The Response of Juvenile Olive Turtle, *Lepidochelys olivacea*: The Inclusion of Dried Seaweed and Seagrass in Fish Based-Formulated Feed

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Abstract. A common efforts to improve the survivorship of sea turtle in Indonesia is cage nursery program and fed single food type i.e. fresh fish. Such effort, however, has some constrains such as availability and the sea turtle would tend to be habituated because of single-continuous feeding type. Eventually, it could have impact on their feeding behaviour and survivorship when it is released to the nature. This study concerns on using easily found dried material such as fish, sea grass and sea weed. Four feeds type i.e. Fish Pellet (FP); F-*Syringodium* sp pellet (FSp); Fish-Ulva sp pellet (FUp) and fresh fish (FF)- control, were tested. Completely Randomised Design (CRD) with three replication were applied (totally 9 units + 1 unt for control), four individuals for each experiment unit (totally 40 individuals). The feed are given twice a day (i.e. 8 am and 16 pm). The experiment was run for 8 weeks and sampling of growth response were 2 weeks interval. The results showed that the Fish pellet (FP) resulted in superior response for all criterion (body weight, length and width of carapace) compared to by fish fresh fish (FF), F-*Syringodium* sp pellet (FSp) and Fish-Ulva sp pellet (FUp). Daily growth (body weight) were significantly different between diet i.e. 0.71±0.19 g/d, 0.54±0.40 g/d, 0.35±0.77 g/d and 0.24±0.30 g/d for fish pellet (FP), fresh fish (FF), F-*Syringodium* sp pellet (FSp) and Fish-Ulva sp pellet (FUp) consecutively (P < 0.05). Nevertheless, the response of carapace length and carapace wide were not significantly different between diets (P > 0.05). The food ratio were FCR > 6 classified low, the efficient feed type (lowest FCR) was shown by fish pellet (FP) feed (i.e. 6.79) and the least efficient (high FCR) was shown by pellet of fish + Ulva sp (FUp) i.e. 24.6. The analysis of body weight – carapace length relationship showed negative allometric (b < 3). The economical analysis on feed production budget indicated that pellet of fish feed (FP) was the highest price, such high cost is due to high price of binder (i.e. CMC).

Overall, the study emphasised that fish pellet feed (FP) was potential feed type to substitute conventional feed i.e. fresh fish for pre released juvenile Olive sea turtle at cage nursery.

1. Introduction

Sea turtle has been listed in the Red Data Book of IUCN and included at Appendix I CITES (Convention on International Trade in Endangered Species) [1]. One of those conserved sea turtle is Olive Turtle, *Lepidochelys olivacea*, known as° penyu lekang or Kempi°- Bali language. The turtle has been listed as one of conserved animal in Indonesia Goverment Rule no. 7/ Agriculture Ministry of Republic of

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*Note: The content is a natural representation of the text without any additional annotations or questions.*
Indonesia [2]. As most of sea turtle conservation center in Bali, *Lepidochelys olivacea* at “Kurma Asih” Sea Turtle Conversation Center is also fed with single feed type i.e. fresh fish (*Sardinella* sp.).

Such continuous single feed type leads the sea turtle to feeding conditioning (habituation) that could disadvantage the animal and to some extent could decrease the animal survivorship when the animal is released to the nature. In the nature, the animal will tend to forage the same feed type fed previously [3] and the post released turtle must switch into other feed source because fish is fast swimmer and low catch ability, otherwise the sea turtle will face starved condition. The other constrain of fresh fish feeding method is the quality and availability of the fish. It is crucial, therefore, to develop alternative feed based on material that are plenty available, low cost production and result in maximum growth response for juvenile Olive turtle. The preliminary study on adults green turtle showed that algae (*Ulva* sp.) and Seagrass (*Syringodium* sp) were well responded [4]. This finding assume that these materials are palatable and nutritious feed materials for omnivorous sea turtle as Olive sea turtle and the other benefit is that the materials are plenty in the nature The objective of this study was addressed to develop dry formulated fish-based feed (pellet) as an alternative feed for juvenile Olive turtle at conservation center. The benefit of dried feed are storable and the nutrition value can be arrange as the need of the animal [5].

