**Dietary mannan oligosaccharide improves health status of the digestive system of marron, *Cherax tenuimanus* (Smith, 1912).**

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## Abstract

The aim of this experiment was to determine the effects of dietary supplementation of mannan oligosaccharide, Bio-Mos® (MOS) (Alltech, USA) on the gut microbiota, histology of gut and hepatopancreas of marron, *Cherax tenuimanus*. Marron were fed six different diets supplemented with 0%, 0.05%, 0.1%, 0.2%, 0.4% and 0.8% of MOS for a duration of 112 days. Total bacteria in the gut were highest when marron were fed diets which included 0.1% to 0.4% MOS, whereas total bacteria/*Vibrio* spp. ratio was the highest in the marron fed 0.4% MOS diet only. The marron fed 0.2% and 0.4% MOS diets showed normal morphology and higher densities of microvilli, and thicker layer of gut epithelium. The health of hepatopancreas of marron was also better when marron were fed 0.1% to 0.4% MOS diets. The results suggest that application of 0.2% to 0.4% of MOS in the diet improves the digestive system health of marron.

*Keywords*: Bio-Mos®, mannan oligosaccharide, marron, gut health, hepatopancreas

**1. Introduction**

The financial success in aquaculture depends on the advanced understanding of the biology, nutrient requirement and environmental management during the production of the target species. During the production cycle, the nutrient assimilation by the aquaculture species and their health are intimately linked (Wedemeyer, 1997). For the past ~~several~~two decades, antibiotics and growth promoters have been included in animal feeds worldwide at sub-therapeutic concentrations due to their positive effects on weight gain and feed utilization (Rosen, 1996). However, antibiotic use has been intensively criticized for potential development of antibiotic-resistant bacteria and destruction of environmental microbial flora (Gatlin, 2002). In addition, the increasing economical and social concern to reduce the use of antibiotics and other therapeutic chemicals used in aquaculture has encouraged more environmentally friendly approaches to improve growth and disease control (Hansen and Olafsen, 1999). Therefore, there has been an increased interest in the use of alternatives to antibiotics to control potential pathogens. Two main groups of additives to fulfill this role are probiotics and prebiotics (Gatesoupe, 1999, Torrecillas et al., 2007).

Prebiotics have been defined as selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health (Gibson et al., 2004). Those compounds help to alter the microbial community in the intestine to one dominated by beneficial bacteria (Manning and Gibson, 2004). Some of the more common prebiotics used include fructooligosaccharide (FOS), transgalactooligosaccharide (TOS), and inulin (Burra et al., 2005). Recently, one prebiotic named mannan oligosaccharide (MOS) (Bio-Mos®, Aqua Grade, Alltech,

USA) derived from the cell wall of *Saccharomyces cerevisiae* has been used in the husbandary practices of poultry (Hooge, 2004), ~~husbandry of~~ pigs and rabbits (Rozeboom et al., 2005, Mourão et al., 2006) and aquaculture (Torrecillas et al., 2007) as a diet supplement. This compound has been shown to affect gut health by pathogen adsorption and immune modulation in the target species (Staykov et al., 2007). In aquaculture, incorporation of MOS in the diet leads to the improvement of the gut health of sea bream (*Diplodus sargus*) (Dimitroglou et al., 2005), sole (*Solea senegalensis*) (Sweetman and Davies, 2007) and rainbow trout (*Oncorhynchus mykiss*) (Dimitroglou et al., 2008) by making the gut microvilli more uniform and longer. Supplementation of MOS in the diet did not show any change in the hepatopancreas of the tiger shrimp, *Penaeus semisulcatus* (Genc et al., 2007). Despite the potential benefits to health and performance noted in some aquatic species, the use of MOS in crustacean aquaculture has been poorly studied and no information is available for marron, *Cherax tenuimanus* (Smith 1912).

The marron is one of the important species for aquaculture industry for over 30 years in Western Australia (O'Brien and Davies, 2000). Although various studies have been conducted on nutrient requirements (O'Brien, 1995, Fotedar et al., 1996, Fotedar et al., 1999, O'Brien and Davies, 2000), the information on the effects of any supplements in the marron diets is very limited. This study aimed to evaluate the effects of MOS supplementation on the gut microbiota, histology of gut and hepatopancreas of marron.

## 2. Materials and methods

### 2.1. Preparation of test diets

Six isonitrogenous and isocaloric diets supplemented with 0% (D1), 0.05% (D2), 0.1% (D3), 0.2% (D4), 0.4% (D5) and 0.8% (D6) mannan oligosaccharide (MOS)

were formulated. Feed ingredients and supplements used to formulate the diets were supplied by Specialty Feeds Pty Ltd in Western Australia except for Bio-Mos® which was supplied by Alltech, USA. The proximate compositions of the ingredients and supplements were used as a basis to form the required formulae using the software FeedLIVE version 1.52 (Table 1).

