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Research Article

Apparent Nutrient Digestibility of Balanced Diets with Soy Bean Extract or Meat and Bone Meal in the Nile Tilapia (*Oreochromis niloticus* L.) Infected with *Vibrio anguillarum*

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Abstract: Nile tilapia, *Oreochromis niloticus* L., infected with *Vibrio anguillarum* or not infected were fed balanced diets containing vegetal (solvent extracted soy bean meal (SBM)) or animal (meat and bone meal (MBM)) protein sources in order to demonstrate the apparent nutrient and energy digestibility. Regardless of protein sources in the diets, feed intake was lower in the infected group. Apparent nutrient (AD_{DM} and AD_{CP}) and energy digestibility (AD_{kJ}) values of Nile tilapia fed the SBM diet were significantly better ($P < 0.05$) than those fed the CONT and MBM diets and changed little between the measurement days in both groups (non-infected and infected). Although no visible symptoms associated with vibriosis were evident on either the skin or internal organs, the significantly lower ($P < 0.05$) AD_{DM}, AD_{CP}, and AD_{kJ} values obtained between day 1 and 10 (specifically on days 3 and 10) in the infected group fed the CONT and MBM diets might have implicated the pathogenicity of the bacteria. Further research concentrating on higher amounts of solvent extracted soy bean and meat and bone meal, different environmental conditions and doses of infection with *V. anguillarum* and immune response parameters including physiological and cellular stress responses is needed in order to identify the overall apparent nutrient digestibility of plant and animal protein sources in Nile tilapia infected with *V. anguillarum*.

Key Words: Nile tilapia, solvent extracted soy bean, meat and bone meal, vibriosis, digestibility coefficients

Vibrio anguillarum ile Enfekte Edilmiş Nil Tilapyalarında (*Oreochromis niloticus* L.) Soya Fasulyesi Küspesi ve Et-Kemik Unu ile Hazırlanmış Dengeli Yemlerin Görünür Besin Maddeleri Sindirilebilirlikleri

Özet: *Vibrio anguillarum* ile enfekte edilen ve edilmeyen Nil tilapyaları (*Oreochromis niloticus* L.) görünür besin maddeleri ve enerji sindirilebilirlik katsayılarının belirlenmesi amacıyla bitkisel (soya fasulyesi küspesi, SFK) ve hayvansal (et-kemik unu, EK) protein kaynakları içeren dengeli yemlerle beslenmişlerdir. Yemlerdeki protein kaynağına bakmaksızın, denenen tüm yem gruplarında yem alımı enfekte edilmiş balıklarda daha az gerçekleşmiştir. SFK içeren dengeli yemlerle beslenen Nil tilapyalarında görünür besin maddeleri (GSD_{KM} ve GSD_{HP}) ve enerji (GSD_{kJ}) sindirilebilirlik değerleri KONT ve EK yemleri ile beslenen balıklardan daha yüksek olduğu bulunmuş ve her iki grupta da (enfekte edilmiş ve edilmemiş) yemler arasında çok az bir değişiklik göstermiştir. KONT ve EK yemleri ile beslenen enfekte edilmiş balıklarda 1. gün ile 10. gün arasında (özellikle 3. ve 10. günde) oluşan önemli oranda düşük ($P < 0,05$) GSD_{KM}, GSD_{HP} ve GSD_{kJ} sindirilebilirlik değerleri bakterinin patojenitesinin bir göstergesi olarak düşünülebilir. *V. anguillarum* ile enfekte edilmiş Nil tilapyalarında soya fasulyesi küspesi ve et-kemik unu görünür sindirilebilirliklerinin tamamiyle anlaşılabilmesi için gelecekte daha fazla bu protein kaynaklarını ihtiva eden yemler, farklı çevre koşulları ve farklı bakteri enfeksiyon dozları, fizyolojik ve hücresel stres dahil bağışıklık sistemine ait parametrelerin dikkate alındığı çalışmalara ihtiyaç duyulmaktadır.

