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Effect of Various Doses of Fermented Herbal Extracts as a Feed Additive on Growth Performance and Feed Efficiency of Mud Crabs (*Scylla olivacea*) Cultivated in Ponds

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Key words: Crab, growth, feed efficiency, herbal extracts, molting

Abstract

This study aimed to analyze the effect of various dosages of herbal extracts on growth, molting, and the feed efficiency of orange mud crabs (*Scylla olivacea*) cultivated in ponds. The herbal extract used was a combination of *Morus alba*, *Curcuma xanthorrhiza*, and *Boesenbergia rotunda*. 240 crabs were used in this study for four dosages of herbal extract, namely 0, 200, 400, 600 mg kg⁻¹ of feed. Furthermore, each dosage treatment consisted of 60 crabs kept individually in a crab box which was placed on a floating bamboo raft. This was to enable floating on the surface of the pond water which had a depth of ±80 cm. The feed given during this study was formulated feed at a dose of 5% body weight daily. Based on the one-way anova, the results showed that the dose of herbal extract influences the growth and feed efficiency of the mud crabs. A higher dose of herbal extract in the feed (600 mg kg⁻¹ of feed) provided higher growth and feed efficiency (LSD Test: P<0,1). Significant growth occurred after molting, and crabs of the control (without herbal extract) treatment received an average weight gain of ±43,73%, while the treatment of 600 mg kg⁻¹ of herbal extract got an average weight gain of ±60,73 from the initial weight. However, in non-molting crab, ±5,44 and ±6,42 in weight gain was observed

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respectively. Significant differences also occurred between treatments for the specific growth rate, feed consumption, feed efficiency, and condition factors. These results provided information that it is very important to manage molting in crab cultivation. Furthermore, herbal extracts is potentially used to stimulate molting, improve growth, and increase feed efficiency in crab cultivation.

Introduction

Mud crab is one of the crab species with high economic value worldwide which are generally marketed alive, while some are sold as frozen or cooked products. There are four species of mud crab that are the focus of commercial fisheries and aquaculture production, namely *Scylla serrata*, *S. tranquebarica*, *S. paramamosain* and *S. olivacea*. These are in great demand in both export and domestic markets with prices ranging from 8 to 25 USD depending on the size and season (Lalramchhani et al., 2019). Prices are also influenced by the shape of the product such as soft-shell crab (Fujaya et al., 2020). Waiho et al. (2021) stated that soft-shell crab products are one of the crab culture products with world market demand that continues to increase at high prices. Currently, there are three different culture systems including crab fattening, grow-out, and soft-shell crab production in practice that target different tiers of the global crab market (Rahman et al. 2020). In Indonesia, grow out and fattening are the most widely practiced methods of crab cultivation, and both are generally carried out in earthen ponds. Often, there are no special innovations in the cultivation other than feeding crabs with trash fish, chicken intestines, and others. In recent years, crab farmers have also started producing soft-shell crabs, either using horizontal farming methods with bamboo and HDPE boxes, or vertical farming.

When it comes to rearing, fattening, and producing soft-shell crabs, growth is the main focus during the rearing period. If growth does not occur optimally, aquaculture as part of economic activity will suffer losses, as crab growth is affected by molting. Molting and growth in decapoda crustaceans such as crabs, lobsters, shrimps cannot be separated, and they influence each other. Molting is a precursor to growth and vice-versa. Therefore, various efforts have been made to stimulate growth and molting in crab culture, such as limb autotomy, eyestalk ablation, ecdysteroid and phytoecdysteroid application, control of physical parameters such as temperature and salinity, biogenic amines application, methyl farnesoate application, and molt-inhibiting hormone (MIH) inhibition (Waiho et al., 2021).

Herbal extracts are one of the promising natural medicines that are used as functional feed additives to stimulate growth and molting in crabs. Herbal extracts generally contain terpenoids, alkaloids, saponins, flavonoids which act as anti-oxidants, anti-bacterial, immune-nutrition (Singh et al., 2013). Klein (2004) stated that phytoecdysteroids are present in several plants, and one of their roles is to stimulate protein synthesis. Fujaya et al. (2018) also administered spinach and mulberry leaf extracts to stimulate molting in soft-shell crab production. The use of herbs in the aquaculture industry is expected to be friendlier to the environment and cultivated animals. According to Bharati et al. (2019), increasing aquaculture growth cannot be separated from the development of quality feed, which requires appropriate and essential feed additives. Furthermore, the development of these additives shows promising results for aquaculture. These do not only improve fish growth performance but also their health performance. These functional feed additives originate from a variety of organic sources and are environmentally friendly for fish and the environment. Examples of these additives that have been developed include prebiotics, probiotics, seaweed, fungi, microalgae, enzymes, organic acids, mycotoxin binders, photogenic or Phyto biotic compounds and yeast.

This study examined the response of mud crabs to the various doses of mixed herbal extracts (combination of *Morus alba*, *Curcuma xanthorrhiza*, and *Boesenbergia rotunda*) as a functional feed additive on growth, molting, feed consumption, feed efficiency, condition factor and survival of mud crabs. The three medicinal plants are expected to synergize to improve the health and growth of crabs through the active compounds they contain. Furthermore, this combination of herbal extracts is expected to be one of the natural medicines that can be applied to juvenile crabs for growing out, fattening, and production of soft-shell crabs.

Materials and Methods

Experimental crab

The study was carried out at the Educational Farm of Hasanuddin University, Bojo Village, Mallusetasi District, Barru Regency. There were 240 mud crabs (*Scylla olivaceae*) with a bodyweight of 107.08 ± 11.93 g and carapace width of 80.91 ± 4.22 mm and were obtained from local fishermen's captures. Furthermore, weighing was carried out using an electric scale with an accuracy of 0.1 g and measuring the width of the carapace using a caliper with an accuracy of 0.1 mm as initial data. All crabs were then divided into 4 treatments and 6 replicates. There were four dosages of herbal extract, namely 0 mg kg⁻¹ of feed (A), 200 mg/kg of feed (B), 400 mg/kg of feed (C), and 600 mg/kg of feed (D). Each dosage treatment consisted of 60 crabs, while each replicate consisted of 10 crabs.

The container used in this study was a crab box with a length, width, and height of 30 × 20 × 15 cm. The test animals were kept for 31 days individually in the crab box and given a label (tag) on each box as a marker of the treatment of the tested crab. Afterwards, each was placed on a bamboo raft with a float, to enhance floating on the surface of the pond water which had a depth of ± 80 cm.

Experimental Feed

The container used in this study was a crab box with a length, width, and height of 30 × 20 × 15 cm. The test animals were kept for 31 days individually in the crab box and given a label (tag) on each box as a marker of the treatment of the tested crab. Afterwards, each was placed on a bamboo raft with a float, to enhance floating on the surface of the pond water which had a depth of ± 80 cm.

The feed used in this study was fermented artificial feed in form of pellets, as recommended by Aslamyah et al. (2021). The feedstuff and results of feed proximate analysis are shown in **Table 1**.

Table 1 The composition (% dry matter) of feedstuff and results of feed proximate analysis

Composition	Percentage (%)
Fish flour	25
Fresh fish	20
Fish silage	10
Crab shell flour	5
Soy flour	8
Corn starch	10
Coconut meal flour	8
Pollard flour	10
Vitamin & mineral mix ¹	4
Amount	100
Water (%)	8.9
Ash (% dry weight)	13.49
Protein (% dry weight)	41.93
Fat (% dry weight)	7.43
Crude fiber (% dry weight)	7.82
NFE (% dry weight)	29.33
DE (kcal/kg) ²	2767.63
C/P (DE/g Protein)	6.76

¹ The composition of vitamins & mineral mix.

Every 10 kg contains 12,000,000 IU of Vitamin A; Vitamin D 2,000,000 IU; Vitamin E 8,000 IU; Vitamin K 2,000 mg; Vitamin B1 2,000 mg; Vitamin B2 5,000; Vitamin B6 500 mg; Vitamin B12 12,000 g; Ascorbic acid 25,000 mg; Calcium-D-Phantothenate 6000 mg; Niacin 40,000 mg; Choline Chloride 10,000 mg; Methionine 30,000 mg; Lysine 30,000 mg; Manganese 120,000 mg; Iron 20,000 mg; Iodine 200 mg; Zinc 100,000 mg; Cobalt 200,000 mg; Copper 4,000 mg; Santoquin (antioxidant) 10,000 mg; Zinc bacitracin 21,000 m g.