All the dry feed ingredients and supplements were passed through a 100 µm mesh sieve in order to obtain a uniform particle size. The feed ingredients and supplements for each diet were thoroughly mixed. Approximately 100 mL of ~~reasonable amount of~~ freshwater ~~(approximately of 100mL water~~ per kg of the mixed ingredients and supplements~~)~~ was added and thoroughly mixed to obtain the glutinous mixture. The mixtures~~s~~ were then passed through a mince mixer to obtain pellets (1 mm diameter). The pellets were dried in the direct sunlight for six hours and were allowed to cool at room temperature for half an hour then packed and stored.

Analyses of the dry matter, crude protein and ash of the diets were performed according to the standard methods of the Association of Official Analytical Chemists (AOAC, 1995).

### 2.2. Experiment design

#### 2.2.1. Animals

Marron juveniles (4.44 ± 0.20 g total weight) were purchased from Aquatic Resource Management Pty Ltd, Western Australia and shipped to the Curtin Aquatic Research Laboratory (CARL). The marron were stocked in tanks provided with aerated, recirculating filtered freshwater and acclimated to the culture conditions for 2 weeks. During the acclimation period, the marron were fed a commercial diet on alternate days,

supplied by Enviroplus, Australia (26% protein, 47-50% carbohydrate, 9% fats and 8.9% ash) at the rate of 3% total weight.

**2.2.2. Culture system**

Plastic cylindrical tanks (800 mm diameter, 500 mm high, 250 L capacity) were used for the experiment. Sufficient PVC pipes and oyster net of appropriate sizes were placed in each tank to provide shelters for the marron. The tank was supplied with aerated recirculating freshwater at a rate of approximately 3L $min^{-1}$. The water in each tank was filtered through both mechanical and biological filter. During the experiment, water temperature ranged between 19.2 and 21.7 $^{o}C$, and pH was between 7.58 and 8.26.

**2.2.3. Feeding trial**

The experimental set up consisted ~~by~~of 18 tanks organized in 3 blocks. Each block ~~consisted of~~had 6 randomly placed tanks ~~whe~~and ~~re~~ each tank represented one dietary treatment . The marron from the acclimation tanks were randomly distributed among the culture tanks at the density of 15 marron per tank. Each tank was fed one of the test diets for 16 weeks so that each diet was represented in triplicate.

The feed was initially provided at the rate of 10% body weight every second day (a standard industry practice) which was reduced to 5-6% depending on the amount of uneaten food left in the tanks. Uneaten food and faeces were siphoned before feed was provided. The amount of water lost during siphoning was added into each tank to retain the water level.

**2.3. Sample analysis and data collection**

**2.3.1. Gut bacteria**

At the end of culture period, guts of 5 marron from each tank were dissected out and weighed to four decimal places (Wgut) and placed in sterile test tube. Five mL of Phosphate Buffered Saline (PBS) was added to the tube and homogenized using glass rod and homogenizer to obtain a solution. Several serial 10-fold (10x, 100x, 1000x, 10000x) dilutions were prepared using PBS solution. Aliquot of each diluted homogenized solution was incubated in Nutrient Agar plate (0.1 mL/plate, replicated in 3 plates for each dilution, for 24 h at 25$^{o}$C in order to determine the total bacteria. Similarly, diluted solutions were incubated in Thiosulfate Citrate Bile Sucrose Agar (TCBS) plates for 46 h to determine the total *Vibrio* spp. The plates having 20-200 colonies were counted for the bacterial count determination.

**3.2.2. Gut micrograph assessment**

Guts of 5 marron from each treatment at the end of culture period were dissected and prepared for scanning electron microscope (SEM) following the procedure described by Dunlap and Adaskaveg (1997) with some modification. The guts of marron were immersed in 3% Glutaraldehyde in 0.1 M cacodylate buffer over night. The guts were then washed in 3 changes of the cacodylate buffer and 3 changes in distill water for 5 minutes per change. After that, the guts were immersed in 2% $OsO_4$ for 2 hours following by 3 washes in the distill water for 5 minutes per wash. The samples were then dehydrated using a series of 50%, 75%, 95% ethanol solutions for 5 minutes and finally for 3 times in 100% ethanol for 5 minutes per change. The samples were then chemically dried by washing in a series of 50%, 75% and 100% (twice) hexamethyldisilizane (HMDS) in ethanol solutions for 5 minutes per change. Finally, the samples were dried at room temperature and mounted on a stub using carbon tape,

coated with gold and viewed under a pressure scanning electron microscope (Philips LX- 30, Eindhoven, Holland). The images obtained from SEM were described for number of villii per group and densities of the villi in the gut. Mean ~~value of~~ number of villi/group was calculated based on the number of villi from 20 ~~random~~randomly selected ~~villi~~ groups. For the purpose of calculating villi density, ~~of villi~~, three 10 x 10 µm random areas ~~of 10 x 10 µm~~ in each image were chosen and then number of villi in each area were counted. The m~~for the number of villi in each area. M~~ean of these three values was considered as the ~~the~~ 'density of villi/100 µm$^2$'.