Anahtar Sözcükler: Nil tilapyası, soya fasulyesi küspesi, et-kemik unu, vibriosis, sindirilebilirlik katsayısı

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Introduction

Infectious diseases are the major cause of fish losses in the aquaculture industry (1). Costs and impacts of chemotherapy on the environment have prompted researchers to investigate the effects of nutritional modifications on the prevention or reduction of the severity of fish epizootics (1). Several studies demonstrated that mammals when fed animal protein were more resistant to stress than those fed vegetal or mixed protein sources (2,3). Néji and de la Noüe (4) demonstrated that apparent digestibility values for dry matter, crude protein, and gross energy in the rainbow trout were significantly lower specifically on days 5 and 9 of *Aeromonas salmonicida* infection than the values measured with non-infected fish regardless of the dietary protein source.

Growth parameters and nutrient digestibility of diets consisting of vegetal and animal protein sources were all measured in the Nile tilapia previously (5-9). However, little is known about how different protein sources affect feed intake and nutrient digestibility in infected fish as a whole (10).

Although vibriosis caused by *Vibrio anguillarum* is considered a disease that generally occurs in marine or estuarine environments, it could be a health problem in freshwater environments as well (11). Recently, *Vibrio* spp. was demonstrated to be the dominant bacterial population in the Nile tilapia in brackish water ponds in Saudi Arabia (12). Since the susceptibility of Nile tilapia to *V. vulnificus* was reported (13), the vibriosis could also become an important issue in tilapia farming specifically when co-cultured with other fish species like eels. Therefore, this study aims to demonstrate how the nutrient digestibility of balanced diets consisting of vegetal (solvent extracted soybean (SBM)) and animal (meat and bone (MBM)) meals is affected over the first 15 days of infection in Nile tilapia experimentally infected with *V. anguillarum*.

Materials and Methods

Fish and Maintenance

The Nile tilapia used in this digestibility experiment were supplied by the Fish Culture Unit at the Faculty of Fisheries and Aquaculture, Mersin University. Prior to the experiment, all the fish was kept in 1000-l square tanks with a central drainage system and fed commercial trout pellets (Çamlı Fish Feed Company, İzmir, Crude Protein: 42%, 2-mm dry pellets). The experiment was conducted using 9 glass aquaria measuring 40H × 100L × 40W cm. Two weeks before the experiment, 20 fish (42.4 ± 2.3 g average initial wet mass) randomly selected from the stock tanks were housed in aquaria following individual weighing and acclimatized to the experimental diets.

Because the experimental system did not allow 3 experimental diets to be offered to Nile tilapia infected and not infected with *V. anguillarum* simultaneously in triplicate, the digestibility trial was conducted in 2 separate 15-day periods. The fish not infected with *V. anguillarum* were investigated in the first 15-day period and designated as controls for the infected fish in the second experiment. The second experiment was conducted using 14 fish (42.3 ± 2.1 g average wet mass). Fish were fed at 4% and 3% live weight in 2 equal meals from 0900 to 1000 hours and from 1700 to 1800 hours, respectively, during the 2-week acclimation period to experimental diets and in the actual trials.

Feces from each aquarium were collected by sedimentation (14). Fish were prevented from escaping using perforated plastic sheeting on top of each aquarium. Aeration to the experimental system was supplied by a central aeration system using air stones. The dissolved oxygen level in each aquarium was not allowed to fall below 5.5 mg l^{-1} during the experiment. Water temperature was kept at 26 ± 2 °C in the system using heaters with thermostatic control. Uneaten feed was collected from each aquarium following feeding during the experiment. Approximately two-thirds of the water in each aquarium was then replaced with aerated and heated clean freshwater from two 1000-l reservoir tanks. For each replicate, food consumption was measured before the fecal collection days (days 1, 3, 5, 7, 10, and 14). The average physical and chemical water quality parameters during the experiment were not allowed to fall below the required levels for Nile tilapia (15).

Diet formulation and preparation

Two experimental diets were formulated to replace 30% of fish meal crude protein with solvent-extracted SBM and MBM. These alternative protein sources were both supplied by a commercial feed company located in Adana. The locally produced fish meal, supplied by another commercial feed company, was used in the experimental diets. Commercial cod liver oil and dextrin were used as oil and carbohydrate sources respectively in the diets. Diets were formulated to contain similar crude protein and gross energy on a dry matter basis. The amount of mineral and vitamin mixes incorporated into diets was chosen in accordance with the dietary requirements of Nile tilapia (16) (Table 1).