² Calculation results based on the energy equation (NRC, 1988): 1 g carbohydrate = 2.5 kca 1DE; 1 g protein = 3.5 kcal DE; 1 g fat = 8.1 kcal DE

The stages of making feed begin with preparing feedstuff, including dry ingredients, fish silage, and fresh fish. Dry feed ingredients were ground into flour, except for the vitamin and mineral mix. The ingredients were weighed and mixed starting from the smallest to the largest amount, and stirred until homogeneous. Afterwards, the ingredients were inoculated with 10 mL 100/g of feedstuff microorganism mix, which had previously

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been diluted with water in a ratio of 1: 3. The mixture was stirred evenly, stored in airtight plastic, and after 72 hours of incubation, opened and dried. Fish silage was prepared by removing fish scales, before washing and draining. The fish was grounded with a meat milling machine until smooth and placed in a closed bucket that was previously lined with a plastic bag. 1.5 L of formic acid was then added to 50 kg of fish grinder and stirred until evenly mixed. Stirring was performed 3 to 4 times daily until the 4th day, and on the 5th day, the water at the top was removed. Furthermore, the mixed microorganisms were inoculated with a dose of 10 mL 100/g of feedstuff and incubated for 7 days in a tightly closed and vacuum. Fresh fish was prepared by removing scales and washing thoroughly. The fish was then drained dried, and grounded until smooth.

Making feed was carried out by weighing all the ingredients used and mixing until homogeneous. The feed dough was printed using a pellet printer for crabs with a diameter of 1 cm and steamed for \pm 40 minutes. Pellets were cut 2 cm long and dried in the sun. Furthermore, the dried feed was then cooled at room temperature or aerated, placed in a plastic bag and stored in a dry place, until use for the next test.

The artificial feed was coated with fermented herbal extracts that have been prepared previously. These fermented extracts were made up of three herbal extracts, including mulberry leaf extract (*Morus alba*), temulawak (*Curcuma xanthorrhiza*), and temu kunci (*Boesenbergia rotunda*), at equal concentrations, and fermented with *Lactobacillus casei* and *Saccharomyces cereviceae*. Approximately 1 ml of probiotic and 1 ml of molasses were added into distilled water containing 500 mg of herbal extracts of Stock FHE fermentation time: 1 month, and fermentation condition under room temperature. Furthermore, the dilution of stock FHE for each treatment was as follows: treatment A (100 ml of distilled water); treatment B (20 ml FHE stock + 80 ml distilled water); treatment C (60 ml FHE Stock + 40 ml distilled water); and treatment D (100 ml FHE stock; without distilled water). Feed was administered in the afternoon at a dose of 5% of body weight with a frequency of once every 2 days. Feed containing herbs were given at 1-day intervals compared to feed without herbs (control). The rest of the feed was then measured before the next feeding. The remaining feed was collected during the rearing period, dried, and weighed at the end of the study.

Visual observations were made daily to control the development of the test crabs. If a dead crab was found, it was recorded and weighed. If molting crabs were found, they were recorded and weighed, and placed back in the crab box for rearing until they reach the end of cultivation. Furthermore, observations of water quality including temperature, salinity, dissolved oxygen, and pH were carried out every day during cultivation, while ammonia was measured at the beginning and end of cultivation. The temperature was determined using a thermometer, salinity with a hand refractometer, dissolved oxygen with a DO meter, pH with a pH meter, and ammonia in the laboratory.

Growth performance

The growth involving absolute weight gain and specific growth rate. Absolute weight gain was measured using the formula:

$$WM = W_t - W_0$$

Where:

WM: absolute weight growth (g),

W_t: body weight (g),

W₀: initial body weight (g).

Meanwhile, the specific growth rate was calculated using the formula:

$$SGR = \frac{\ln W_t - \ln W_0}{T} \times 100$$

Where:

SGR: Specific Growth Rate (%)

Wt: average weight of crabs at the end of the study (g)

Wo: average weight of crabs at the beginning of the study (g)

T: maintenance time in days.

Percentage of molting

The percentage of molting (%) was measured by comparing the number of molting test crabs with live test crabs multiplied by 100.

Survival rate

The survival rate (%) of mud crabs was calculated using the formula:

$$S = \frac{N_t}{N_o} \times 100$$

Where:

S: the survival rate (%)

Nt: the number of live crabs at the end of rearing (tails)

No: the number of crabs at the beginning of rearing (tails).

Feed consumption

Feed consumption (KP) was calculated by determining the difference in the amount of feed given to the amount of feed remaining. This calculation was based on the dry weight of the feed.

Feed efficiency

Feed efficiency (EP) was calculated using the formula as proposed by Watanabe (1988):

$$EP = [(W_t + D) - W_0] / F \times 100$$

where:

EP: feed efficiency

Wt: the average weight of crabs at the end of the study (g)

Wo: the average weight of crabs at the beginning (g)

D: the weight of crabs that died during the study (g).

Condition Factor

The F or Condition factor (K) was calculated using Fulton's condition factor formula, namely

$$FC = 100 \times W / L^3$$

where:

FC: the condition factor

W: the weight of the test crab,

L: the width of the carapace of the test crab.

To determine the level of change in the condition factor of the test crab at the beginning of the study compared to the end, it was calculated using the formula:

$$TPFK = (FK_b - FK_a) / FK_a \times 100$$

where:

TPFK: the rate of change in condition factor (%)

FKa: the initial condition factor

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FKb: the final condition factor.

Statistical analysis

Data were presented using tables and images. The effect of treatment on growth, feed consumption, feed efficiency and condition factors were analyzed using Analysis of Variance (ANOVA). Further tests were carried out using the LSD test at a 90% confidence level. A difference of $P < 0.1$ is stated to be statistically significant. Furthermore, to analyze the molting and non-molting crab data, an independent sample t-test was used. The analysis was performed using Excel software and SPSS version 16.0.

Results

Feed consumption, feed efficiency, growth, and changes in condition factors

The results showed that the tested crabs consumed feed well, which contained herbal extracts. Although the molting percentage was without significant difference between treatments after 31 days of rearing, there were differences in crab responses in terms of feed consumption, feed efficiency, growth, and changes in condition factors. Overall, crabs have a high survival rate above 90% in **Table 2** (below).

Table 2 Description of crabs used at the beginning of the study, molting percentage, and survival rate.

TC	N	W0 (g)	CL0 (mm)	CW0 (mm)	CF0	MP (%)	SR (%)
A	60	106,88±11,46	58±4,3	81,28±4,5	19,88±2,13	16,67	93,33
B	60	106,7±12,11	58,72±3,3	81,05±3,9	20,27±2,62	21,67	91,67
C	60	107,13±12,40	57,73±3,0	80,62±3,0	20,42±2,12	10,00	95,00
D	60	107,05±12,11	58,47±3,7	80,48±5,1	21,06±6,15	15,00	96,67

Treatment Code (TC); 0 mg kg⁻¹ of feed (A); 200 mg kg⁻¹ of feed (B); 400 mg kg⁻¹ of feed (C); and 600 mg kg⁻¹ of feed (D); Number of juvenile (N); Initial Weight (W0); Initial Carapace Length (CL0); Initial Carapace Width (CW0); Initial Condition Factor (CF0); Molting percentage (MP) and Survival Rate (SR).

Based on the independent sample t-test analysis, it was observed that there was a very significant difference between molting and non-molting crabs, both in terms of growth, condition factors, feed consumption and feed efficiency (**Figure 1**). Therefore, molting and non-molting crab data were separated to reduce the bias in data analysis. Changes in unique condition factors due to molting caused crabs to enlarge, however, conditional factors were decreased (**Tables 3 and 4**).

