



## Effect of the seaweed *Ulva lactuca* as a feed additive on growth performance, feed utilization and body composition of Nile tilapia (*Oreochromis niloticus* L.)

W. Nafity<sup>1\*</sup>, M. Droussi<sup>2</sup>, N. Berday<sup>1</sup>, A. Araba<sup>1</sup>, M. Benabid<sup>2</sup>

<sup>1</sup>Hassan II Institute of Agronomy and Veterinary Medicine, Rabat, Morocco

<sup>2</sup>National Center of Hydrobiology and Fish Culture, Azrou, Morocco

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### Abstract

The present study investigates the potential of the supplementation of the seaweed *Ulva lactuca*, as a dietary additive ingredient in fish feed, to improve the Nile tilapia (*Oreochromis niloticus*) growth performance, feed utilization and carcass composition. The experiment was conducted in the Deroua fish farming facilities (Beni Mellal, Morocco), on male Nile tilapia of a mean weight of  $50 \pm 1g$ . Three experimental diets composed of 32% of dietary protein were prepared using dried algae meal ingredient incorporated at levels of 0% (control), 5% and 10% of fish feed. By the end of the experiment, fish growth parameters, feed utilization, somatic indexes and carcass composition were evaluated. Fish fed on 5% seaweed diet tended to have higher growth performance than those fed to the control and 10% seaweed diet ( $p > 0.05$ ). Feed utilization by fish seemed not to be affected by seaweed meal rates change ( $p > 0.05$ ). The viscerosomatic (VSI), hepatosomatic (HSI) and gonadosomatic (GSI) indexes showed no significant difference between diets. Crude protein and crude lipid contents in the fish carcass were not statistically significant ( $p > 0.05$ ), whereas significant differences ( $p < 0.05$ ) in ash content was observed between treatments. In addition, *Ulva lactuca* meal can be included as supplement ingredient in the diet without impairing growth performance, feed utilization and body composition of Nile Tilapia.

\* Corresponding Author: Nafity Widad ✉ [nafityw@gmail.com](mailto:nafityw@gmail.com)

## Introduction

Nile tilapia (*Oreochromis niloticus*) is the most important aquaculture species in the world. Morocco has recently introduced this fish as a targeted species in his aquaculture program. The promotion of this activity will improve inland fisheries production in the country. The challenges facing tilapia production today is to improve feed formulation with natural feed ingredients in order to enhance the fish growth, carcass quality, maximize feed efficiency, minimize fish mortality and reduce production cost.

Several nutritional trials with partially incorporated macro-algae meal were conducted, aiming to the improvement of fish viability, disease resistance and carcass quality (Hamauzu and Yamanaka, 1997). The supplementation of the fish diet with seaweed ranged between 2% up to 28%. Differences between levels of seaweeds may be variable depending on the feeding habits, age and the species of both algae and fish (Güroy *et al.*, 2007; El-Tawil, 2010). Furthermore, low-level of dietary incorporation of algal meal produced a significant increase in fish growth and feed utilization (Mustafa *et al.*, 1995a, and b).

The marine green seaweed (*Ulva lactuca*) could be supplemented to Nile tilapia diet at an optimum level of 15% and lead to a significant improvement in growth performance without any negative effect on feed efficiency or survival rate (Güroy *et al.*, 2007; El-Tawil, 2010). These authors found that the highest values for weight gain of Nile tilapia fed on diet supplemented with various levels of *Ulva* meal were achieved when fish were fed at a level of 5 to 10% of *Ulva*. However, Yone *et al.* (1986) reported that a large amount of algae supplement suppressed excessively the absorption of nutrients and resulted in a decrease in growth and feed efficiency. Nakagawa *et al.* (1993) reported that optimum feed efficiency and protein efficiency was attained in black sea bream when the supplementation level of *Ulva* meal was 2.5-5.0% of the diet. Nevertheless, information concerning seaweed use in fish culture remains very limited and needs further investigation.

The present work aims to investigate the effect of the seaweed *U. lactuca*, collected from the Moroccan Atlantic coast, as a dietary supplement on growth performance, feed utilization and body composition of Nile tilapia, and to determine the adequate level of supplementation for optimum fish response.