Finely ground dry ingredients were mixed with a small kitchen mixer for about 45 min before cod liver oil and mineral and vitamin mixes were added to the mixture. Following the inclusion of Cr_2O_3 (10 g.kg diet^{-1}), the mixtures were further mixed for 30 min. Diets were manufactured as strands using a kitchen meat grinder after distilled water was added. Following drying all the diet strands were broken into equal size pellets by hand in accordance with the mouth size of Nile tilapia. Dried diets were individually bagged and stored at –20 °C until used.

Table 1. Formulation and chemical composition of the experimental diets.

Ingredients (g.kg diet^{-1})	Diets		
	CONT	SBM	MBM
Fish meal	613.0	429.0	429.0
L-lysine	0.0	3.0	6.0
Soybean meal	0.0	302.8	0.0
Meat and bone meal	0.0	0.0	434.5
Fish oil	83.0	95.0	45.0
Dextrose	50.0	50.0	47.0
Bentonite	175.5	41.7	0.0
Carboxymethylcellulose	50.0	50.0	10.0
Mineral mix†	12.5	12.5	12.5
Vitamin mix‡	5.0	5.0	5.0
Stab-C§	1.0	1.0	1.0
Cr_2O_3	10.0	10.0	10.0
Chemical composition			
Dry matter (DM g.kg diet^{-1})	937.5 ± 6.3	942.4 ± 3.8	954.6 ± 7.5
Crude protein (g.kg DM^{-1})	407.6 ± 4.9	415.9 ± 6.7	415.4 ± 8.7
Crude fat (g.kg DM^{-1})	128.4 ± 3.1	128.2 ± 2.8	127.5 ± 1.8
NFE+crude fiberll (g.kg DM^{-1})	216.2	323.6	219.3
Ash (g.kg DM^{-1})	247.8	132.3	237.8
Gross Energy (MJ.kg DM^{-1})	16.8 ± 0.01	18.6 ± 0.01	16.5 ± 0.8

† Mineral mixture (mg/kg food): Mn; 1000, Fe; 437.5, Zn; 625.0, Cu; 62.5, I; 25.0, Co; 5.0, Se; 1.9.

‡ Vitamin mixture (mg/kg food): Vitamin A; 114,000 IU, Vitamin D_3; 14,250 IU, Vitamin E; 1140.0, Vitamin K_3; 57.0, Vitamin B_1; 142.5, Vitamin B2; 199.5, Vitamin B_6; 142.5, Vitamin B_{12}; 0.342, Biotin; 2.85, Folic acid; 57.0, Niacin; 1254.0, Pantothenic acid; 285.0, Vitamin C; 1425.0, Inositol; 1140.0, Antioxidants; 712.5. Vitamin mixture was supplied by Hoffman La Roche, İstanbul, Turkey.

§ Stab-C (L-Ascorbyl-2-polyphosphate)

‖ NFE=Nitrogen-free extractives. Calculated as the remainder of crude protein + crude fat + ash.

The experimental infection procedure

V. anguillarum was injected into juvenile Nile tilapia intraperitoneally. In the experimental infection procedure 24-h fresh cultures of bacteria were used. Following the 24-h incubation period, bacteria were centrifuged for 10 min at 3500 × g. The supernatant was then re-centrifuged and washed twice with phosphate buffered saline (PBS) solution. After being diluted with PBS, this bacterial suspension was prepared in 4 different doses (17). The dilution consisting of bacterial numbers as 1×10^6 CFU (colony forming units) was chosen and injected into the fish using plastic syringes (two 10 cc syringes per 14 fish in each aquarium at a rate of 1 cc per fish).

Sampling and calculation of apparent nutrient digestibility

Fecal samples were collected from aquaria between 2000 and 0700 hours on days 1, 3, 5, 7, 10 and 14 in each trial throughout the experiment. The samples were then dried in an oven at 40 °C in plastic jars. Following drying, samples were ground and kept at –20 °C until used for nutrient, gross energy, and chromic oxide analysis (see chemical analysis). The crude protein and chromic oxide levels of the samples were analyzed using fecal matter collected from each replicate of treatments, whereas gross energy was analyzed using the remaining fecal matter of each treatment after being pooled equally. The apparent nutrient digestibility of the experimental diets was calculated using the standard formula:

$AD (\%) = 100 - [100(\%I_{diet}/\%I_{feces}) \times (\%N_{feces}/\%N_{diet})]$ (18), where I is the inert marker and N is the nutrient.