Table 3 Growth, Feed consumption and Feed Efficiency of molting crab

TC	WG (g)	CLG (mm)	CWG (mm)	SGR	CoCF (%)	FC (g)	FE (%)
A	43,73±13,30 ^a	8,7±3,6 ^a	12±5,4 ^a	12,9±1,25 ^a	-4,13±6,89 ^a	88,79±5,58 ^a	53,29±17,22 ^a
B	40,03±10,77 ^a	4,9±2,2 ^{ab}	8,6±2,2 ^{ab}	12,63±0,75 ^a	4,32±11,13 ^a	88,22±4,30 ^a	50,24±12,60 ^a
C	53,9±14,58 ^{ab}	9,4±2,2 ^{ac}	14,4±4,2 ^{ac}	13,51±0,73 ^a	-5,10±16,01 ^a	90,52±0,83 ^a	63,72±15,25 ^{ab}
D	60,73±11,41 ^{bc}	7,6±3,5 ^a	11,1±3,7 ^a	13,96±0,80 ^b	9,38±7,54 ^b	79,6±9,16 ^b	81,97±15,53 ^b

Different letters in the column are statistically different according to the LSD test ($p < 0.1$). Treatment Code (TC); 0 mg kg⁻¹ of feed (A); 200 mg kg⁻¹ of feed (B); 400 mg kg⁻¹ of feed (C); and 600 mg kg⁻¹ of feed (D); Weight gain (WG); Specific Growth Rate (SGR), Change of Condition Factor (CoCF); Feed Consumption (FC); Feed Efficiency (FE)

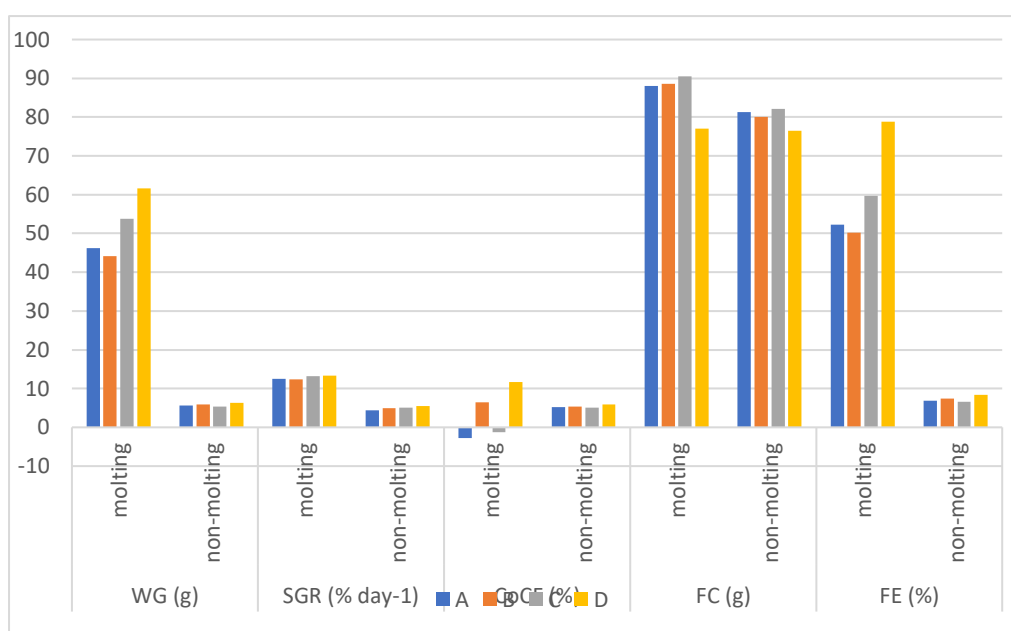


Figure 1 Comparison of growth, feed consumption, and feed efficiency of molting and non-molting crabs. $P < 0.05$ based on independent sample t-test; Treatment: 0 mg/kg of feed (A), 200 mg/kg of feed (B), 400 mg/kg of feed (C), and 600 mg/kg of feed (D); Weight gain (WG); Specific Growth Rate (SGR), Change of Condition Factor (CoCF); Feed Consumption (FC); Feed Efficiency (FE).

Table 4 Growth, Feed consumption and Feed Efficiency of non-molting crab

TC	WG (g)	SGR	CoF (%)	FC (g)	FE (%)
A	5,44±2,11 ^a	4,3±1,32 ^a	5,03±2,09 ^a	81,57±2,80 ^a	6,61±2,74 ^a
B	5,93±1,28 ^a	4,95±0,56 ^a	5,22±1,76 ^a	88,87±20,95 ^{ab}	7,67±2,22 ^a
C	5,11±0,62 ^{ab}	4,8±0,51 ^a	4,81±0,69 ^a	81,95±2,04 ^a	6,26±0,94 ^{ab}
D	6,42±1,52 ^{ac}	5,52±0,65 ^b	5,96±1,37 ^a	76,71±7,12 ^{ac}	8,41±1,95 ^{ac}

Different letters in the column are statistically different according to the LSD test ($p < 0.1$). Treatment Code (TC); 0 mg kg⁻¹ of feed (A); 200 mg kg⁻¹ of feed (B); 400 mg kg⁻¹ of feed (C); and 600 mg kg⁻¹ of feed (D); Weight gain (WG); Specific Growth Rate (SGR), Change of Condition Factor (CoCF); Feed Consumption (FC); Feed Efficiency (FE).

Water quality

During the study, water quality was in fairly good condition, except for low dissolved oxygen in the morning. Temperatures ranged between 27 – 32 °C, salinity 28-35 ppt, pH 6.9 – 8.9, dissolved oxygen 2.1 – 5.6 ppm, and ammonia 0.002 ppm.

Discussion

In aquaculture, especially in crab farming, growth is very important, as it is related to time and maintenance. If growth is stimulated in a shorter period, farmers can harvest faster with lower maintenance costs, which enhances profitability. Functional feed additive treatments were derived from three herbal extracts, namely mulberry (*M. alba*) leaf extract, temulawak (*C. xanthorrhiza*) and temu kunci (*B. rotunda*) rhizome. These treatments obtained a positive effect in promoting growth and increasing feed efficiency. The results showed that the higher the dose of herbal extract was used, the higher the growth and feed efficiency. Furthermore, a dose of 600 mg of ethanol extract from a combination of three types of herbs per kg of feed obtained the highest growth and feed efficiency.

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The results of this study also showed that the active compounds contained in the combination of extracts synergize to stimulate growth. Fujaya et al., (2018) stated that mulberry (*M. alba*) extract contains phytoecdysteroids, which are ecdysteroid mimic hormones naturally found in some plants (Klein, 2004; Dinan et al., 2009). Ecdysteroids in crustaceans and crabs are important hormones that control the molting process. This compound plays a role in activating protein synthesis by increasing mRNA synthesis (Preston-mafham & Dinan, 2002). This activity is also strengthened by the role of extracts of temulawak (*Curcuma xanthorrhiza*) and temu kunci (*Boesenbergia rotunda*) which are known to contain curcumin and essential oils. Both compounds act as antioxidants and increase appetite. This enables the supply of raw materials for crab nutrition to be fulfilled.

The bioactive phytochemicals and secondary metabolites of the mulberry extract include steroids, saponins, alkaloids, glycosides, and phenolic compounds including terpenoids, flavonoids, anthocyanins, and tannins. These bioactive compounds of mulberry extract exhibit outstanding anti-oxidative, anti-diabetic, anti-stress, nephroprotective, antimicrobial, anti-mutagenic, anticancer, anxiolytic, hepatoprotective, anthelmintic, antimicrobial, immunomodulatory effects and has cholesterol-lowering effects (Hussain et al., 2017). The anti-stress effect was significantly demonstrated in experiments on rats subjected to restraint. Chronic stress restraint will result in cognitive dysfunction, altered behavioral parameters, increased leukocyte count, levels of superoxide dismutase (SOD), lipid peroxidation (LPO), glucose and corticosterone, with concomitant decreases in catalase (CAT) activity, and glutathione reductase (GSH). Furthermore, gastric ulcers, adrenal glands and spleen were also used as stress indexes. All these restraint-induced disturbances were attenuated by ethyl acetate soluble (EASF) from *Morus alba* (Nade & Yadav, 2010). This effect was certainly very necessary for crabs in the cage or crab box.

The response of crabs to temulawak extract appears to be similar to that of other animals. According to Hosseini et al., (2016), *C. xanthorrhiza* (CX), known as temulawak, contains essential oils rich in phenols. The antioxidant activity of CXEO mainly refers to xanthorrhizol, arcurcumene, and curcumene. It has been widely documented that these phenolic compounds can enhance gut health resulting in better food absorption activities.