**2.3.3. Gut and hepatopancreas histology**

Histological analysis of marron gut and hepatopancreas was performed following the method described by Genc et al.(2007). Hepatopancreas and gut of the marron at the end of trial were dissected and fixed in 4% buffered formalin for 24 hours. After dehydrating by passing the tissue through a series of alcohol solutions of 70, 85 and 98%, the samples were vacuum embedded in paraffin. The histological sections (4 – 5 µm) were stained for general morphological purposes with hematoxylin and eosin (H&E). The samples were analysed by Olympus BX 50 microscope under100X and 400X magnifications.for (i) number of bacteria in the hepatopancreas, (ii) condition of hepatopancreatic cells, (iii) condition of epidermal cells, (iv) condition of epithelium layer of the gut. After analysis all samples were photographed.~~ically analysed and documented for number of bacteria and condition of cells of the hepatopancreas, and epithelium layer and epidermal cells of the gut using the Olympus BX 50 microscope at 100X and 400X magnifications.~~

**2.4. Data analysis**

The normality of data was assessed by the Shapiro-Wilk test (Winer, 1991) and the homogeneity of variance was assessed by Levene test (Winer, 1991) prior to analysis. Normal data were subjected to one-way analysis of variance using post-hoc LSD test for data of homogenous variance, and Tamhane's test (Winer, 1991) for data of non-homogenous variance. Non-normal data was subjected to non-parametric testing using Kruskal-Wallis H test (Winer, 1991). Differences were considered significant at 5% level of probability.

## 3. Results

### 3.1. The test diets

There was no significant difference ($P > 0.05$) in gross energy, crude protein, moisture and ash content among the formulated diets with different supplemented levels of MOS (Table 1).

### 3.2. Gut bacteria

At the end of experiment, total bacteria in the gut of marron were significantly affected ($P < 0.05$) by different MOS supplemented diets. The highest concentration of bacteria was in marron fed D5 and the lowest was in marron fed D2. Marron fed D3, D4, and D5 have more bacteria in the gut than the marron fed D1, D2 and D6. Total bacteria in the gut showed no significant difference between marron fed D1 and D6 (Table 3).

*Vibrio* spp. in the gut of marron was significantly higher ($P < 0.05$) in the gut of marron fed D3 than marron fed other diets. Marron fed D1, D2, D4, D5 and D6 showed no difference ($P > 0.05$) in total *Vibrio spp.* in the gut after 112 days of culture. Total bacteria/*Vibrio* spp. ratio was the highest in marron fed D5 whereas D1, D4 and D6

resulted in similar gut bacteria/*Vibrio* spp. ratio. The lowest total bacteria/*Vibrio* spp. ratios were in the marron fed D2 and D3 (Table 2). The relationship between total bacteria and *Vibrio* spp. in the gut of marron was expressed by the following equation: total bacteria count = 8.7938 x vibrio count + 0.2591 with the $R^2 = 0.0193$.

**3.2.2. Gut morphology and histology**

Scanning electron micrographs of middle section of the hindgut of marron fed the different diets are shown in Figure 1. Villi in the gut were presented in groups of 3 to 11 villi. The villi distribution was thorough and uniformed in the inner surface of the gut. Villi of marron fed MOS supplemented diets ~~seem~~ were observed to be longer than villi of marron fed control diet. The number of villi per group of marron fed D1 was significantly lower than marron fed all MOS supplemented diets. Marron fed D4 and D5 had the highest number of villi per group followed by marron fed D2, D3 and D6. Marron fed D1, D2 and D6 had the same density of villi, which were significantly lower ($P < 0.05$) than the villi density of marron fed D3, D4 and D5 (Table 3).

Transversal sections of marron gut showed that, the marron fed D4, D5 and D6 have thicker epithelium layer than the marron fed D1, D2 and D3 diets (Figure 2). The epidermal cells of marron fed D4, D5 and D6 concentrated at higher density in the gut surface than marron fed D1, D2 and D3 (Figure 3).

**3.3. Hepatopancreas histology**

The lowest bacteria number was observed in the hepatopancreas of marron fed D5 followed by D3 and D4. Hepatopancreas of marron fed D1 and D6 revealed the cell damage syndrome while the hepatopancreatic cells of marron fed D3, D4 and D5 were in the better condition indicated by the clear cell walls in the histological sections. The

hepatopancreatic cell size of marron fed D3, D4 and D5 were larger than marron fed the other diets. The lipid storage in hepatopancreas of marron fed D3, D4 and D5 were also higher than in marron fed D1, D2 and D6 (Figure 4).

**4. Discussion**

The increased concern on the use of antibiotics and chemicals in aquaculture has promoted research toward the safe alternative products to improve the growth, food conversion efficiency, health and disease resistance of the host (Daniels et al., 2006). In agriculture, prebiotic and probiotic substances have been used as dietary supplements to activate organisms' innate immune response in order to reduce the risk of disease. Prebiotics are indigestible carbohydrates which stimulate the growth and activity of beneficial bacteria in the intestine and can activate the innate immune responses of cultured organisms when used as dietary supplements (Hooge, 2004). Prebiotics have also increased the efficiency of the digestive tract in many organisms through increasing the regularity, height and integrity of the gut villi (Hooge, 2004). Evidence has shown that microbiota in the digestive tract stimulated by prebiotics plays an integral role in several processes such as growth, digestion, immunity, and disease resistance of the host organisms as demonstrated in poultry (Patterson and Burkholder, 2003) and in humans (Gibson and Roberfroid, 1995). However, the application of prebiotics in aquaculture is rather limited but does hold a considerable potential (Gatlin, 2002). This work is the first attempt to investigate the role of prebiotic, MOS, on the gut health and hepatopancreas histology of marron.