Chemical analysis

Diet and fecal samples were analyzed for crude protein (Kjeldahl, selenium catalyst; %N × 6.25). Gross energy in diets and feces was analyzed using a bomb calorimeter (IKA® – WERKE, C2000 Autobomb, basic model, Staufen, Germany, calibrated with benzoic acid). Crude fat in diets was analyzed according to the soxhlet method (19). Dry matter (g per kg DM) and ash in diets were analyzed using standard methods (20). Chromic oxide was determined according to Furukawa and Tsukahara (21).

Statistical analysis

All data were subjected to one-way ANOVA using SPSS 10.0 and reported as mean ± sd throughout the text. Student's t-test was also used to identify the significant differences between non-infected and infected groups for diets and individual sampling day. The apparent digestibility values for dry matter, crude protein, and gross energy calculated for each diet were arcsin-transformed prior to analysis and normality and homogeneity of variance were confirmed for each parameter. When a significant treatment effect was observed, a Tukey-Kramer HSD test was used to compare means. A probability of 0.05 or less was considered significant.

Results

There was no mortality during the trials. Feed intake was reduced in fish infected with *V. anguillarum* compared to the control group (non-infected fish) regardless of dietary protein sources used in the diets. Apparent dry matter digestibility (AD_{DM}) values for infected and non-infected (control group) fish varied between 56% and 74% and between 48% and 76%, respectively (Table 2). The AD_{DM} of fish infected and fed with CONT diet was 48% and this value was significantly lower ($P < 0.05$) than the AD_{DM} of the non-infected fish fed the same diet (Table 2). The average AD_{DM} values for fish fed the MBM diet in both groups followed a similar trend to the SBM diet but they were 8%-10% lower. It was also evident that the AD_{DM} values obtained on experimental diets (CON, SBM, and MBM) in both groups were significantly different ($P < 0.05$) from each other (Table 2).

The average sampling day AD_{DM} values obtained on the CONT diet (a) in both groups were not significantly different ($P > 0.05$) (Figure 1). However, the average AD_{DM} value calculated on day 5 for the infected group was significantly lower ($P < 0.05$) than the value calculated on the same day for the control group (Figure 1). The magnitude of the average sampling day AD_{DM} values obtained on the MBM diet (c) were also not significantly different ($P > 0.05$) in both groups but the values measured on days 3 and 10 in the infected group were significantly lower ($P < 0.05$) than the values measured for the same sampling days in the control group (Figure 1).

Apparent digestibility values for crude protein (AD_{CP}) obtained on experimental diets generally varied between 70% and 85% in the non-infected and infected Nile tilapia (Table 2). Although the AD_{CP} values obtained on

Table 2. Apparent dry matter (AD_{DM}), crude protein (AD_{CP}), and gross energy (AD_{kJ}) digestibility values (%) of nutritionally balanced diets containing animal (meat and bone meal (MBM)) and vegetal (solvent extracted soybean meal (SBM)) protein sources in the Nile Tilapia not infected and infected by *V. anguillarum*.

Diets	Control (non-infected)	Infected	F*	P*
AD_{DM}				
CONT	55.13 ± 7.11^{c}	$48.84 \pm 5.86^{1,c}$	16.486	< 0.001
SBM	73.38 ± 3.94^{a}	75.00 ± 3.22^{a}	1.648	0.205
MBM	66.49 ± 5.13^{ab}	67.10 ± 11.82^{b}	3.142	0.086
F**	29.148	29.744		
P**	< 0.001	< 0.001		
AD_{CP}				
CONT	76.13 ± 6.60^{b}	73.18 ± 3.73^{ab}	0.779	0.385
SBM	84.74 ± 2.92^{a}	85.49 ± 3.27^{a}	0.186	0.669
MBM	78.84 ± 6.31^{ab}	$70.51 \pm 11.61^{1,bc}$	4.133	0.045
F**	4.515	6.210		
P**	0.016	0.004		
AD_{kJ}				
CONT	80.58 ± 3.83	77.74 ± 3.11^{abc}	2.796	0.106
SBM	82.07 ± 3.70	83.55 ± 2.92^{a}	1.740	0.197
MBM	77.37 ± 6.55	74.63 ± 8.24^{b}	0.961	0.334
F**	3.099	5.639		
P**	> 0.05	0.007		

Each value is the mean (±sd) of triplicate aquaria (n = 18). Means in the same column with different superscripts are significantly different (Tukey-Kramer HSD, P < 0.05).