## Materials and methods

The present study was carried out in the Deroua Fish farming plant facilities during a period of 60 days. The fish farm (32°19'N, 6°33'W) is located in the forest of Deroua at 20 km south west of the city of Beni Mellal. Emberger (1930) classified this region as a semi-arid area with temperate winter.

### *Ulva meal preparation*

The seaweed *U. lactuca* biomass was collected from El Jadida coastline (33°15'N, 8°30'W), located on the Atlantic shore of Morocco from June to July. Fresh thalli were harvested at the intertidal zone during low tide. The algal biomass was washed with seawater, sun-dried and milled using a laboratory blender of a mesh of 3.2 mm diameter to prepare a seaweed meal. And then, the proximate chemical composition of *Ulva* meal was determined through laboratory analysis according to AOAC (2003) as shown in table 1.

**Table 1.** Physico-chemical composition of *U. lactuca* meal (% dry matter).

Parameter	Dry matter*	Crude Protein	Ash	NDF	ADF	Crude fiber
Ulva meal	91.0	14.2	41.6	26.4	12.1	4.4

\*(% as fed)

### *Fish feed formulation and preparation*

Three fish feed diets of crude protein content of 32.75 ± 0.55% were formulated using the seaweed (*U. lactuca*) meal as a dietary ingredient supplemented at levels of 0% (control diet), 5% (5%S diet) and 10% (10%S diet) of fish feed (Table 2). The ingredients for each diet were mixed, oil and 35% distilled water were added, after which the experimental diets were pelleted with a laboratory pelleting machine. The moist pellets were sun dried, and stored at -20°C until utilization.

**Table 2.** Chemical composition and energy content of the experimental fish diets.

Experimental fish diets composition	Control (0%S)	5%S Diet	10%S Diet
Dry matter (% as fed)	92.9	93.1	93.4
Crude protein (% DM)	33.0	32.2	33.3
Ash (% DM)	12.5	14.6	10.7
NDF <sup>1</sup> (% DM)	23.1	20.9	21.1
ADF <sup>2</sup> (% DM)	5.2	5.8	4.9
Crude fiber (% DM)	4.1	3.6	3.9
NFE <sup>3</sup> (% DM)	34.3	33.8	36.5
GE (kcal/kg diet) <sup>4</sup>	416.5	409.4	400.1
DE (kcal /kg diet) <sup>5</sup>	312.4	307.0	300.0

S: seaweed

<sup>1</sup>NDF: neutral detergent fiber

<sup>2</sup>ADF: Acid detergent fiber

<sup>3</sup>NFE: Nitrogen-free extractives = 100 – (ash + ether extract + crude protein + gross fiber).

<sup>4</sup>GE (gross energy) was calculated using the conversion factors of 23.6, 39.5 and 17.0 KJ/g for protein, lipid and nitrogen free extract (NFE), respectively (Brett and Groves (1979).

<sup>5</sup>DE (digestible energy) was calculated by applying the coefficient of 0.75 to convert gross energy to digestible energy according to Hepher *et al.* (1983).

#### Chemical analysis

Proximate composition of all ingredients used for feed preparation, experimental diets and fish carcass were determined according to the procedures of the Association of Official Analytical Chemists (AOAC, 2003). Dry Matter (DM) was determined within weight change calculation before and after 105°C drying, crude protein (CP) was determined by the Kjeldhal method, ash was measured after drying at 105°C, calcinations in muffle oven at 500°C, and fat was determined by ethyl ether extraction (Soxhlet technique).

#### Characteristics of experimental fish raising facilities

The experiment was performed in three treatments using three concrete tanks. To triplicate the trial, each tank was divided with a net into three equal compartments of (L/W/H=3.50/3.55/1.60 m) and an efficient water volume of about 20 m<sup>3</sup>.

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#### The experimental fish stock

A total number of 180 male Nile tilapia of an initial average weight of 50 ± 1 g were used to conduct this study. The fish were selected from the stock available in the farm in order to ensure homogeneous size for the whole biomass. The fish were randomly equally distributed in the three tanks treatments, with each tank stocked in triplicate. Thereafter, the three tanks treatments received each one kind of diet (Control = (0%S diet), (5%S diet), and (10%S diet). The fish were acclimated to the new breeding environment and to the experimental diets during 7 days. Then fish were fed until complete satiation three times a day (9h00 AM, 14h00 PM and 17h00 PM) and daily records were made for consumed feed for each tank.