The total bacteria and *Vibrio* spp. in the gut of marron before feeding the control and MOS fed has been analysed. There was not significantly different between them. Further, the same has been verified by other researchers working on western king

prawns (*Penaeus latisulcatus*) as a target species (Hai and Fotedar, 2009). It is clear that MOS has altered the microbial community in the gut of marron. The total bacteria in the marron gut showed an increase with the increase in dietary MOS concentration except for the MOS supplemented at high concentration of 0.8%. However, the incidence of *Vibrio* spp. was lower in 0.4% MOS treated marron and at this application rate, the total bacteria in the digestive tract of marron have dominated over the *Vibrio* spp. which was specifically grown using TCBS medium. When marron were fed equal to or higher than 0.2% MOS there was no correlation ($R^2 = 0.58$) between total bacteria and *Vibrio* spp. in the gut of the marron. Increase in *Vibrio* spp. when marron were fed 0.05% and 0.1% MOS showed that at a low supplementation level, MOS has stimulated the growth of harmful bacterial community. The same similarility between ratio of total bacteria/*Vibrio* spp. in the gut of marron fed the control and the 0.8% MOS diets indicated that high level of MOS in the diets did not change the gut bacterial community profile. The current results suggested that 0.4% is the most appropriate concentration of MOS to be supplemented in the diet for stimulating the development of the total bacterial community in the digestive tract of marron. The results are consistent with previous studies on the effects of MOS on fish by Zhou and Li (2004) who reported that the intestinal microbial populations in Jian carp (*Cyprinus carpio*), were significantly affected by the dietary inclusion of 0.24% of MOS. The presence of *Escherichia coli* in the intestinal digesta was significantly decreased while significant increases were observed in *Bifidobacterium* and *Lactobacillus* (Zhou and Li, 2004). Recent work by Dimitroglou et al. (2008) demonstrated that MOS reduced the bacterial load in the gut of both rainbow trout (*Oncorhynchus mykiss*) and sea bream (*Diplodus sargus*) by reducing the total aerobically cultivated bacteria. In rainbow trout, MOS fed fishes had

reduced numbers of *Micrococcus* spp., *Staphylococcus* spp., *Aeromonas/Vibrio* spp. and other unidentified Gram + bacteria and increased *Acinetobacter* spp., *Pseudomonas* spp. and *Enterobacter* spp. Therefore MOS promoted the colonization of beneficial bacteria associated with the natural gut flora of healthy rainbow trout (Dimitroglou et al., 2008).

Results from the current experiment have also implied that~~,~~ the inclusion of MOS in the diets affected the gut morphology indicated by the higher number of villi/group and density of villi. Higher density of the villi in the hindgut indicates that the marron have a greater ability to irrigate the gut, to protect the cuticle layer and/or in smooth movement of faeces and intake of environmental water (Chisaka et al., 1999). The ~~better~~ higher protection from higher number of villi/group and density of villi in the gut of marron fed 0.2 and 0.4% MOS diets could result in thicker gut epithelium layer and higher density of epidermis cells than marron fed other diets. Those better conditions of epithelium layer indicates the better ability in the transport, assimilation, storage of nutrients (Ferna´ ndez et al., 2002) and transport of ions and water (Mykles, 1979) in the digestive tract. The current results are totally in agreement with the previous studies that application of MOS has increased microvilli in both density and length in sea bream (*Diplodus sargus*) (Dimitroglou et al., 2005), sole (*Solea senegalensis*) (Sweetman and Davies, 2007) and rainbow trout (*Oncorhynchus mykiss*) (Dimitroglou et al., 2008).

Hepatopancreas histology is considered to be an important tool used in nutrition studies of crustaceans (Vogt et al., 1985). In the current study, hepatopancreas of marron was also affected by harmful bacterial community of the gut. The healthy hepatopancreas has higher ability in absorption of nutrients, storage of lipids and production of digestive enzymes by the digestive gland (Johnson, 1980).

The results presented here reveal MOS can be an effective dietary tool for improving the performance of marron. Our unpublished data showed that when MOS ~~were~~was supplemented~~-applied~~ at the rate of 0.2% and 0.4% in the diets, the mortality of cultured marron was significantly reduced by approximately 55% and 80% respectively and immune responses ~~such as~~as indicted by total haemocyte count, proportion of granular cells ~~proportion~~ and bacteraemia were improved. In addition, the resistant ability of marron fed 0.2% and 0.4% MOS supplemented diets, to bacterial infection and other stressors such as exposure to~~, to~~ $NH_3$ and air~~-exposure stressors~~ were also higher ~~in marron fed 0.2% and 0.4% MOS supplemented diets~~ (Sang et al., 2009). Those possible effects are directly related to the better condition of digestive system of marron when MOS ~~were~~ was applied at the rate of 0.2% and 0.4% in the diet. The role of MOS in ~~improvement~~ improving~~of the~~ survival, immune system and digestive system has also been demonstrated in many aquaculture species. However, the further research is needed to understand the mechanism of the observed changes in health of digestive system when MOS is applied in the dietary of marron.