[1] Indicates significant difference compared with the control (non-infected) (Student's t-test, P < 0.05)

the SBM diet in both groups were higher than those of the CONT and MBM diets, a significant difference was only found with CONT and MBM diets in the non-infected and infected groups, respectively (Table 2). Moreover, the AD_{CP} values obtained on the MBM diet in the infected group were significantly lower than those in the non-infected group (Table 2).

The average sampling day AD_{CP} values obtained on the SBM diet (b) on days 1, 3, and 5 in the infected group were higher than those in the non-infected group and the difference between values measured on day 1 in both groups was statistically significant (P < 0.05) (Figure 2). There were significant differences (P < 0.05) between some of the AD_{CP} values obtained on the SBM diet for different sampling days in the non-infected (control) group but these differences were not observed in the infected group. The AD_{CP} measured on day 3 in the infected group was significantly lower (P < 0.05) than the same day measurement in the non-infected group (Figure 2). There was no significant difference (P > 0.05) between the AD_{CP} values obtained on the MBM diet (c) for different sampling days in both groups but the AD_{CP} values measured on days 1, 3, and 10 in the infected group were significantly lower (P < 0.05) than their counterparts in the non-infected (control) group (Figure 2).

The overall AD_{kJ} values obtained on different experimental diets varied between 77% and 83% in the non-infected (control) group (Table 2). The AD_{kJ} values obtained on diets in the infected group were significantly different (P < 0.05) compared with the control group. Specifically, the AD_{kJ} values measured in fish fed the MBM diet in the infected group were significantly lower (P < 0.05) than those in fish on the SBM diet (Table 2).

The differences between AD_{kJ} values by sampling days obtained on the CONT (a) diet in the non-infected group were statistically significant (P < 0.05) but no significant

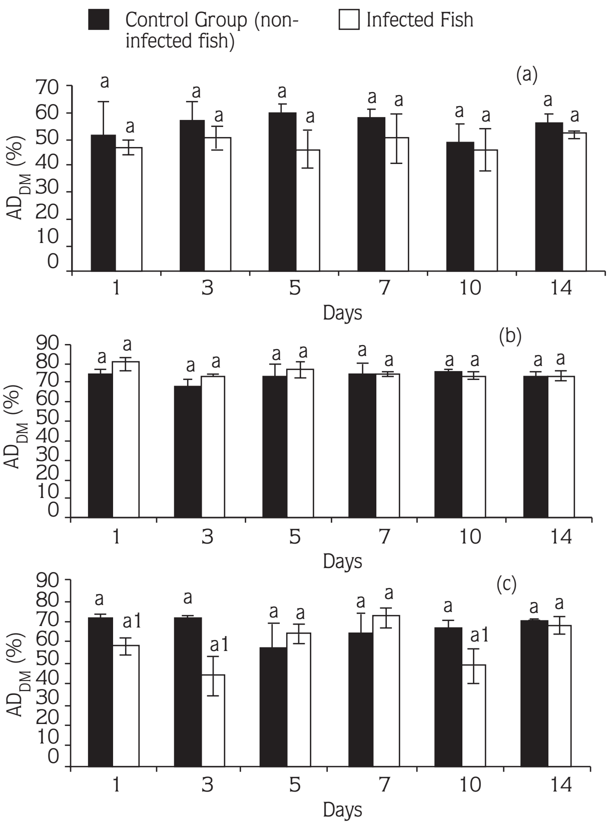

Figure 1. The AD_{DM} values of a) CONT, b) SBM, and c) MBM diets in Nile tilapia infected or not infected with *V. anguillarum*. Means ± sd (n = 3). Multiple comparisons were made separately for each group and means with different superscripts in groups are significantly different ($P < 0.05$). [1]Indicates a significant difference ($P < 0.05$) between means recorded for the same day in each group.