#### Water quality handling

Water quality parameters including temperature (°C), dissolved oxygen (DO), pH, NO<sub>2</sub><sup>-</sup> (mg/l), NO<sub>3</sub><sup>-</sup> (mg/l) and PO<sub>3-4</sub><sup>-</sup> (mg/l) were surveyed during all the study period to maintain the fish breeding environment suitable for optimal growth. Water analysis was performed according to Rodier (1996) as illustrated in table 3.

**Table 3.** The fish tanks water quality during the experimental breeding period.

Temperature (°C)	DO (mg/l)	pH	NO <sub>2</sub> <sup>-</sup> (mg/l)	NO <sub>3</sub> <sup>-</sup> (mg/l)	PO <sub>3-4</sub> <sup>-</sup> (mg/l)
27.0	7.9	7.6	0.6	3.7	0.5

During the experiment, water was exchanged at a daily rate of 30% to keep good water quality parameters within the suitable range for fish rearing. Every two weeks, the tanks water was removed and tanks were cleaned, and then were filled with new fresh water.

#### The fish stock survey

The fish weight was measured fortnightly after a 24 h starvation period. By the end of experiment, 15 fishes were randomly selected from each treatment (5 fishes from each compartment) for chemical analysis. Livers, viscera and gonads of 15 fishes per treatment

(5 fishes per compartment) were removed and weighed.

*Fish growth performance and feed nutritive value calculations*

Growth performance and feed nutritive value were determined in terms of final individual fish weight (g), body weight gain (BWG, g), specific growth rate (SGR, g/day), relative growth rate (RGR,%), survival rate (%), feed conversion ratio (FCR), protein efficiency ratio (PER), hepatosomatic index (HSI), viscerosomatic index (VSI) and gonadosomatic index (GSI). The following formulae were used for calculation:

Body weight gain =  $W_2 - W_1$  Where:  $W_2$ : final body weight;  $W_1$ : initial body weight;

Specific growth rate =  $(\ln W_2 - \ln W_1) \times 100 / \text{period in day}$ ;

Relative growth rate =  $(\text{weight gain} / \text{initial body weight}) \times 100$ ;

Survival rate:  $N_1 / N_0 \times 100$ , where:  $N_1$ : total number of fish survival in pond at the end of experiment,  $N_2$ : total number of fish in ponds at the beginning of experiment;

Feed conversion ratio (FCR) =  $\text{total feed consumption (g)} / (W_2 - W_1)$ ;

Protein efficiency ratio (PER) =  $(W_2 - W_1) / \text{protein intake (g)}$ ;

HSI:  $(\text{liver wet weight (g)} / \text{weight of fish (g)}) \times 100$ ;

GSI:  $(\text{gonads wet weight (g)} / \text{weight of fish (g)}) \times 100$ ;  
 VSI:  $(\text{intestine wet weight (g)} / \text{weight of fish (g)}) \times 100$ .

*Statistical analysis*

Data were analyzed using one-way analysis of variance (ANOVA). A Duncan's multiple range test was performed at a 5% level (Zar, 2001), to detect any

significant difference among treatments. This Statistical analysis was performed using SAS software version 9.1.

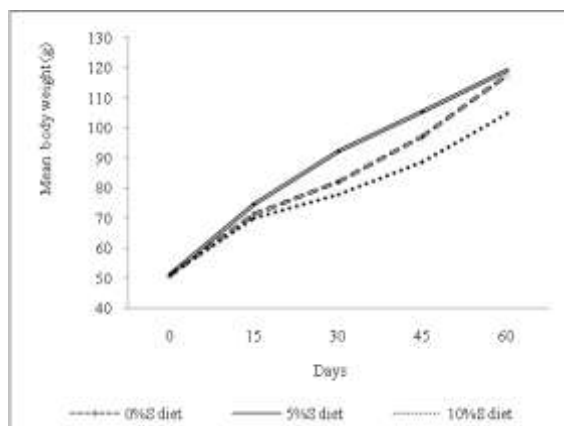
**Results**

*Fish growth performance and feed utilization*

Results obtained for growth performance and feed utilization during the experiment are summarized in Fig. 1 and Table 4. The three experimental diets were well consumed by fish. The 5%S diet allowed the highest fish growth performance when compared to control and 10%S diets. However, differences between treatments were not statistically significant ( $p > 0.05$ ). For 5%S diet, the fish final body weight increased from 51.1 to 119.2 g, leading to a body weight gain of 68.9 g, a relative weight gain of 35%, a specific growth rate of 1.4 g/day, while fish fed on 10%S diet recorded the lowest growth parameters with a specific growth rate of 1.2 g/day.