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In conclusion, the use of MOS has improved the digestive tract health of marron by increasing the beneficial bacteria community, villi density and epithelium layer thickness of the gut, and increasing hepatopancreatic lipid storage, especially when supplemented at the level of 0.2% and 0.4% in the diet. Hepatopancreas histology and gut health evidence from the current experiment show that the MOS inclusion of 0.2% to 0.4% in the diet is the most appropriate concentration to improve the health of the digestive system of marron.

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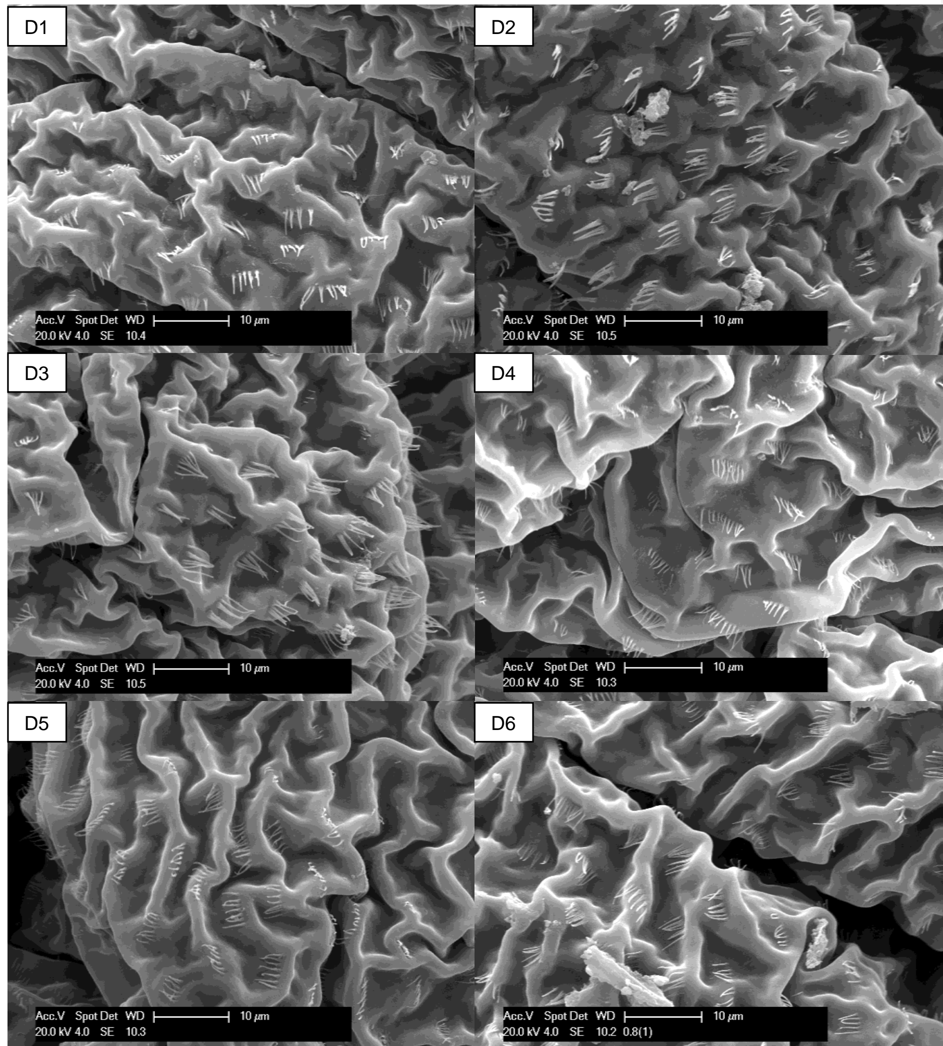


Figure 1. Gut micrographic of marron fed different MOS supplemented diets. D1: MOS free; D2: MOS- 0.05%; D3: MOS - 0.1%; D4; MOS - 0.2%; D5; MOS - 0.4%; D6: MOS - 0.8%.