2. Materials And Method

The observation was carried out at the Center of Sea Turtle Conservation “Kurma Asih”, Perancak Village, Jemberana Bali Indonesia. Four formulated fish based feed (pellet) were tested i.e. three dried feed formulations (fish; Fish + Ulva sp and Fish + *Syringodium* sp) and fresh fish feed – as the control (table 1). The experiment design was Completely Randomised Design (CRD) with three replication, except control was only 1 experiment unit, totally 10 experiment units (4 individual @ experiment unit). The *L. olivacea* were 7 days post-hatched (totally 40 individual with initial range 21-22.33g, 4.67 – 4.77 mm and 4.11 – 4.17 for body weight, carapace length and carapace wide consecutively.

The experiment was run for 8 weeks (56 days) and the turtle was fed ad libitum twice a day (at 8 am and 16 pm). The remaining uneaten feed was removed and measured after blotting treatment. The water was regularly substituted twice a day i.e. before feeding time and the water quality was weekly checked by means of tools kit for NO$_2$, NO$_3$, pH, temperature and salinity.

The sampling for growth monitoring was carried out by two weeks interval. The variable measured were body weight, carapace length and carapace wide. The data were calculated and statistically analysed.

Body weight (W) = $W_t - W_0$

Daily Growth Rate (g/day) = $(W_t - W_0)/D$

Carapace length (CL) = $CL_t - CL_0$

Carapace Wide (CW) = $CW_t - CW_0$

Note:

$W_t$ = The average body weight (by sampling time) (gr)

$W_0$ = The average initial body weight (Gr)

$CL_t$ = The average carapace length (by sampling time) (mm)

$CL_0$ = The average initial carapace length (mm)

$CW_t$ = The average carapace wide (by sampling time) (mm)

$CW_0$ = The average initial carapace wide (mm)

D = total experiment period (day)

Body length- Weight Correlation, $W = aL^b$

Note

W = Body Weight (gr)

L = Carapace length (mm)

Feed intake (FI), $FI = \sum_{t=0}^{n}(FI_t)$

Daily Consumption rate *(Daily Feed intake / Fl)*

$FL_t = (FW_t X (CF)) - (FW_t)$

$CF = \frac{FW_t}{FW_0}$
3.

3.1. Growth

The statistical analysis of growth response of *L. olivacea* at Table 2 and Figure 1 (a,b and c). The average initial weight of juvenile *L. olivacea* was 21g and the carapace length and carapace wide were 4.67 mm and 4.11 mm respectively. Body weight increase was significantly affected by feed type. Juvenile *L. olivacea* fed with fish pellet and fresh fish diet showed superior growth response compared to those animal fed pellet composed mixed fish-Ulva sp and fish-Syringodium sp. (Table 2). Though fish pellet and fresh fish consistently showed superiority compared to fish-Ulva sp. pellet and fish-Syringodium sp. pellets, these feed type showed non significantly affect on to either carapace length or carapace wide (*P > 0.05*; Table 2).

The analysis of body weight and carapace length correlation is at Figure 2. The overall constant (b value) of body weight – carapace length correlation were 2.1968; 0.3809; 2.9807, 2.1919 for Fp,
FUp, FSp and FF feed type consecutively (Figure 2). All “b-value” were less than three (b < 3) indicating that the growth is negatively alometric.

Table 2. The individual average increase of body weight (BW), carapace length (CL) and carapace wide (CW) of _L. olivacea_

| No | Feed Type | BW increase (g/indv) | Daily BW increase (g/day) | CL increase (mm/indv) | Daily CL increase (mm/day) | CW increase (mm/indv) | Daily CW increase (mm/day) |
|----|-----------|----------------------|--------------------------|----------------------|---------------------------|----------------------|---------------------------|
| 1  | Fp        | 39.70±3.69b          | 0.71±0.19                | 2.01±0.75a           | 0.04±0.04a                | 1.76±0.65a           | 0.03±0.03                 |
| 2  | FUp       | 13.33±5.72a          | 0.24±0.30                | 0.79±0.85a           | 0.01±0.04a                | 0.65±0.75a           | 0.01±0.04                 |
| 3  | FSp       | 19.83±14.40b         | 0.35±0.77                | 1.14±2.08a           | 0.02±0.11a                | 1.02±1.83a           | 0.02±0.09                 |
| 4  | FF        | 30.75±22.56b         | 0.54±0.40                | 1.86±2.80a           | 0.03±0.05a                | 1.80±2.55a           | 0.03±0.04                 |

Note:

