol* . **76**:6494–503. doi:10.1128/AEM.01137-10
5. Talebi A, Amirzadeh B, Mokhtari B, Gahri H.(2008). Effects of a multi-strain probiotic (PrimaLac) on performance and antibody responses to Newcastle disease virus and infectious bursal disease virus vaccination in broiler chickens. *Avian Pathol* **37**:509–12.
6. Salianeh N, Shirzad MR, Seifi S. (2011). Performance and antibody response of broiler chickens fed diets containing probiotic and prebiotic. J Appl Anim Res. 39:65–7. doi:10.1080/09712119.2011.565222
7. Mohan B, Kadirvel R, Natarjan A, Bhaskaran M.(1996) Effect of probiotic supplementation on growth, nitrogen utilization and serum cholesterol in broilers. Br Poult Sci.37:395–401. doi:10.1080/00071669608417870
8. SD-17-00300