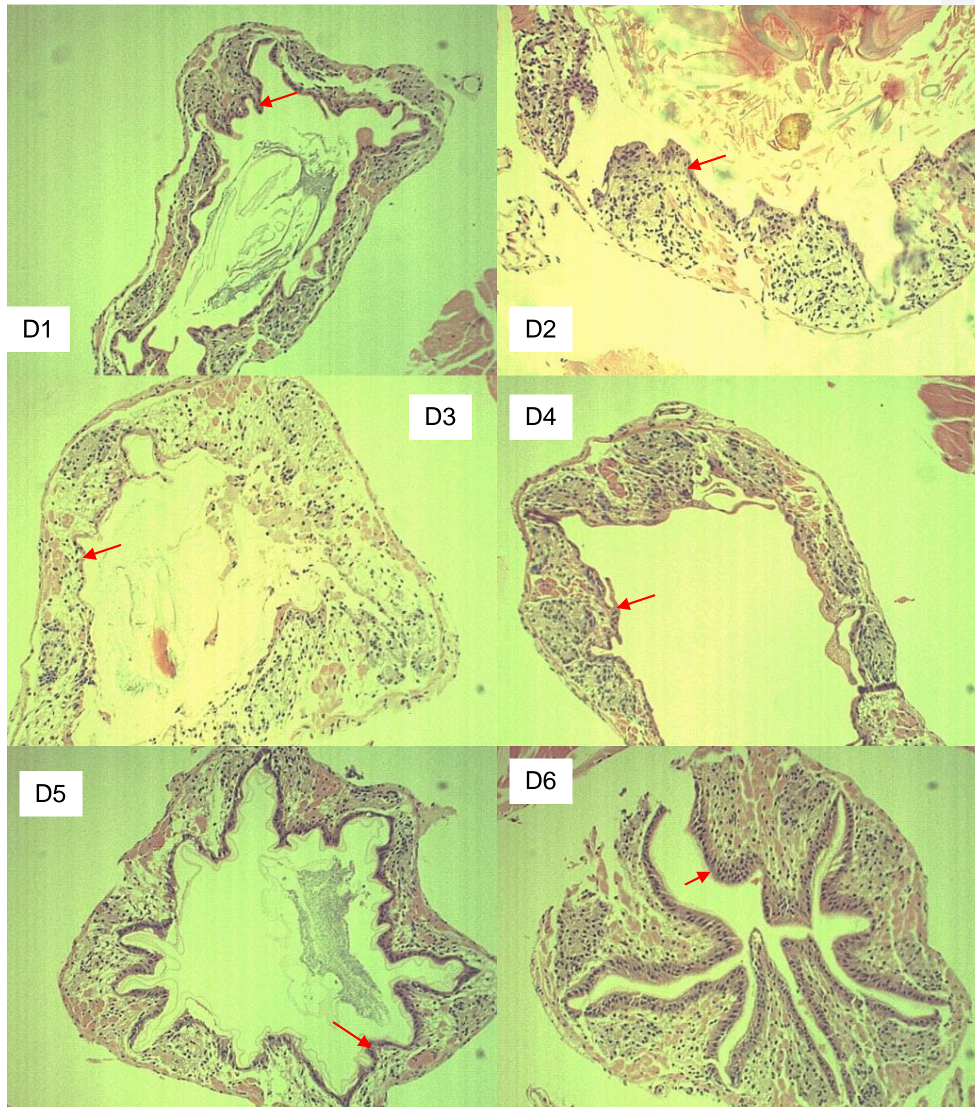


Figure 2. Transversal sections of the gut of marron, fed the different MOS supplemented diets at 100X magnifications (Sections were all taken from the same general area of the gut, H&E stained. The arrow points the epithelium layer of the gut). D1: MOS free; D2: MOS - 0.05%; D3: MOS - 0.1%; D4; MOS - 0.2%; D5; MOS - 0.4%; D6: MOS - 0.8%.

