ชื่อเรื่อง (ภาษาไทย) ผลของการเสริมซินไบโอติค เยรูซาเล็ม อาร์ติโชคและแลคโตบาซิลลัส แรมโนซัสในอาหารต่อการเจริญเติบโต การตอบสนองทางภูมิคุ้มกันและการป้องกันเชื้อแอโรโมแนส เวโรนิไอ ในปลาทับทิมวัยอ่อน

ชื่อเรื่อง (ภาษาอังกฤษ) Efficacy of synbiotic Jerusalem artichoke and Lactobacillus rhamnosus GG supplemented diets on growth performance, serum biochemical parameters, intestinal morphology, immune parameters and protection against *Aeromonas veronii* in juvenile red tilapia (*Oreochromis* spp.)

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Introduction

Synbiotics, a synergistic combination of probiotics and prebiotics, are currently regarded as one of the most practical nutritional supplements in tilapia farms. In this study, the effect of supplementing the diet of red tilapia (*Oreochromis* spp.) with Jerusalem artichoke (*Helianthus tuberosus*) and *Lactobacillus rhamnosus* GG (LGG) was evaluated. Growth performance, serum biochemical parameters, intestinal morphology, goblet cell counts, immune parameters and protection against *Aeromonas veronii* challenge were determined.

Materials and methods

1. **Probiotic and prebiotic preparation :** The probiotic bacterium, *L. rhamonsus* GG (ATCC 53103) was cultured in a MRS broth at 37 °C for 48 h. Then, the density of the bacterial suspension in the phosphate-buffered saline was determined and bacteria were mixed into commercial dry pellets in feed for probiotic group. JA samples were obtained from Phetchabun Research Station, Agro-Ecological System Research and Development Institute, Kasetsart University, Thailand. The JA tubers were cleaned and thin pieces were sliced. These slices were then ground into powder using a hammer grinder. The samples were dried at 50 °C for 24 h and kept at 4 °C until use. The proximate composition of JA was analyzed using the standard methods of AOAC (1990). The four treatment diets were as follows: a control diet (C), 10.0 g kg−1 JA-supplemented (JA) diet, 10^8 CFU g−1 LGG-supplemented (LGG) diet and 10.0 g kg−1 JA+10^8 CFU g−1 LGG-supplemented (JA + LGG) diet.
2. **Fish culture :** Two hundred and forty male fish (average body weight 14.05 ± 0.42 g) were obtained from a Good Aquaculture Practice certified farm, Thailand. The mono males of red tilapia in this experiment were produced by hormonal sex reversal. The fish were divided into eight 1000-L tanks (30 each) and allowed to acclimatize for two weeks. Fish were hand-fed approximately 5% of their body weight twice a day. The experiment was carried out in duplicate (i.e. two tanks for each experimental diet).
3. **Growth performance :** After 30 days of feeding, the final weight, weight gain (WG), specific growth rate (SGR), and feed conversion ratio (FCR) were calculated according to standard formulae.
4. **Blood collection and measurement of serum biochemical parameters :** After 30 days of feeding, blood samples were collected from six fish from each tank. Samples were taken from the caudal vein using a hypodermic syringe. Blood samples were allowed to clot at 4 °C for at least 3 h and were centrifuged at 2600×g for 10 min at room temperature to obtain serum samples. The samples were analyzed by using an automate chemistry analyzer (AU400, Olympus, Tokyo, Japan). The following parameters were measured: glucose, triglyceride, cholesterol, total protein, albumin, blood urea nitrogen (BUN), total bilirubin (Tbilirubin), direct bilirubin (D-bilirubin), serum alanine transaminase (ALT) and serum aspartate aminotransferase (AST)
5. **Measurement of villous height, villous width, absorptive area and goblet cells :** After 30 days of feeding, six fish from each tank were randomly sampled and anesthetized with clove oil. Three parts of the intestine, the foregut (after the pyloric part of the stomach to the spiral part of the intestines), the midgut (the spiral part of the intestines), and the hindgut (after the spiral part to 2 cm before the anus) were collected and fixed in neutral buffered 10% formalin. Samples were processed, embedded in paraffin, sectioned at 5 μm, stained with hematoxylin and eosin and examined by light microscopy. Villous height and width were measured using I-Solutions DT software (Image & Microscope Technology Inc., USA). For the villus height measurement, the ten highest intact villi were selected per section and their height was measured from the tip to the bottom. The average was expressed as the mean villus height for each section. The absorptive area was calculated. The goblet cells in the intestine were counted and classified by special staining as Periodic Acid-Schiff (PAS) staining for neutral mucin, Alcian blue (AB) staining (pH 2.5) for acid mucin, and AB-PAS double-staining for mixed type. The goblet cells from each section were counted using a high-power field (HPF; 400× magnification) and calculated (goblet cell numbers/HPF).
6. **Immunological assay :** Immune parameters, including lysozyme activity, alternative complement haemolytic 50 (ACH50) activity and total immunoglobulin (Ig) were measured.
7. **Mortality test :** The mortality test was carried out by *A. veronii* isolated from naturally diseased Nile tilapia (*Oreochromis niloticus*) in Nong Khai province, northeastern Thailand. The concentration of *A. veronii* was selected based on the results from previous study. After the challenge, any clinical signs of infection or mortalities were recorded for 15 days. The cumulative mortality were calculated by the following calculation: (Total mortality in each treatment after challenge/Total number of fish challenged for same treatment) × 100.
8. **Statistical analysis :** Results were analyzed by one-way analysis of variance (ANOVA) using SPSS version 22 software for Windows (SPSS Inc., Chicago, USA). Statistically significant differences between the groups were determined by Duncan's multiple range tests with a significance level of $P < 0.05$. The significance level was also set at 5% ($P < 0.05$) using SPSS version 22 software for Windows (SPSS Inc., Chicago, USA).

