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A novel plant extract mix, **Grazix™**, is capable of binding endotoxin

C.C. Wu¹, G. Wu²

¹ School of Veterinary Medicine, National Taiwan University, Taipei, China

² LiveLeaf Bioscience, San Carlos, CA, USA

E-mail: ccwu1980@hotmail.com



Phytobiotics are a group of feed additives that consist of plant-derived ingredients for promoting livestock health and well-being and improving livestock growth and production efficiency. The mechanisms of phytobiotics have not been entirely understood but their benefits to overall health in animals have been noted. The objective of the present study was to determine if a novel plant extract mix (GRAZIX™, LiveLeaf Bioscience, San Carlos, CA) as feed additive could bind polysaccharide (LPS), the major component of endotoxin. Endotoxin is present in Gram-negative bacterial outer membrane and can contribute to life-threatening inflammatory reactions, diarrhea, and shock. A novel Polymyxin B/LPS-fluorescein isothiocyanate (FITC) binding inhibition assay was developed to determine the binding ability of GRAZIX™ to LPS. GRAZIX™ and a polyphenol TANIN compound were diluted to 1000x, 100x, 10x, 1x, and 0.1x in distilled water, respectively. Diluted compound was mixed with LPS-FITC and incubated at room temperature (RT). Polymyxin B was then added and incubated at RT. The supernatants containing compounds free in the supernatants

and compounds bound by LPS-FITC were discarded after centrifugation. The pellets containing Polymyxin B bound by LPS-FITC (Polymyxin B/LPS-FITC) were re-suspended in distilled water. The fluorescence of the suspension mixture was measured in a microplate reader. The percentage (%) of Polymyxin B/LPS-FITC binding inhibition is calculated by using 100% minus the percentage of Polymyxin B/LPS-FITC binding detected. GRAZIX™ was shown to have 97.9 and 85.4% of Polymyxin B/LPS-FITC binding inhibition when diluted to 100x and 10x, respectively, while the polyphenol TANIN compound had 98.2 and 97.8% of Polymyxin B/LPS-FITC binding inhibition when diluted to 100x and 10x, respectively. The results clearly indicated that GRAZIX™ can bind LPS by their near 100 % inhibition of the binding between Polymyxin B and LPS; thus, GRAZIX™, a plant extract mix containing polyphenols, is capable of binding endotoxin. This is likely one of the mechanisms that GRAZIX™ improves performance and health of livestock.

Keywords phytobiotics;feed additive;plant extract;Grazix;LPS;binding