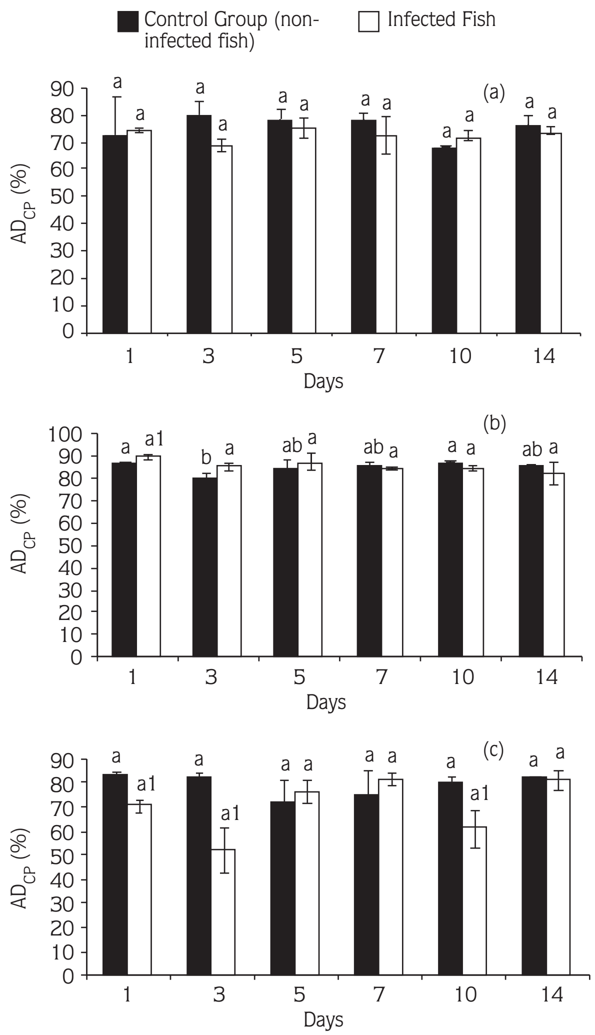

Figure 2. The AD_{CP} values of a) CONT, b) SBM, and c) MBM diets in Nile tilapia infected or not infected with *V. anguillarum*. Means ± sd (n = 3). Multiple comparisons were made separately for each group and means with different superscripts in groups are significantly different ($P < 0.05$). [1]Indicates a significant difference ($P < 0.05$) between means recorded for the same day in each group.

differences were observed in the infected group (Figure 3). Significant differences ($P < 0.05$) were also detected between the average sampling day AD_{kJ} values obtained on the SBM (b) diet in both groups. The AD_{kJ} measured for day 1 in the infected group was significantly lower ($P < 0.05$) than that in the non-infected group (Figure 3).

Discussion

This study showed that Nile tilapia had better nutrient digestibility values when fed the SBM diet regardless of experimental infection with *V. anguillarum*. In line with our findings, several authors were previously able to demonstrate that the apparent crude protein digestibility (AD_{CP}) of soy products (both solvent extracted and full fat meals) ranged between 90% and 95%, indicating that the protein in these meals is highly digestible in Nile tilapia (5,6,9). In addition, Schneider et al. (9) reported that Nile tilapia (55 g) had better growth and nutrient digestibility values when fed solvent extracted soybean (15% of total feed ingredients) than when fed full fat soybean meal. The AD_{DM}, AD_{CP}, and AD_{kJ} measured for solvent extracted soybean used in their study were 80%, 91%, and 87%, respectively. It has also been demonstrated that growth performance and carcass chemical composition of the Nile tilapia were not negatively affected when they were fed