Results

The results showed that fish fed with synbiotic-supplemented diets had a significantly higher ($P < 0.05$) feed conversion ratio (FCR), specific growth rate (SGR), and average daily gain (ADG) than fish fed with a control diet. The synbioticsupplemented diet increased glucose, total protein and the total cholesterol levels. The absorptive area of the proximal and distal intestine of fish fed on the synbiotic diet was significantly higher ($P < 0.05$) than in those fed with probiotics (LGG), prebiotic-supplemented diets (JA), and the control diet. Goblet cell counts revealed that the numbers of acid mucous cells, neutral mucous cells and double-staining mucous cells of fish fed the synbiotic-supplemented diet (JA + LGG) were significantly higher ($P < 0.05$) in the proximal and distal intestine. Fish fed the synbiotic-supplemented diets also exhibited significantly higher ($P < 0.05$) lysozyme activity. The cumulative mortalities of fish fed with a synbiotic-supplemented diet were significantly lower than those of fish fed other diets.

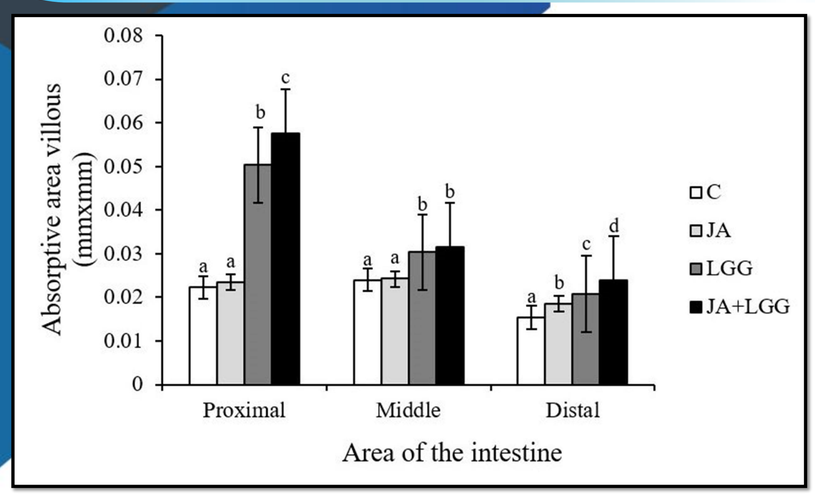

Fig. 1. Absorptive area of three parts of the intestine of red tilapia fed with the control diet, JA - 10.0 g kg−1 JA-supplemented diet, LGG - 10^8 CFU g−1 LGG supplemented diet, and JA + LGG - 10.0 g kg−1 JA+10^8 CFU g−1 LGG-supplemented diet for 30 days.

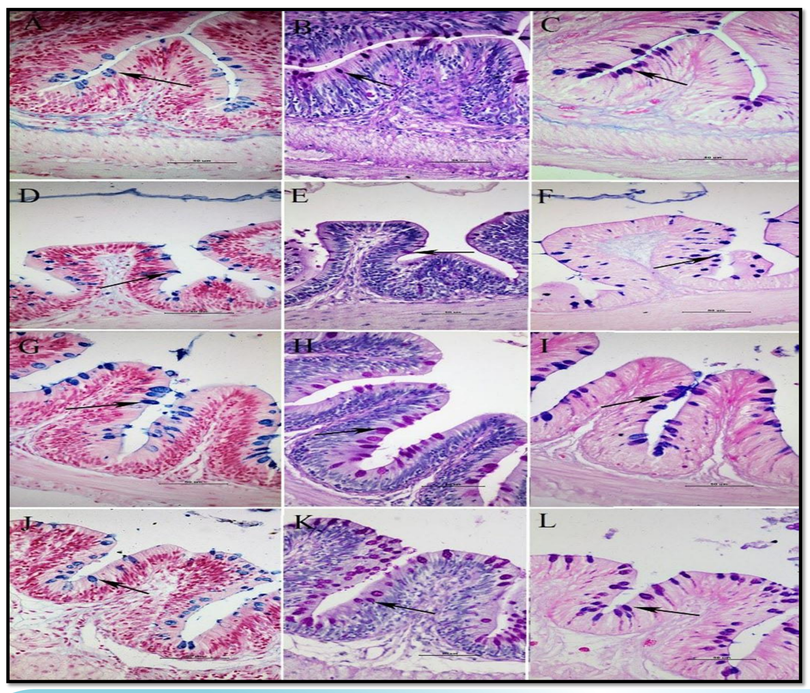

Fig. 2. Distal intestinal goblet cells (arrows) of red tilapia fed with the control diet (A, B and C), JA - 10.0 g kg−1 JA-supplemented diet (D, E and F), LGG - 10^8 CFU g−1 LGG-supplemented diet (G, H and I), and JA + LGG - 10.0 g kg−1 JA+10^8 CFU g−1 LGG-supplemented diet (J, K and L) for 30 days after three types of staining: AB staining (A, D, G and J), PAS staining (B, E, H and K), and AB-PAS double-staining (C, F, I and L).

Conclusion

Taken together, the data confirmed the beneficial effect of JA and LGG symbiotic diet on growth performance and health status of red tilapia. Direct administration of JA and LGG in fish feed can be used as a practical nutritional supplement in red tilapia.