- *a* Feed type tested were as dry feed (pellet), except FF: Fresh fish (feed given as fresh feed)
- *b* The value at the same column give the same notification indicates non significance (P > 0.05)
- *c* Fp: fish pellet; FUp: Fish+ Ulva sp pellet; FSp = Fish+ Syringodium pellet, FF= fresh fish
- *d* BW: body weight; CL: Carapace length; CW: Carapace wide; idv: individual

3.2. Consumption

Feed consumption rate (FI) varies between diets and the individual feed intake of sea turtle fed fresh fish showed higher feed intake to those fed the three other feed (table 3). The sea turtle fed fish pellet (Fp) showed superior FCR to those fed other feed types which show relatively similar FCR (table 3). The economical analysis on to budget required for feed production-based on the material price showed that Fp was costly than the other feed type. (Table 3)

**Fig 1.** The growth rate: body weight (BW), carapace length (CL) and carapace wide (CW) of _L. olivacea_ over 8 weeks. (Fp: fish pellet; FUp: Fish+ _Ulva_ sp pellet; FSp = Fish+ _Syringodium_ pellet, FF= fresh fish)
Fig 2. The correlation body weight (BW) and carapace length (CL) of L. olivacea over 8 weeks at different feeding type (Fp : fish pellet; FUp : Fish+ Ulva sp pellet; FSp = Fish+ Syringodium pellet , FF= fresh fish)

Table 3. Consumption and Feed intake (FI) of (L. olivacea) over experiment period (56 days)

| No | Feed type | FI (g)      | Daily FI rate (g/d) | Individual FI (g/indv) | FCR       | Feed production! (IDR/100 g) |
|----|-----------|-------------|---------------------|------------------------|-----------|-------------------------------|
| 1  | Fp        | 289.83±11.52| 5.18±0.20           | 24.15±0.96             | 6.79±0.26 | 1,730                         |
| 2  | FUp       | 238.83±8.81 | 4.26±0.15           | 19.00±0.73             | 10.24±0.37| 1,235                         |
| 3  | FSp       | 295.14±12.25| 5.27±0.21          | 24.60±1.02             | 11.88±0.49| 1,235                         |
| 4  | FF        | 206.22±7.76 | 3.68±0.13           | 51.56±1.94             | 11.00±0.41| 1,200                         |

Note:

a The feed production was calculated based on the assumtion price when the study was carried out; (Sardinella sp per kg is IDR 12,000, fresh seagrass and alga are IDR 1,000; CMC (binder) is IDR 90,000 and vitamin mix is IDR 40,000)

b FI : feed intake; FCR : food conversion ratio

c Fp : fish pellet; FUp : Fish+ Ulva sp pellet; FSp = Fish+ Syringodium pellet , FF= fresh fish)

d Feed production was the budget required for producing the feed and composed based on the material price

3.3. Survival Rate (SR)

The overall survival rate (SR) varies between diet, and was 75 % for fish pellet and fresh fish, 58.33 and 16.67 for FSp and FUp respectively (Table 4) and water quality was showed at Table 5
Table 4. Survival Rate (SR) of juvenile L. olivacea

| No | Feed type                  | SR(%)    |
|----|----------------------------|----------|
| 1  | Fish Pellet (Fp)           | 75.00±10.57 |
| 2  | Fish – Ulva sp pellet (FUp)| 16.67±32.74 |
| 3  | Fish – Syringodium sp pellet (FSp) | 58.33±16.00 |
| 4  | Fresh Fish (FF)            | 75.00±10.83 |

Table 5. Water quality of experiment pond

| Parameter          | Measured | Reference                                  |
|--------------------|----------|--------------------------------------------|
| pH                 | 8.50     | 6.5 – 8 (Campbell and Busack, 1979)        |
| Water temperature  | 25-28    | 29 - 32°C (Campbell and Busack, 1979)      |
| Salinity (ppt)     | 12       | 29 - 34 ppt (Campbell and Busack, 1979)    |
| Nitrit (NO₂) (mg/L)| 0.00     | -                                          |
| Nitrat (NO₃) (mg/L)| 0.00     | -                                          |

In the nature, instinctively animal will forage and prefer feed material have been consumed previously. Thus fresh fish-habituated sea turtle will forage fish when it is released to the nature. It is unlikely that the animal would be successful because fish is fast swimmer. The animal, therefore, must switch to other feed, otherwise the animal will not survive because of starvation. The switching feeding behaviour to some extent is influenced by previous feeding experience i.e. initiation treatment to various feed type at cage nursery [3]. Therefore, the aim of the current study was to investigate alternative feed for post hatched sea turtle at cage nursery to introduce various feed type and to find optimum feed type. To achieve this objective, the inclusion of macroalgae (Ulva sp) and seagrass (Syringodium sp) which commonly found in marine ecosystem was carried out. The materials are nutritious feed material [6] and [7]. The Ulva sp. have widely been used as feed material for such as abalone [5], the later feed material was also well responded by green sea turtle [4]. This study seem to be pioneer applying dry feed (pellet) including non animal material for post hatched L olivacea compare to other studies used animal animal materials such as shrimp, Tuna sp [8], Decapetru spp and mollusca [9] and Ebi shrimp [10].