Similarly, temu kunci (*B. rotunda*) which is one of the main components of the herbal extract used in this study also contains essential oils. Chahyadi et al., (2014) also emphasized that the rhizome of *B. pandurata* contains essential oils and many flavonoid compounds which showed many interesting pharmacological characteristics, such as antifungal, antibacterial, and antioxidant. Eng-Chong et al., (2012) stated that *B. rotunda* has great medicinal potential, as nearly a hundred compounds were isolated from this plant which is also known as "Thai Ginseng". These compounds have many roles, including anti-microbial, anti-parasitic, antioxidant, and also inhibit the formation of biofilms by intestinal pathogens.

In this study, the condition factor was used to describe the level of obesity. There was a difference in the value of the condition factor in the test crabs. The initial condition factor of the crabs in this study varied between 14 - 54 based on the ratio of weight and width of the carapace. Furthermore, at the end of the study, the condition factor of crabs varied between 15 - 60, there were crabs whose condition factors increased while in some, this factor decreased. The decreasing condition factor occurred in crabs that have just molted. The observations by Moslen & Miebaka, (2018) showed that the variation in condition factors was caused by eating behavior, biological factors, and responses to the environment. A low condition factor value means the crabs are light for their length, an indication of low feeding intensity and the presence of spawning activity. A high K value is the assumption of high feeding intensity and a gradual increase in fat accumulation, which also indicates preparation for a new reproductive period (Suryandari et al., 2018).

In this study, the dynamics of conditional factors were influenced by molting activity. Crabs that had just molted had a lower condition factor than other crabs before molting. Therefore, crabs that had just molted were classified as thin, while crabs before molting were classified as fat. As the growth of crab weight was not always followed by the growth of the width or length of the carapace, the growth indicator using the condition factor value

was significantly helpful. In portunid crabs, the molting process is divided into four main stages, namely intermoult, premoult, ecdysis and postmoult (Waiho et al., 2021). The intermoult is the stage after cuticle formation, and the exoskeleton has hardened after mineralization has fully occurred. Meanwhile, the intermolt stage is the crab muscle growth stage or the fattening stage. The premoult is the preparation stage for molting, consisting of the early, mid, and late premoult stages. In the premoult stage, a new exoskeleton begins to be formed under the old hard exoskeleton, while the new exoskeleton is retracted. This enables the old and new exoskeletons to separate with the help of enzymes. The process of releasing the crab from the old exoskeleton is called ecdysis, where the crab slowly pulls itself out of the old exoskeleton through a crack at the back of the carapace that borders the abdomen. The crabs that have just been released from the old exoskeleton have a soft shell which expands as more water is absorbed to stretch the new cuticle. At this time, the size of the crabs was enlarged but had a low condition factor resulting in the light weight and were classified thin.

There was a significantly different level of change in conditional factors between molting and non-molting crabs (Table 3). The existence of a minus value illustrates that the crab decreased in the condition factor after molting. This also showed that the crabs have molted at the end of the rearing period, therefore, they have not had time to grow to fill their enlarged carapace. According to Fujaya, (2011), molting will naturally occur when body size increases due to growth. This phenomenon always occurs because the crab's body is wrapped by a rigid carapace and cannot develop following the development of body weight. Molting is also influenced by external and internal factors. External factors include light, temperature, food availability, tides, while internal factors include body size and hormones. Both of these factors influence the brain and stimulate the Y-organ to produce molting hormones.

The results of this study showed the importance of molting management in crab culture. Molting is not only important for the production of soft-shell crabs, but also for the rearing and cultivation of fattening crabs. Herbal extracts can be used to help stimulate molting, increase growth, and increase feed efficiency, thereby enables farmers to maximize profits in crab culture.

In conclusion, the best dose to stimulate growth, feed efficiency, and the level of fatness of mud crabs was 600 mg kg⁻¹ of feed, which was the highest dose applied. Therefore, further study is required to obtain optimal doses of herbal extracts (*M. alba*, *C. xanthorrhiza*, and *B. rotunda*) in stimulating the growth and molting of cultured mud crabs.

Acknowledgements

The authors are grateful to the Hasanuddin University Research and Community Service Institute for funding this study through the Unhas Applied Research Grant for the 2020 fiscal year with the contract number 1585/UN4.22/PT.OI.03/2020.

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Is there any previous research on the use of *Morus alba*, *Curcuma xanthorrhiza*, and *Boesenbergia rotunda* as a functional feed additive of mud crabs?

Why choose these herbs?

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Siti Aslamyah Feb 6, 2022 - 7:46 pm SST


Dear Dr. S. Hofstetter

Sorry for a bit late response due to too many activities in our faculty. Herewith, I sent you the revision of our manuscript (with red sentences). The team has decided to revision the title for this publication in order to adjust with the parameters that had been tested. Do hope by this time our manuscript is fulfilling the journal necessary requirements. Thank you so much for your positive comments and cooperation.

Regards with the best hope,

Dr. Siti Aslamyah and Team
Fisheries Department
Faculty of Marine and Fisheries
Hasanuddin University, Makassar City
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Attachments

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Utilization of Feed and Growth Performance of Mud Crabs: The Effect of Herbal Extracts as Functional Feed Additives

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Key words: Crab, growth, herbal extracts, molting, utilization of feed

Abstract

This study aimed to analyze the effect of herbal extracts as functional feed additives on utilization of feed and growth performance of orange mud crabs (*Scylla olivacea*). The test animals used were 60 crabs for each treatment dose. The crabs are kept individually in crab box which was placed on a floating bamboo raft. This was to enable floating on the surface of the pond water which had a depth of ± 80 cm. The treatments tested were four doses of a combination herbal extract from *Morus alba*, *Curcuma xanthorrhiza*, and *Boesenbergia rotunda*, namely 0, 200, 400, 600 mg kg⁻¹ of feed. Formulated feed with protein content of 41.93%, fat 7.43%, Nitrogen Free Extract (NFE) 29.33%, and crude fiber 7.82% was given at a dose of 5% body weight everyday. Based on one-way ANOVA, the results showed that the dose of herbal extracts influences the feed utilization and growth performance of mud crabs. The dose of herbal extract 600 mg kg⁻¹ of feed provided higher feed utilization and growth performance (LSD Test: $P < 0.1$). Significant differences were found between feed utilization and growth performance of mud crabs after molting and non-molting. In molting mud crabs, treatment with herbal extract 600 mg kg⁻¹ obtained an average weight gain of ± 60.73 from the initial weight, compared to control crabs (without herbal extracts) gaining an average weight gain of $\pm 43.73\%$. However, in non-molting crabs, ± 5.44 and ± 6.42 weight gain was observed, respectively. Significant differences also occurred between treatments for specific growth rates, feed consumption, feed efficiency, and condition factors. These results provided information that herbal extracts are potentially as feed additives to increase feed utilization and growth performance, as well as stimulate molting in crab cultivation.

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Introduction

Mud crab is one of the crab species with high economic value worldwide which are generally marketed alive, while some are sold as frozen or cooked products. There are four species of mud crab that are the focus of commercial fisheries and aquaculture production, namely *Scylla serrata*, *S. tranquebarica*, *S. paramamosain* and *S. olivacea*. These are in great demand in both export and domestic markets with prices ranging from 8 to 25 USD depending on the size and season (Lalramchhani et al., 2019). Prices are also influenced by the shape of the product such as soft-shell crab (Fujaya et al., 2020). Waiho et al. (2021) stated that soft-shell crab products are one of the crab culture products with world market demand that continues to increase at high prices. Currently, there are three different culture systems including crab fattening, grow-out, and soft-shell crab production in practice that target different tiers of the global crab market (Rahman et al. 2020). In Indonesia, grow out and fattening are the most widely practiced methods of crab cultivation, and both are generally carried out in earthen ponds. Often, there are no special innovations in the cultivation other than feeding crabs with trash fish, chicken intestines, and others. In recent years, crab farmers have also started producing soft-shell crabs, either using horizontal farming methods with bamboo and HDPE boxes, or vertical farming.