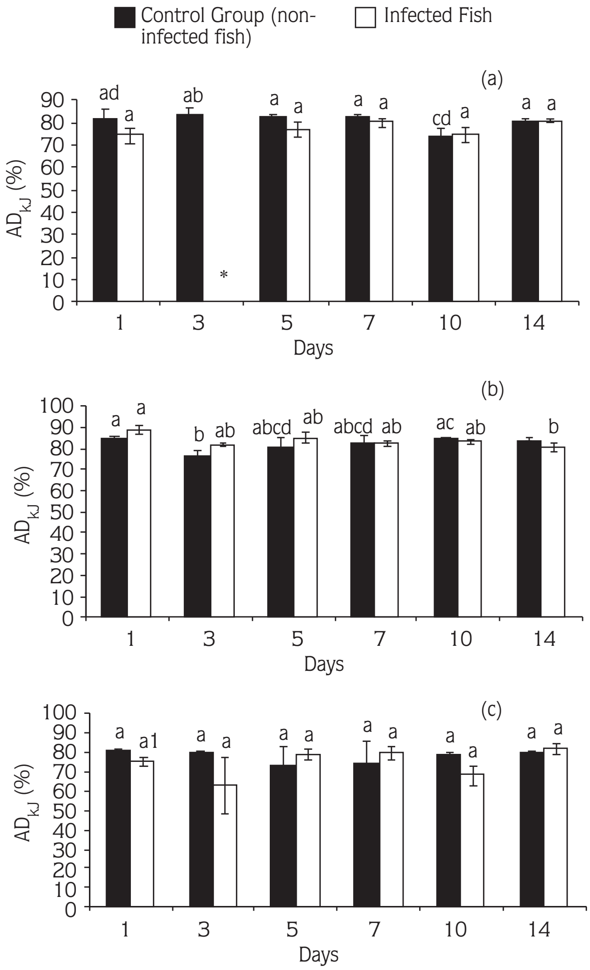

Figure 3. The AD_{kJ} values of a) CONT, b) SBM, and c) MBM diets in Nile tilapia infected or not infected with *V. anguillarum*. Means ± sd (n = 3). Multiple comparisons were made separately for each group and means with different superscripts in groups are significantly different ($P < 0.05$). [1]Indicates a significant difference ($P < 0.05$) between means recorded for the same day in each group. *Not enough feces were available for the analysis.

solvent extracted soy bean meal (64% of the total feed ingredient) and supplemented with L-lysine and L-threonine and corn and wheat flour as only other protein sources compared with a fish meal based control diet (22).

The similar MBM AD_{CP} and AD_{kJ} values to those of the SBM and CONT diets found in this study might suggest equal digestion and metabolism of MBM proteins as SBM and fish meal proteins. Limited information is available on the bioavailability of nutrients in meat and bone meal in the Nile tilapia (8). According to Wu et al. (8), juvenile Nile tilapia showed similar weight gain increment, feed efficiency ratio, and net protein utilization values to a fish meal based control diet when fed a diet that mainly consisted of plant proteins (corn gluten, corn flour consisting high amount of lysine and solvent extracted soy bean meals) and 7% meat and bone meal supplemented with synthetic amino acids as protein sources. Although not significantly different, the 8% to 9% lower AD_{DM} than that of the SBM diet obtained in the non-infected group might be attributed to the high amount of ash in the MBM diet (Table 2). The high amounts of acid detergent fiber (ADF), which constitutes the cell wall fraction of plant proteins, and crude ash in meat and bone meals were demonstrated to lower the nutrient and energy digestibility in a wide range of carnivorous farmed fish species (23-25). A strong negative correlation between the amount of MBM in diets and AD_{CP} values was also reported for gilthead sea bream (*Sparus aurata*) (23) and their findings of 79% crude protein digestibility when MBM replaced 30% of total crude protein content of the feed are in good agreement with the 80% crude protein digestibility found in this study for the Nile tilapia. However, the higher nutrient and energy digestibility obtained on the SBM diet compared with the other diets in the non-infected group suggested that the Nile tilapia could utilize higher amounts of plant proteins than other important farmed fish species (5,7,9). It is also expected that the amount of protein synthesized depends not only on the amount of protein absorbed but also on the amino acid balance of the particular protein and the digestible energy intake (26).