Daily growth rate of fish pellet (Fp) (i.e. 0.71 g/day) was higher than conventional feed i.e. fresh flesh of Sardinella sp (i.e. 0.54 g/day). The growth rate and the conversion ratio of individual fed fish pellet and fresh showed non significance even give higher lead to optimistic result of this study. This study confirm that fish pellet (Fp) is recommended for feed of Olive sea turtle at cage nursery. The fish pellet is not only simpler than fresh fish feed, the pellet result in more growth response and more efficient (lowest FCR). In accordance with the production budget indicating that fish pellet (Fp) is costly, the total budget is cumulative of component price and it is likely that that the binder (CMC) causes the raise of production budget. Therefore, it is crucial to observe other palatable and cost-effective binder.

The result of this study was compares favourable to study of [8] showed the highest response of juvenile to mixed fresh feed with growth rate ± 30.3 per 6 weeks equally ±0.72 per day. The finding of this study, to some extent, fish pellet is favourable because fish pellet feed can be stocked. This investigation result also showed higher growth response of post hatched Olive sea turtle fed fresh fish of Decaperus spp with daily growth rate ± 0.61 [9] or ebi shrimp with daily growth rate ± 0.42 [10].

In the nature, plenty animal such as molusca life as associated organism by attached to algae and seagrass [11]. Feeding stimulation by including macro-algae and seagrass material in pre released-Olive turtle feed is expected stimulate the response of the sea turtle to algae and seagrass where which various fauna live in. Thus juvenile Olive sea turtle released to the nature would not face starving period and more survive. Though Olive sea turtle is omnivorous animal, the result (Table 2) showed that the
inclusion of macroalga and seagrass in feed formula showed less growth response compared to fresh fish (FF) or fish pellet (Fp) (Table 2). Such lower growth rate response is likely related to differences in protein level. The inclusion of Ulva spp with 5.26% protein level [6] and Syringodium sp with 3.26% protein level [7] in the fish-base pellet formula of Sardinella spp, with 20.36 protein level [12] could reduce protein mixed feed up to 11.46% and 10.5 for Fish-Ulva spp pellet (FUp) and Fish-Syringodium sp pellet (FSp) consecutively. Protein is main nutrient in animal feed, it has significant effect onto animal growth [13]. The daily growth response to fish-Syringodium sp pellet (i.e. 0.35) to some extent is lower than those fed fresh feed Tuna sp [8] however the response was similar to those fed Shrimp ebi [8] [10]. This finding showed Syringodium sp as promising material for composing dry feed of just-hatched Olive sea turtle. The positive growth response to fresh Syringodium sp feed also showed by green adult sea turtle [4].

Consumption rate data could be used for analysing palatability of feed tested, the individual feed intake (FI) (Table 3) showed that fresh fish feed was more palatable compared to other feed type. This could be due to completeness of nutrients in fresh fish or attractant content, feed processing, such as heat drying, could diminish nutrients content of feed material. The overall FCR showed more than 6, the FCR of fish pellet feed (Fp), however, showed higher efficiency compared to other feed type i.e. 6.79 ± 0.26 (Table 3). This low FCR will increase the cost-effective feed.

The animal tested indicated normal response feeding and fed actively on all pellet given during study, nevertheless some dead animal indicated symptom of infection. It is assumed, therefore, that the overall mortality is unlikely affected by diet. Throughout the experiment, water quality parameters were mostly in the range of sea turtle tolerance, except water pH and salinity. Such low salinity because the water for nursery caged at “Kurma Asih” sea turtle conservation center was from soil water (infiltrated seawater) and not taken from sea water thus the salinity is low. This choice seems as cost efficiency consideration of management. In accordance to the discussion above, it is likely that fish pellet feed is promising for pre-released juvenile sea turtle at cage nursery and further research is crucial to develop cheaper binder and to get optimum protein level and energy requirement.

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