When it comes to rearing, fattening, and producing soft-shell crabs, growth is the main focus during the rearing period. If growth does not occur optimally, aquaculture as part of economic activity will suffer losses, as crab growth is affected by molting. Molting and growth in decapoda crustaceans such as crabs, lobsters, shrimps cannot be separated, and they influence each other. Molting is a precursor to growth and vice-versa. Therefore, various efforts have been made to stimulate growth and molting in crab culture, such as limb autotomy, eyestalk ablation, ecdysteroid and phytoecdysteroid application, control of physical parameters such as temperature and salinity, biogenic amines application, methyl farnesoate application, and molt-inhibiting hormone (MIH) inhibition (Waiho et al., 2021).

The use of herbs in the aquaculture industry is expected to be friendlier to the environment and cultivated animals. According to Bharati et al. (2019), increasing aquaculture growth cannot be separated from the development of quality feed, which requires appropriate and essential feed additives. Furthermore, the development of these additives shows promising results for aquaculture. These do not only improve fish growth performance but also their health performance. These functional feed additives originate from a variety of organic sources and are environmentally friendly for fish and the environment. Examples of these additives that have been developed include prebiotics, probiotics, seaweed, fungi, microalgae, enzymes, organic acids, mycotoxin binders, photogenic or Phyto biotic compounds and yeast.

Herbal extracts are one of the promising natural medicines that are used as functional feed additives to stimulate growth and molting in crabs. Herbal extracts generally contain terpenoids, alkaloids, saponins, flavonoids which act as anti-oxidants, anti-bacterial, immune-nutrition (Singh et al., 2013). The phytoecdysteroids are present in several plants, and one of their roles is to stimulate protein synthesis (Klein, 2004). One of them is the mulberry plant (*Morus alba*). The ecdysteroids contained in *Morus alba* are of the 20-hydroxyecdysone group. At present, mulberry leaf extract has been successfully trialed as a growth and moulting stimulant for mud crabs with an optimal dose of 2.4 mg g⁻¹ feed or 240 mg kg⁻¹ feed (Fujaya et al., 2018). In *Morus* spp. a wide range of the phytochemicals are present in leaves, fruit, roots and wood. Therefore, *Morus* spp. has various biological functions (antidiabetic, antiobesity, anti-inflammatory, anticancer, antibacterial, antioxidant, antiviral, cytoprotective and neuroprotective etc.). This plant is used traditionally for the development of different food products as well as the treatment of various diseases (Dhiman et al., 2020). The content of phenolic compounds, especially flavonoids and anthocyanins, causes *Morus alba* to have strong antioxidant activity, thereby potentially eliminating free radical production and cellular damage caused by free radicals. (Hussain et al., 2017).

To complement the function of mulberry leaf extract (*Morus alba*), as a growth and molting stimulant, as well as an herb to maintain crab health. Added extracts of temulawak (*Curcuma xanthorrhiza*) and temu kunci (*Boesenbergia rotunda*), which are known to have

various physiological functions, including appetite stimulant, hepatoprotector, antimicrobial, anti-inflammatory, analgesic, antipyretic, chloretic, and others. the latter is used as a "herbal" to improve intestinal health (Eng-Chong et al., 2012), and medicinal plants containing curcumin, xanthorrhizol, dan pinostrobin (Qomaladewi & Cho, 2021)

This study examined the response of mud crabs to the various doses of mixed herbal extracts (combination of *Morus alba*, *Curcuma xanthorrhiza*, and *Boesenbergia rotunda*) as a functional feed additive on growth, molting, feed consumption, feed efficiency, condition factor and survival of mud crabs. The three medicinal plants are expected to synergize to improve the health and growth of crabs through the active compounds they contain. Furthermore, this combination of herbal extracts is expected to be one of the natural medicines that can be applied to juvenile crabs for growing out, fattening, and production of soft-shell crabs.

Materials and Methods

Experimental crab

The study was carried out at the Educational Farm of Hasanuddin University, Bojo Village, Mallusetasi District, Barru Regency. There were 240 mud crabs (*Scylla olivaceae*) with a bodyweight of 107.08 ± 11.93 g and carapace width of 80.91 ± 4.22 mm and were obtained from local fishermen's captures. Furthermore, weighing was carried out using an electric scale with an accuracy of 0.1 g and measuring the width of the carapace using a caliper with an accuracy of 0.1 mm as initial data. All crabs were then divided into 4 treatments and 6 replicates. There were four dosages of herbal extract, namely 0 mg kg⁻¹ of feed (A), 200 mg/kg of feed (B), 400 mg/kg of feed (C), and 600 mg/kg of feed (D). Each dosage treatment consisted of 60 crabs, while each replicate consisted of 10 crabs.

The container used in this study was a crab box with a length, width, and height of 30 × 20 × 15 cm. The test animals were kept for 31 days individually in the crab box and given a label (tag) on each box as a marker of the treatment of the tested crab. Afterwards, each was placed on a bamboo raft with a float, to enhance floating on the surface of the pond water which had a depth of ± 80 cm.

Experimental Feed

The container used in this study was a crab box with a length, width, and height of 30 × 20 × 15 cm. The test animals were kept for 31 days individually in the crab box and given a label (tag) on each box as a marker of the treatment of the tested crab. Afterwards, each was placed on a bamboo raft with a float, to enhance floating on the surface of the pond water which had a depth of ± 80 cm.

The feed used in this study was fermented artificial feed in form of pellets, as recommended by Aslamyah et al. (2021). The feedstuff and results of feed proximate analysis are shown in **Table 1**.

Table 1 The composition (% dry matter) of feedstuff and results of feed proximate analysis

Composition	Percentage (%)
Fish flour	25
Fresh fish	20
Fish silage	10
Crab shell flour	5
Soy flour	8
Corn starch	10
Coconut meal flour	8
Pollard flour	10
Vitamin & mineral mix ¹	4
Amount	100
Water (%)	8.9
Ash (% dry weight)	13.49
Protein (% dry weight)	41.93
Fat (% dry weight)	7.43

Crude fiber (% dry weight)	7.82
NFE (% dry weight)	29.33
DE (kcal/kg) ²	2767.63
C/P (DE/g Protein)	6.76

¹ The composition of vitamins & mineral mix.

Every 10 kg contains 12,000,000 IU of Vitamin A; Vitamin D 2,000,000 IU; Vitamin E 8,000 IU; Vitamin K 2,000 mg; Vitamin B1 2,000 mg; Vitamin B 2 5,000; Vitamin B6 500 mg; Vitamin B12 12,000 g; Ascorbic acid 25,000 mg; Calcium-D-Phanthothenate 6000 mg; Niacin 40,000 mg; Choline Chloride 10,000 mg; Methionine 30,000 mg; Lysine 30,000 mg; Manganese 120,000 mg; Iron 20,000 mg; Iodine 200 mg; Zinc 100,000 mg; Cobalt 200,000 mg; Copper 4,000 mg; Santoquin (antioxidant) 10,000 mg; Zinc bacitracin 21,000 m g.

² Calculation results based on the energy equation (NRC, 1988): 1 g carbohydrate = 2.5 kca 1DE; 1 g protein = 3.5 kcal DE; 1 g fat = 8.1 kcal DE

The stages of making feed begin with preparing feedstuff, including dry ingredients, fish silage, and fresh fish. Dry feed ingredients were ground into flour, except for the vitamin and mineral mix. The ingredients were weighed and mixed starting from the smallest to the largest amount, and stirred until homogeneous. Afterwards, the ingredients were inoculated with 10 mL 100/g of feedstuff microorganism mix, which had previously been diluted with water in a ratio of 1: 3. The mixture was stirred evenly, stored in airtight plastic, and after 72 hours of incubation, opened and dried. Fish silage was prepared by removing fish scales, before washing and draining. The fish was grounded with a meat milling machine until smooth and placed in a closed bucket that was previously lined with a plastic bag. 1.5 L of formic acid was then added to 50 kg of fish grinder and stirred until evenly mixed. Stirring was performed 3 to 4 times daily until the 4th day, and on the 5th day, the water at the top was removed. Furthermore, the mixed microorganisms were inoculated with a dose of 10 mL 100/g of feedstuff and incubated for 7 days in a tightly closed and vacuum. Fresh fish was prepared by removing scales and washing thoroughly. The fish was then drained dried, and grounded until smooth.