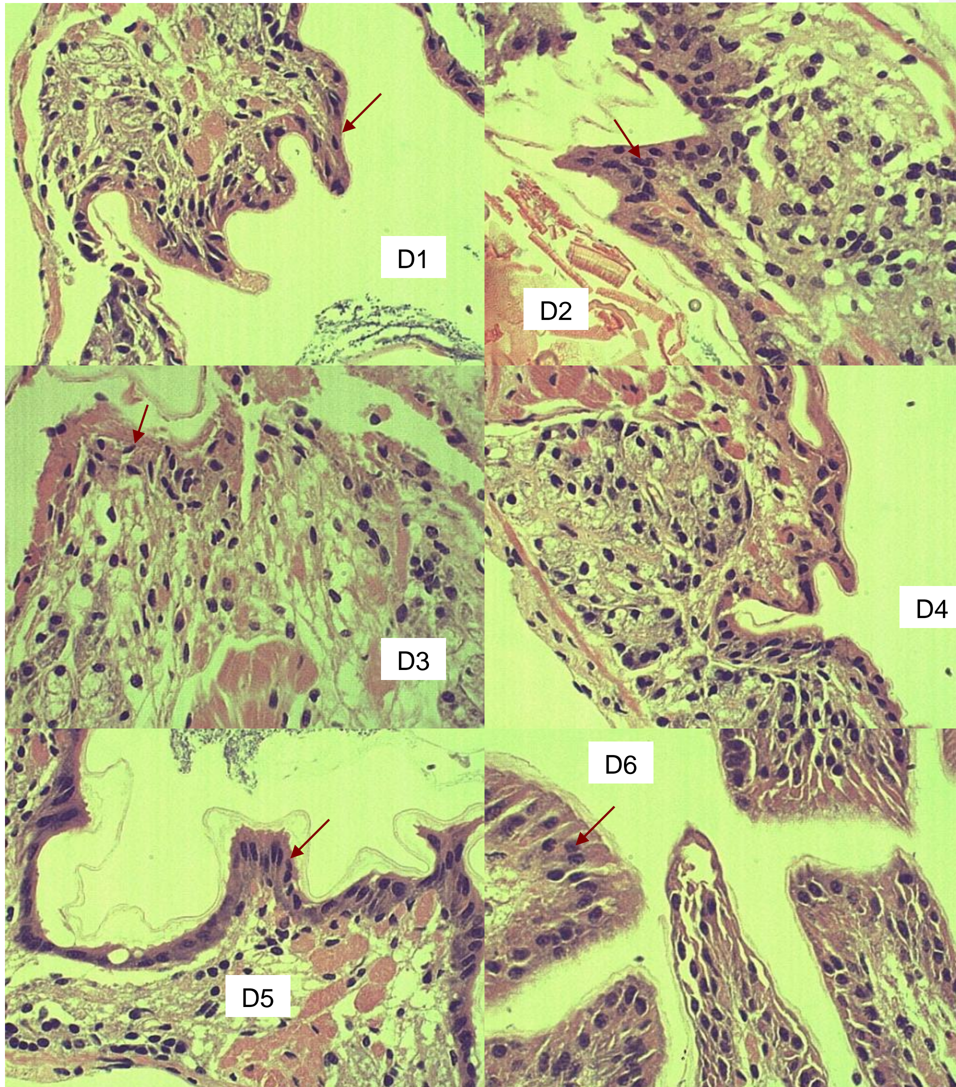


Figure 3. Transversal sections of the gut of marron, fed the different MOS supplemented diets (H&E stained, 400X magnification) (The arrow points the epidermis cell of the inner gut lining). D1: MOS free; D2: MOS - 0.05%; D3: MOS - 0.1%; D4; MOS - 0.2%; D5; MOS - 0.4%; D6: MOS - 0.8%.