The fish mortality that occurred during the trial was mostly related to handling during sampling. The highest survival rate (97.5%) was obtained using 5%S diet, followed by 10%S diet (94.4%), and then by control diet (91.9%). However, the difference between treatments was not significant ( $p > 0.05$ ).

The lowest feed conversion ratio and the highest protein efficiency ratio (2 and 3.7 respectively) were obtained using the 5%S diet, followed by the control, and then by the 10%S diet. The difference between the treatments was not significant ( $p > 0.05$ ).



**Fig. 1.** Growth response of Nile tilapia fed on experimental diets.

**Table 4.** Growth performance and feed utilization of the Nile tilapia fed *U. lactuca* as feed additive in diets.

Parameters	Control (0%S diet)	(5%S diet)	(10%S diet)	±SE
Growth performance				
Initial body weight (g)	50.9 <sup>a</sup>	51.1 <sup>a</sup>	51.2 <sup>a</sup>	±0.9
Final body weight (g)	117.4 <sup>a</sup>	119.2 <sup>a</sup>	104.8 <sup>a</sup>	±1.4
Body weight gain (g)	66.5 <sup>a</sup>	68.0 <sup>a</sup>	53.6 <sup>a</sup>	±8.9
Specific growth rate (% day-1)	1.3 <sup>a</sup>	1.4 <sup>a</sup>	1.2 <sup>a</sup>	±0.1
Relative growth rate (%)	130.6 <sup>a</sup>	133.1 <sup>a</sup>	104.6 <sup>a</sup>	±17.4
Survival rate (%)	91.9 <sup>a</sup>	97.5 <sup>a</sup>	94.4 <sup>a</sup>	±1.5
Feed utilization				
Feed conversion ratio	2.3 <sup>a</sup>	2.0 <sup>a</sup>	2.4 <sup>a</sup>	±0.1
Protein efficiency ratio	3.5 <sup>a</sup>	3.7 <sup>a</sup>	3.4 <sup>a</sup>	±0.9
Somatic indexes				
Hepatosomatic index	1.8 <sup>a</sup>	1.3 <sup>a</sup>	1.6 <sup>a</sup>	±0.3
Gonadosomatic index	1.2 <sup>a</sup>	1.8 <sup>a</sup>	2.1 <sup>a</sup>	±0.4
Viscerosomatic index	6.4 <sup>a</sup>	5.1 <sup>a</sup>	6.4 <sup>a</sup>	±0.5

\*Means in same row followed by same superscript letter are not significantly different (p>0.05).

Regarding somatic indexes, the 5%S diet realized the lowest HSI and VSI (1.3 and 5.2, respectively), while GSI tended to increase for fishes fed on diets supplemented with seaweed meal (1.9 and 2.2, for diets 5%S and 10%S, respectively). No significant difference was noticed for the aforementioned results (p>0.05).

#### Carcass composition

Results of diet effect on Nile tilapia body chemical composition at the end of the feeding trial are summarized in table 5. A slight difference between the three treatments was observed, but remained non-significant (p>0.05). The body crude protein content remained nearly similar for all treatments, with a slight increase for the 5%S diet treatment. A slight decrease of body crude lipids content was noticed for fish fed on seaweed supplemented diets, accompanied with a slight increase of body moisture, compared to control treatment. Body ash composition registered the highest values for control and 10%S diets and remained low for 5%S diet.