Making feed was carried out by weighing all the ingredients used and mixing until homogeneous. The feed dough was printed using a pellet printer for crabs with a diameter of 1 cm and steamed for \pm 40 minutes. Pellets were cut 2 cm long and dried in the sun. Furthermore, the dried feed was then cooled at room temperature or aerated, placed in a plastic bag and stored in a dry place, until use for the next test.

The artificial feed was coated with fermented herbal extracts that have been prepared previously. These fermented extracts were made up of three herbal extracts, including mulberry leaf extract (*Morus alba*), temulawak (*Curcuma xanthorrhiza*), and temu kunci (*Boesenbergia rotunda*), at equal concentrations, and fermented with *Lactobacillus casei* and *Saccharomyces cereviceae*. Approximately 1 ml of probiotic and 1 ml of molasses were added into distilled water containing 500 mg of herbal extracts of Stock FHE fermentation time: 1 month, and fermentation condition under room temperature. Furthermore, the dilution of stock FHE for each treatment was as follows: treatment A (100 ml of distilled water); treatment B (20 ml FHE stock + 80 ml distilled water); treatment C (60 ml FHE Stock + 40 ml distilled water); and treatment D (100 ml FHE stock; without distilled water). Feed was administered in the afternoon at a dose of 5% of body weight with a frequency of once every 2 days. Feed containing herbs were given at 1-day intervals compared to feed without herbs (control). The rest of the feed was then measured before the next feeding. The remaining feed was collected during the rearing period, dried, and weighed at the end of the study.

Visual observations were made daily to control the development of the test crabs. If a dead crab was found, it was recorded and weighed. If molting crabs were found, they were recorded and weighed, and placed back in the crab box for rearing until they reach the end of cultivation. Furthermore, observations of water quality including temperature, salinity, dissolved oxygen, and pH were carried out every day during cultivation, while ammonia was measured at the beginning and end of cultivation. The temperature was determined using a thermometer, salinity with a hand refractometer, dissolved oxygen with a DO meter, pH with a pH meter, and ammonia in the laboratory.

Fermented Herbal Extract for Mud Crab

Growth performance

The growth involving absolute weight gain and specific growth rate. Absolute weight gain was measured using the formula:

$$WM = W_t - W_0$$

Where:

WM: absolute weight growth (g),

W_t: body weight (g),

W₀: initial body weight (g).

Meanwhile, the specific growth rate was calculated using the formula:

$$SGR = \frac{\ln W_t - \ln W_0}{T} \times 100$$

Where:

SGR: Specific Growth Rate (%)

W_t: average weight of crabs at the end of the study (g)

W₀: average weight of crabs at the beginning of the study (g)

T: maintenance time in days.

Percentage of molting

The percentage of molting (%) was measured by comparing the number of molting test crabs with live test crabs multiplied by 100.

Survival rate

The survival rate (%) of mud crabs was calculated using the formula:

$$S = \frac{N_t}{N_0} \times 100$$

Where:

S: the survival rate (%)

N_t: the number of live crabs at the end of rearing (tails)

N₀: the number of crabs at the beginning of rearing (tails).

Feed consumption

Feed consumption (KP) was calculated by determining the difference in the amount of feed given to the amount of feed remaining. This calculation was based on the dry weight of the feed.

Feed efficiency

Feed efficiency (EP) was calculated using the formula as proposed by Watanabe (1988):

$$EP = [(W_t + D) - W_0] / F \times 100$$

where:

EP: feed efficiency

W_t: the average weight of crabs at the end of the study (g)

W₀: the average weight of crabs at the beginning (g)

D: the weight of crabs that died during the study (g).

Condition Factor

The F or Condition factor (K) was calculated using Fulton's condition factor formula, namely

$$FC = 100 \times W / L^3$$

where:

FC: the condition factor

W: the weight of the test crab,

L: the width of the carapace of the test crab.

To determine the level of change in the condition factor of the test crab at the beginning of the study compared to the end, it was calculated using the formula:

$$TPFK = (FKb - FKa) / FKa \times 100$$

where:

TPFK: the rate of change in condition factor (%)

FKa: the initial condition factor

FKb: the final condition factor.

Statistical analysis

Data were presented using tables and images. The effect of treatment on growth, feed consumption, feed efficiency and condition factors were analyzed using Analysis of Variance (ANOVA). Further tests were carried out using the LSD test at a 90% confidence level. A difference of $P < 0.1$ is stated to be statistically significant. Furthermore, to analyze the molting and non-molting crab data, an independent sample t-test was used. The analysis was performed using Excel software and SPSS version 16.0.

Results

Feed consumption, feed efficiency, growth, and changes in condition factors

The results showed that the tested crabs consumed feed well, which contained herbal extracts. Although the molting percentage was without significant difference between treatments after 31 days of rearing, there were differences in crab responses in terms of feed consumption, feed efficiency, growth, and changes in condition factors. Overall, crabs have a high survival rate above 90% in **Table 2** (below).

Table 2 Description of crabs used at the beginning of the study, molting percentage, and survival rate.

TC	N	W0 (g)	CL0 (mm)	CW0 (mm)	CF0	MP (%)	SR (%)
A	60	106,88±11,46	58±4,3	81,28±4,5	19,88±2,13	16,67	93,33
B	60	106,7±12,11	58,72±3,3	81,05±3,9	20,27±2,62	21,67	91,67
C	60	107,13±12,40	57,73±3,0	80,62±3,0	20,42±2,12	10,00	95,00
D	60	107,05±12,11	58,47±3,7	80,48±5,1	21,06±6,15	15,00	96,67

Treatment Code (TC); 0 mg kg⁻¹ of feed (A); 200 mg kg⁻¹ of feed (B); 400 mg kg⁻¹ of feed (C); and 600 mg kg⁻¹ of feed (D); Number of juvenile (N); Initial Weight (W0); Initial Carapace Length (CL0); Initial Carapace Width (CW0); Initial Condition Factor (CF0); Molting percentage (MP) and Survival Rate (SR).

Based on the independent sample t-test analysis, it was observed that there was a very significant difference between molting and non-molting crabs, both in terms of growth, condition factors, feed consumption and feed efficiency (**Figure 1**). Therefore, molting and non-molting crab data were separated to reduce the bias in data analysis. Changes in

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unique condition factors due to molting caused crabs to enlarge, however, conditional factors were decreased (**Tables 3 and 4**).

Table 3 Growth, Feed consumption and Feed Efficiency of molting crab

TC	WG (g)	CLG (mm)	CWG (mm)	SGR	CoCF (%)	FC (g)	FE (%)
A	43,73±13,30 ^a	8,7±3,6 ^a	12±5,4 ^a	12,9±1,25 ^a	-4,13±6,89 ^a	88,79±5,58 ^a	53,29±17,22 ^a
B	40,03±10,77 ^a	4,9±2,2 ^{ab}	8,6±2,2 ^{ab}	12,63±0,75 ^a	4,32±11,13 ^a	88,22±4,30 ^a	50,24±12,60 ^a
C	53,9±14,58 ^{ab}	9,4±2,2 ^{ac}	14,4±4,2 ^{ac}	13,51±0,73 ^a	-5,10±16,01 ^a	90,52±0,83 ^a	63,72±15,25 ^{ab}
D	60,73±11,41 ^{bc}	7,6±3,5 ^a	11,1±3,7 ^a	13,96±0,80 ^b	9,38±7,54 ^b	79,6±9,16 ^b	81,97±15,53 ^b

Different letters in the column are statistically different according to the LSD test ($p < 0.1$). Treatment Code (TC); 0 mg kg⁻¹ of feed (A); 200 mg kg⁻¹ of feed (B); 400 mg kg⁻¹ of feed (C); and 600 mg kg⁻¹ of feed (D); Weight gain (WG); Specific Growth Rate (SGR), Change of Condition Factor (CoCF); Feed Consumption (FC); Feed Efficiency (FE)