Experimental infection with *V. anguillarum* in Nile tilapia significantly lowered the AD_{DM} and AD_{CP} for fish fed the CONT and MBM diets compared with the fish in the non-infected group. AD_{DM}, AD_{CP}, and AD_{kJ} values of infected fish fed the SBM diet were also unaffected. Néji and de la Noüe (4) reported that rainbow trout experimentally infected with *Aeromonas salmonicida* had significantly lower nutrient and energy digestibility values compared with the control when fed diets either containing 65% and 55% of total feed ingredient as vegetal protein (soy bean extract) or animal by-products (i.e. poultry meal and feather and blood meals) respectively. The AD_{DM}, AD_{CP}, and AD_{kJ} values found for infected Nile tilapia fed the SBM diet in this study contradict the results reported by Néji and de la Noüe (4). However, the higher AD_{DM}, AD_{CP}, and AD_{kJ} values obtained on the SBM diet compared with the animal proteins (CONT and MBM diets) in this study are consistent with their findings (4). The absence of symptoms characterized in vibriosis (27) suggests that the anatomical damage might not be the reason for the impairment of digestibility on different fecal collection days in the infected Nile tilapia fed CONT and SBM diets (4). Therefore, the significantly lower AD_{DM}, AD_{CP}, and AD_{kJ} obtained on the CONT and MBM diets in the infected Nile tilapia could be attributed to the stimulation of the sympathetic nervous system, which controls the gastrointestinal mobility as was demonstrated for mammals under stress (28). However, the activation of this system and its effect on the nutrient digestibility of infected Nile tilapia fed CONT and MBM diets in this study appeared to be on different days of the fecal collection period. This might have been a part of the combat strategy against *V. anguillarum* infection compared with the non-infected group. In this respect, several authors have reported that heat shock proteins known as stress proteins like SP70 were significantly higher in liver and head kidney tissues of coho salmon (*Oncorhynchus kisutch*) infected with chronic *Renibacterium salmoninarum* (29) and rainbow trout (*Oncorhynchus mykiss*) infected with *V. anguillarum* (11). These organs are considered likely places where the pathogen is known to accumulate and induce an inflammatory response in fish (11). The biggest decrease in nutrient and energy digestibility values obtained on CONT and MBM diets in infected fish was between days 1 and 10, where there might have been an inflammatory response compared with the non-infected group. The fact that nutrient and energy digestibility of infected fish fed the SBM diet did not significantly change compared with the control group also suggested that the high amount of non-starch polysaccharides in this diet might have played a role in the resistance of Nile tilapia to *V. anguillarum*. Wang and Wang (30) reported that tilapia (*Oreochromis aureus*) had an increased resistance to *Aeromonas hydrophila* and *Edwardsiella tarda* infections when fed several polysaccharides like glycan (extracted from barley) via activation of the phagocytes. In addition, finding nutrient and energy digestibility values similar to non-infected group values at the end of the sampling period in this study implied that infected fish successfully combated the bacteria as demonstrated in rainbow trout infected with *V. anguillarum* by Ackerman and Iwama (11).

Digestibility measurements can vary greatly among studies conducted even in the same species depending on the methods used for feces collection (31). The Guelph system is mainly used in digestibility studies for juvenile fish and the major shortcoming of this system is the leaching of nutrients from feces into water when allowed to remain for longer periods. Chromic oxide (Cr_2O_3) is also a widely used external marker in fish digestibility studies. Although it is not conclusive, chromic oxide may yield imperfect results in digestibility studies. Both the Guelph system and chromic oxide were used in this study and utmost care was taken in order not to overestimate the digestibility measurements by collecting the fecal samples just after they were defecated. In this respect, similar digestibility values, measured for soy bean extract and meat and bone meal in Nile tilapia compared with the previous findings, justify the methodology used for digestibility measurements in this study. However, improvements in the methodology used in fish digestibility measurements are being considered all the time.

In conclusion, although this study showed that, regardless of infection with *V. anguillarum*, Nile tilapia had better nutrient and energy digestibility when fed the SBM diet than the CONT and MBM diets, a clear conclusion is not possible. Although SBM seems to be a better choice of protein for Nile tilapia (42.4 g) for nutrient utilization, further research investigating the effects of higher amounts of these alternative proteins with different doses of *V. anguillarum* on growth or immunological parameters is needed to evaluate the overall utilization of alternative protein sources in infected Nile tilapia. Moreover, a better understanding of polysaccharides as natural immunoprophylactic substances would also contribute to the wide use of plant proteins as alternative protein sources in omnivorous or herbivorous fish diets in the future.

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