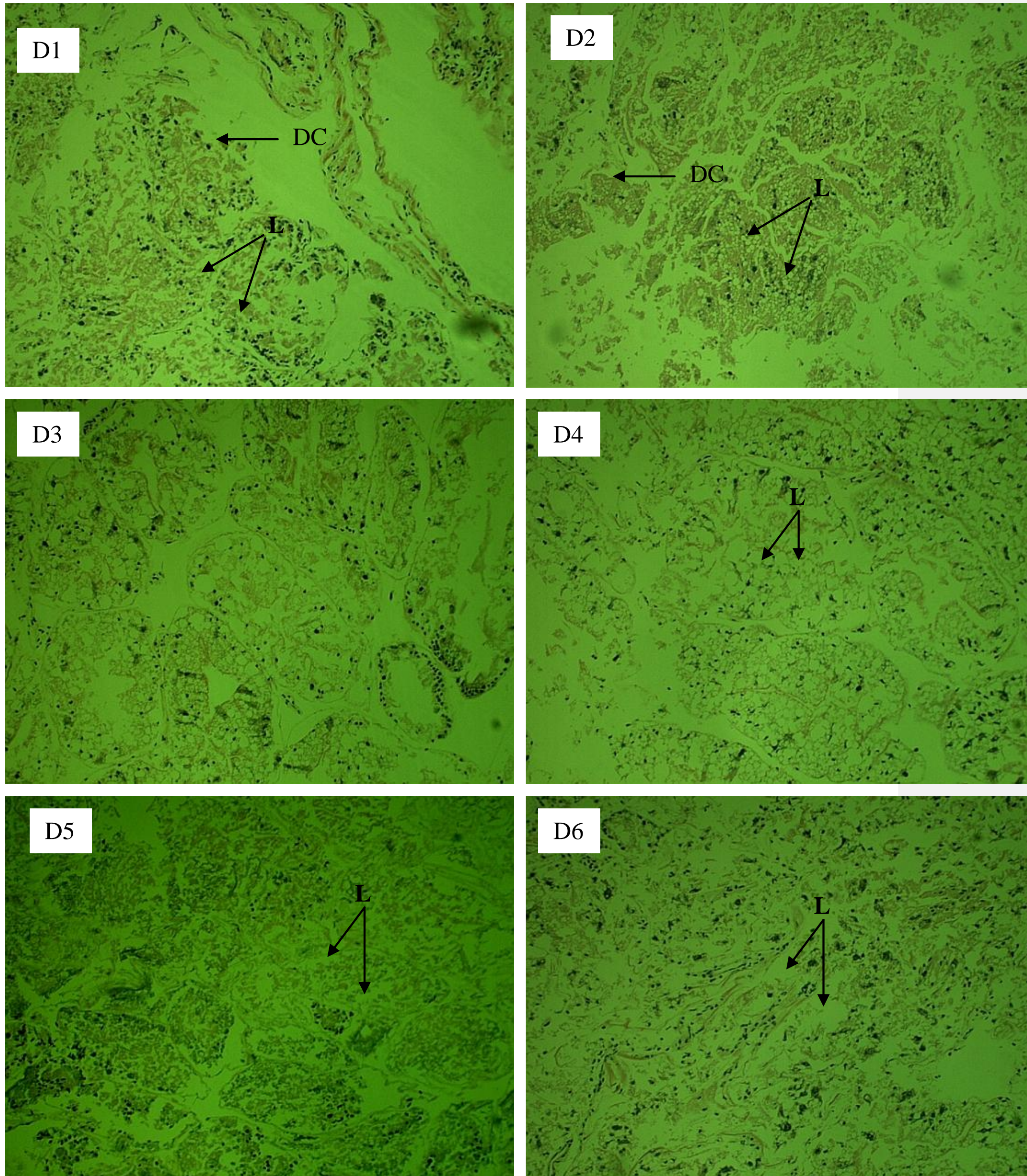


Figure 4. Hepatopancreas histology of the marron fed the different MOS supplemented diets (H&E stained, 100X magnification). DC: Damaged cell, L: lipid. . D1: MOS free; D2: MOS - 0.05%; D3: MOS - 0.1%; D4; MOS - 0.2%; D5; MOS - 0.4%; D6: MOS - 0.8%.

Table 1

Formulation and proximate nutrient components of the test diets for marron

| Ingredients and proximate nutrient components | D1 (%) | D2 (%) | D3 (%) | D4 (%) | D5 (%) | D6 (%) |
|---|---|---|---|---|---|---|
| Fish oil | 3.20 | 3.20 | 3.20 | 3.23 | 3.23 | 3.23 |
| Soybean meal | 10.15 | 10.15 | 10.14 | 10.14 | 10.14 | 10.11 |
| Fishmeal | 33.78 | 33.80 | 33.83 | 33.83 | 33.86 | 34.01 |
| Wheat flour | 49.35 | 49.25 | 49.21 | 49.08 | 48.85 | 48.33 |
| Ascorbic acid | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Betaine | 1.20 | 1.20 | 1.20 | 1.20 | 1.20 | 1.20 |
| Calcium ascorbate | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Premix | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| Cholesterol | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Wheat starch | 1.85 | 1.85 | 1.85 | 1.85 | 1.85 | 1.85 |
| BioMos® | 0.00 | 0.05 | 0.10 | 0.20 | 0.40 | 0.80 |
| Energy (calories/gm) | 4 487.67 | 4 501.67 | 4 501.00 | 4 495.00 | 4 486.00 | 4 485.33 |
| Crude protein (%) | 34.64 | 34.41 | 34.86 | 34.66 | 34.81 | 34.57 |
| Dry matter | 94.56 | 94.01 | 94.13 | 94.60 | 94.13 | 94.61 |
| Moisture content (%) | 5.44 | 5.99 | 5.87 | 5.40 | 5.87 | 5.39 |
| Ash (%) | 6.16 | 6.02 | 5.98 | 6.16 | 6.04 | 6.11 |

Table 2

Bacteria in the gut of marron fed different MOS supplemented diets (Mean ± SE)

| Diets | Total bacteria (million CFU/g) | *Vibrio* (million CFU/g) | Bacteria/*Vibrio* ratio |
|---|---|---|---|
| D1 | $_{a}$974.50 ± 80.66* | $_{a}$0.51 ± 0.09 | $_{a}$2 033.34 ± 384.54 |
| D2 | $_{b}$702.09 ± 71.53 | $_{a}$2.78 ± 0.61 | $_{bc}$293.51 ± 97.34 |
| D3 | $_{c}$2 449.51 ± 58.86 | $_{b}$9.99 ± 1.56 | $_{c}$259.77 ± 47.48 |
| D4 | $_{d}$1 905.06 ± 84.93 | $_{a}$1.46 ± 0.58 | $_{a}$1 816.93 ± 670.60 |
| D5 | $_{e}$3 283.90 ± 72.33 | $_{a}$0.82 ± 0.05 | $_{d}$4 013.93 ± 244.57 |
| D6 | $_{a}$1 216.90 ± 124.64 | $_{a}$1.34 ± 0.39 | $_{abc}$1 144.84 ± 462.75 |

*Value in the same column of each parameter having different subscript letters are significantly different at $P < 0.05$.

Table 3

Villi in the hindgut of marron fed different MOS supplemented diets

| Diets | Number of villi/group (Mean ± SE) | Villi density (villi/100 µm$^2$) (Mean ± SE) |
|---|---|---|
| D1 | $_a$6.13 ± 0.04* | $_a$9.67 ± 0.38 |
| D2 | $_b$7.44 ± 0.20 | $_a$10.93 ± 0.60 |
| D3 | $_b$7.68 ± 0.20 | $_b$13.56 ± 0.48 |
| D4 | $_c$8.85 ± 0.61 | $_b$14.63 ± 0.94 |
| D5 | $_{bc}$8.22 ± 0.27 | $_b$14.04 ± 0.27 |
| D6 | $_{bc}$8.00 ± 0.26 | $_a$10.41 ± 0.72 |

*Value in the same column of each parameter having different subscript letters are significantly different at $P < 0.05$.