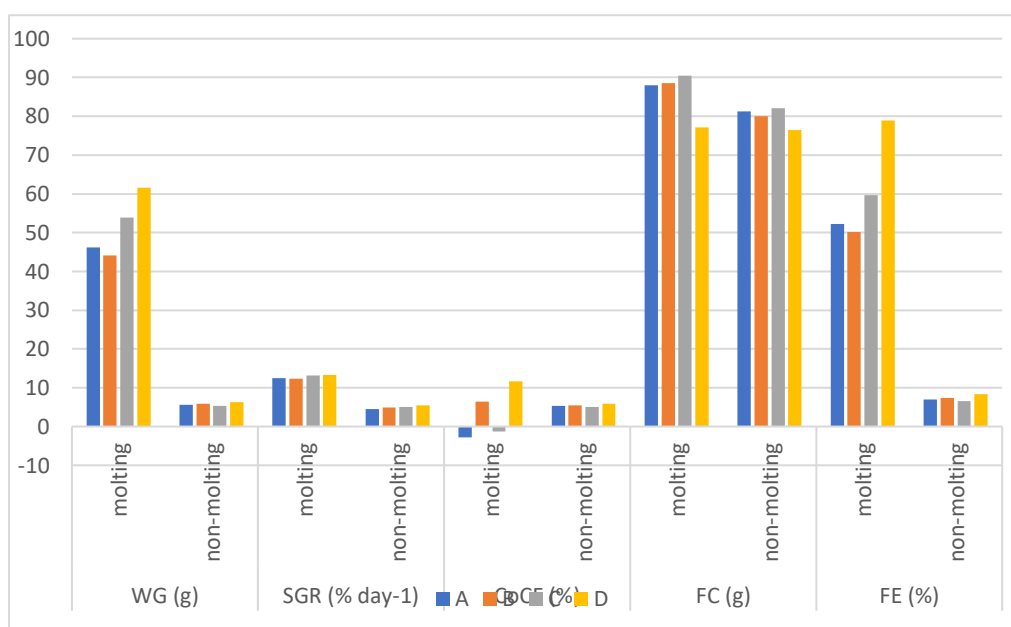


Figure 1 Comparison of growth, feed consumption, and feed efficiency of molting and non-molting crabs. $P < 0.05$ based on independent sample t-test; Treatment: 0 mg/kg of feed (A), 200 mg/kg of feed (B), 400 mg/kg of feed (C), and 600 mg/kg of feed (D); Weight gain (WG); Specific Growth Rate (SGR), Change of Condition Factor (CoCF); Feed Consumption (FC); Feed Efficiency (FE).

Table 4 Growth, Feed consumption and Feed Efficiency of non-molting crab

TC	WG (g)	SGR	CoF (%)	FC (g)	FE (%)
A	5,44±2,11 ^a	4,3±1,32 ^a	5,03±2,09 ^a	81,57±2,80 ^a	6,61±2,74 ^a
B	5,93±1,28 ^a	4,95±0,56 ^a	5,22±1,76 ^a	88,87±20,95 ^{ab}	7,67±2,22 ^a
C	5,11±0,62 ^{ab}	4,8±0,51 ^a	4,81±0,69 ^a	81,95±2,04 ^a	6,26±0,94 ^{ab}
D	6,42±1,52 ^{ac}	5,52±0,65 ^b	5,96±1,37 ^a	76,71±7,12 ^{ac}	8,41±1,95 ^{ac}

Different letters in the column are statistically different according to the LSD test ($p < 0.1$). Treatment Code (TC); 0 mg kg⁻¹ of feed (A); 200 mg kg⁻¹ of feed (B); 400 mg kg⁻¹ of feed (C); and 600 mg kg⁻¹ of feed (D); Weight gain (WG); Specific Growth Rate (SGR), Change of Condition Factor (CoCF); Feed Consumption (FC); Feed Efficiency (FE).

Water quality

During the study, water quality was in fairly good condition, except for low dissolved oxygen in the morning. Temperatures ranged between 27–32 °C, salinity 28-35 ppt, pH 6.9–8.9, dissolved oxygen 2.1–5.6 ppm, and ammonia 0.002 ppm.

Discussion

In aquaculture, especially in crab farming, growth is very important, as it is related to time and maintenance. If growth is stimulated in a shorter period, farmers can harvest faster with lower maintenance costs, which enhances profitability. Functional feed additive treatments were derived from three herbal extracts, namely mulberry (*M. alba*) leaf extract, temulawak (*C. xanthorrhiza*) and temu kunci (*B. rotunda*) rhizome. These treatments obtained a positive effect in promoting growth and increasing feed efficiency. The results showed that the higher the dose of herbal extract was used, the higher the growth and feed efficiency. Furthermore, a dose of 600 mg of ethanol extract from a combination of three types of herbs per kg of feed obtained the highest growth and feed efficiency.

The results of this study also showed that the active compounds contained in the combination of extracts synergize to stimulate growth. Fujaya et al., (2018) stated that mulberry (*M. alba*) extract contains phytoecdysteroids, which are ecdysteroid mimic hormones naturally found in some plants (Klein, 2004; Dinan et al., 2009). Ecdysteroids in crustaceans and crabs are important hormones that control the moulting process. This compound plays a role in activating protein synthesis by increasing mRNA synthesis (Preston-mafham & Dinan, 2002). This activity is also strengthened by the role of extracts of temulawak (*Curcuma xanthorrhiza*) and temu kunci (*Boesenbergia rotunda*) which are known to contain curcumin and essential oils. Both compounds act as antioxidants and increase appetite. This enables the supply of raw materials for crab nutrition to be fulfilled.

The bioactive phytochemicals and secondary metabolites of the mulberry extract include steroids, saponins, alkaloids, glycosides, and phenolic compounds including terpenoids, flavonoids, anthocyanins, and tannins. These bioactive compounds of mulberry extract exhibit outstanding anti-oxidative, anti-diabetic, anti-stress, nephroprotective, antimicrobial, anti-mutagenic, anticancer, anxiolytic, hepatoprotective, anthelmintic, antimicrobial, immunomodulatory effects and has cholesterol-lowering effects (Hussain et al., 2017). The anti-stress effect was significantly demonstrated in experiments on rats subjected to restraint. Chronic stress restraint will result in cognitive dysfunction, altered behavioral parameters, increased leukocyte count, levels of superoxide dismutase (SOD), lipid peroxidation (LPO), glucose and corticosterone, with concomitant decreases in catalase (CAT) activity, and glutathione reductase (GSH). Furthermore, gastric ulcers, adrenal glands and spleen were also used as stress indexes. All these restraint-induced disturbances were attenuated by ethyl acetate soluble (EASF) from *Morus alba* (Nade & Yadav, 2010). This effect was certainly very necessary for crabs in the cage or crab box.

The response of crabs to temulawak extract appears to be similar to that of other animals. According to Hosseini et al., (2016), *C. xanthorrhiza* (CX), known as temulawak, contains essential oils rich in phenols. The antioxidant activity of CXEO mainly refers to xanthorrhizol, arcurcumene, and curcumene. It has been widely documented that these phenolic compounds can enhance gut health resulting in better food absorption activities.

Similarly, temu kunci (*B. rotunda*) which is one of the main components of the herbal extract used in this study also contains essential oils. Chahyadi et al., (2014) also emphasized that the rhizome of *B. pandurata* contains essential oils and many flavonoid compounds which showed many interesting pharmacological characteristics, such as antifungal, antibacterial, and antioxidant. Eng-Chong et al., (2012) stated that *B. rotunda* has great medicinal potential, as nearly a hundred compounds were isolated from this plant which is also known as "Thai Ginseng". These compounds have many roles, including anti-

microbial, anti-parasitic, antioxidant, and also inhibit the formation of biofilms by intestinal pathogens.

In this study, the condition factor was used to describe the level of obesity. There was a difference in the value of the condition factor in the test crabs. The initial condition factor of the crabs in this study varied between 14 - 54 based on the ratio of weight and width of the carapace. Furthermore, at the end of the study, the condition factor of crabs varied between 15 - 60, there were crabs whose condition factors increased while in some, this factor decreased. The decreasing condition factor occurred in crabs that have just moulted. The observations by Moslen & Miebaka, (2018) showed that the variation in condition factors was caused by eating behaviour, biological factors, and responses to the environment. A low condition factor value means the crabs are light for their length, an indication of low feeding intensity and the presence of spawning activity. A high K value is the assumption of high feeding intensity and a gradual increase in fat accumulation, which also indicates preparation for a new reproductive period (Suryandari et al., 2018).