**Table 5.** Whole body composition of Nile tilapia fed on experimental diets (% wet weight).

Carcass composition	Control (0%S diet)	(5%S diet)	(10%S diet)	±SE
Moisture	68.8 <sup>a</sup>	78.4 <sup>a</sup>	77.7 <sup>a</sup>	±2.7
Crude protein	10.8 <sup>a</sup>	11.3 <sup>a</sup>	10.4 <sup>a</sup>	±0.3
Crude lipid	17.5 <sup>a</sup>	14.6 <sup>a</sup>	14.7 <sup>a</sup>	±1.6
Ash	16.3 <sup>a</sup>	14.8 <sup>b</sup>	16.8 <sup>a</sup>	±0.1

\*Means in same row followed by same superscript letter are not significantly different (p>0.05).

#### Discussion

Fish fed on diet supplemented with 5% of *U. lactuca* displayed a slight increase in growth performance, feed utilization and crude protein body content, while these parameters decreased for fish fed on 10% S diet. Studies conducted on *Ulva rigida* species as a supplement in Nile tilapia feed, reported similar results, where the best performance was obtained for a level of inclusion of algae of 5% and decreased at levels of 10 to 20% (Diler *et al.*, 2007; Güroy *et al.*, 2007; Ergun *et al.*, 2009). The same optimum rates of incorporation were recorded by Saleh (2014) for red tilapia and Khalafalla *et al.* (2015) for Nile tilapia fingerlings. The decrease of fish growth and feed utilization at levels of *Ulva* incorporation higher than 5% could be explained by the presence in the algae composition of anti-nutritional factors such as saponins, tannins and phytic acid which are reported to occur in the vegetative tissues of several plants (Francis *et al.*, 2001) and might affect fish growth (Azaza *et al.*, 2008). The presence of indigestible fiber could also impair protein digestibility (Azaza *et al.*, 2008), which contributes to the growth decrease observed during this study. The difference in optimum supplementation level of *Ulva* meal results that could occur is probably related to the differences in fish species, algae species, fish size, and experimental condition in different studies.

Somatic indexes, such as HSI, VSI and GSI, are used to evaluate the nutritional status of fish. The HSI varies as a function of dietary protein, carbohydrate

and lipid level (Lee *et al.*, 1973; Garling *et al.*, 1977; Hilton *et al.*, 1982; Serrano *et al.*, 1992). In this study the HSI, VSI and GSI were not affected by the different levels of *Ulva* in the diets. However, HSI values tended to decrease with fish fed on *Ulva* supplemented diet. Azaza *et al.* (2008) reported the same response. The reason could be due to a reduction of deposition of fat in the liver, which evidently affected its weight.

Cultured fish is known to accumulate highly lipid reserves compared to wild fish. This excessive lipid accumulation leads to the deterioration of carcass quality (Nakagawa *et al.*, 1986). The inclusion of a small amount of algal meal to the fish diet can have significant effects on carcass quality (Mustafa and Nakagawa, 1995; Valente *et al.*, 2006). In the present study, results for the fish carcass moisture, crude protein and crude lipid content were not statistically different. However, carcass composition of Nile tilapia fed with at 5% *Ulva* gave the highest value for crude protein and the lowest value for crude lipid. These results agree with those of Güroy *et al.* (2007); Azaza *et al.* (2008); Ergun *et al.* (2009) who pointed out a reduction in body lipid and an improvement in protein content in the carcass when *Ulva* meal was added to Nile tilapia diets. *Ulva* species have a high content of vitamin C (McDermid and Stuercke, 2003; Ortiz *et al.*, 2006; García-Casal *et al.*, 2007). Benefits of inclusion of *Ulva* meal in practical diets may be interpreted by the presence of vitamin C, which affects lipid metabolism (Miyasaki *et al.*, 1995; Nakagawa *et al.*, 2000; Ji *et al.*, 2003). Dietary algae accelerate the assimilation of ascorbic acid, improving lipid metabolism, especially lipolysis (Nakagawa 1997). Ji *et al.* (2010) reported that ascorbic acid in diet stimulates lipolysis and depresses lipogenesis.

### Conclusion

This work confirms earlier findings showing that *U. lactuca* could be considered as a potential additive in fish feed despite differences in chemical composition related to geographic location. The incorporation of *U. lactuca* up to 5% allows a slight increase in growth performance, feed utilization and body composition

of Nile tilapia. However, long-term effect needs further research to ensure the absence of adverse effect. Future studies should focus as well on digestibility coefficients of *U. lactuca* in order to assess its nutritive value prior to any introduction into the diet.

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