In this study, the dynamics of conditional factors were influenced by moulting activity. Crabs that had just moulted had a lower condition factor than other crabs before moulting. Therefore, crabs that had just moulted were classified as thin, while crabs before moulting were classified as fat. As the growth of crab weight was not always followed by the growth of the width or length of the carapace, the growth indicator using the condition factor value was significantly helpful. In portunid crabs, the moulting process is divided into four main stages, namely intermoult, premolt, ecdysis and postmolt (Waiho et al., 2021). The intermoult is the stage after cuticle formation, and the exoskeleton has hardened after mineralization has fully occurred. Meanwhile, the intermolt stage is the crab muscle growth stage or the fattening stage. The premolt is the preparation stage for moulting, consisting of the early, mid, and late premolt stages. In the premolt stage, a new exoskeleton begins to be formed under the old hard exoskeleton, while the new exoskeleton is retracted. This enables the old and new exoskeletons to separate with the help of enzymes. The process of releasing the crab from the old exoskeleton is called ecdysis, where the crab slowly pulls itself out of the old exoskeleton through a crack at the back of the carapace that borders the abdomen. The crabs that have just been released from the old exoskeleton have a soft shell which expands as more water is absorbed to stretch the new cuticle. At this time, the size of the crabs was enlarged but had a low condition factor resulting in the light weight and were classified thin.

There was a significantly different level of change in conditional factors between molting and non-moulting crabs (Table 3). The existence of a minus value illustrates that the crab decreased in the condition factor after moulting. This also showed that the crabs have moulted at the end of the rearing period, therefore, they have not had time to grow to fill their enlarged carapace. According to Fujaya, (2011), moulting will naturally occur when body size increases due to growth. This phenomenon always occurs because the crab's body is wrapped by a rigid carapace and cannot develop following the development of body weight. Moulting is also influenced by external and internal factors. External factors include light, temperature, food availability, tides, while internal factors include body size and hormones. Both of these factors influence the brain and stimulate the Y-organ to produce moulting hormones.

The results of this study showed the importance of moulting management in crab culture. Moulting is not only important for the production of soft-shell crabs, but also for the rearing and cultivation of fattening crabs. Herbal extracts can be used to help stimulate moulting, increase growth, and increase feed efficiency, thereby enables farmers to maximize profits in crab culture.

In conclusion, the best dose to stimulate growth, feed efficiency, and the level of fatness of mud crabs was 600 mg kg⁻¹ of feed, which was the highest dose applied. Therefore, further study is required to obtain optimal doses of herbal extracts (*M. alba*, *C. xanthorrhiza*, and *B. rotunda*) in stimulating the growth and molting of cultured mud crabs.

Acknowledgements

The authors are grateful to the Hasanuddin University Research and Community Service Institute for funding this study through the Unhas Applied Research Grant for the 2020 fiscal year with the contract number 1585/UN4.22/PT.OI.03/2020.

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Thank you very much for your positive comments and cooperation.

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Utilization of Feed and Growth Performance of Mud Crabs: The Effect of Herbal Extracts as Functional Feed Additives

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Key words: Crab, growth, herbal extracts, molting, utilization of feed

Abstract

This study aimed to analyze the effect of herbal extracts as functional feed additives on utilization of feed and growth performance of orange mud crabs (*Scylla olivacea*). The test animals used were 60 crabs for each treatment dose. The crabs are kept individually in crab box which was placed on a floating bamboo raft. This was to enable floating on the surface of the pond water which had a depth of ± 80 cm. The treatments tested were four doses of a combination herbal extract from *Morus alba*, *Curcuma xanthorrhiza*, and *Boesenbergia rotunda*, namely 0, 200, 400, 600 mg kg⁻¹ of feed. Formulated feed with protein content of 41.93%, fat 7.43%, Nitrogen Free Extract (NFE) 29.33%, and crude fiber 7.82% was given at a dose of 5% body weight everyday. Based on one-way ANOVA, the results showed that the dose of herbal extracts influences the feed utilization and growth performance of mud crabs. The dose of herbal extract 600 mg kg⁻¹ of feed provided higher feed utilization and growth performance (LSD Test: $P < 0.1$). Significant differences were found between feed utilization and growth performance of mud crabs after molting and non-molting. In molting mud crabs, treatment with herbal extract 600 mg kg⁻¹ obtained an average weight gain of ± 60.73 from the initial weight, compared to control crabs (without herbal extracts) gaining an average weight gain of $\pm 43.73\%$. However, in non-molting crabs, ± 5.44 and ± 6.42 weight gain was observed, respectively. Significant differences also occurred between treatments for specific growth rates, feed consumption, feed efficiency, and condition factors. These results provided information that herbal extracts are potentially as feed additives to increase feed utilization and growth performance, as well as stimulate molting in crab cultivation.

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Introduction

Mud crab is one of the crab species with high economic value worldwide which are generally marketed alive, while some are sold as frozen or cooked products. There are four species of mud crab that are the focus of commercial fisheries and aquaculture production, namely *Scylla serrata*, *S. tranquebarica*, *S. paramamosain* and *S. olivacea*. These are in great demand in both export and domestic markets with prices ranging from 8 to 25 USD depending on the size and season (Lalramchhani et al., 2019). Prices are also influenced by the shape of the product such as soft-shell crab (Fujaya et al., 2020). Waiho et al. (2021) stated that soft-shell crab products are one of the crab culture products with world market demand that continues to increase at high prices. Currently, there are three different culture systems including crab fattening, grow-out, and soft-shell crab production in practice that target different tiers of the global crab market (Rahman et al. 2020). In Indonesia, grow out and fattening are the most widely practiced methods of crab cultivation, and both are generally carried out in earthen ponds. Often, there are no special innovations in the cultivation other than feeding crabs with trash fish, chicken intestines, and others. In recent years, crab farmers have also started producing soft-shell crabs, either using horizontal farming methods with bamboo and HDPE boxes, or vertical farming.

When it comes to rearing, fattening, and producing soft-shell crabs, growth is the main focus during the rearing period. If growth does not occur optimally, aquaculture as part of economic activity will suffer losses, as crab growth is affected by molting. Molting and growth in decapod crustaceans such as crabs, lobsters, shrimps cannot be separated, and they influence each other. Molting is a precursor to growth and vice-versa. Therefore, various efforts have been made to stimulate growth and molting in crab culture, such as limb autotomy, eyestalk ablation, ecdysteroid and phytoecdysteroid application, control of physical parameters such as temperature and salinity, biogenic amines application, methyl farnesoate application, and molt-inhibiting hormone (MIH) inhibition (Waiho et al., 2021).

The use of herbs in the aquaculture industry is expected to be friendlier to the environment and cultivated animals. According to Bharati et al. (2019), increasing aquaculture growth cannot be separated from the development of quality feed, which requires appropriate and essential feed additives. Furthermore, the development of these additives shows promising results for aquaculture. These do not only improve fish growth performance but also their health performance. These functional feed additives originate from a variety of organic sources and are environmentally friendly for fish and the environment. Examples of these additives that have been developed include prebiotics, probiotics, seaweed, fungi, microalgae, enzymes, organic acids, mycotoxin binders, photogenic or Phyto biotic compounds and yeast.

Herbal extracts are one of the promising natural medicines that are used as functional feed additives to stimulate growth and molting in crabs. Herbal extracts generally contain terpenoids, alkaloids, saponins, flavonoids which act as anti-oxidants, anti-bacterial, immune-nutrition (Singh et al., 2013). The phytoecdysteroids are present in several plants, and one of their roles is to stimulate protein synthesis (Klein, 2004). One of them is the mulberry plant (*Morus alba*). The ecdysteroids contained in *Morus alba* are of the 20-hydroxyecdysone group. At present, mulberry leaf extract has been successfully trialed as a growth and moulting stimulant for mud crabs with an optimal dose of 2.4 mg g⁻¹ feed or 240 mg kg⁻¹ feed (Fujaya et al., 2018). In *Morus* spp. a wide range of the phytochemicals are present in leaves, fruit, roots and wood. Therefore, *Morus* spp. has various biological functions (antidiabetic, antiobesity, anti-inflammatory, anticancer, antibacterial, antioxidant, antiviral, cytoprotective and neuroprotective etc.). This plant is used traditionally for the development of different food products as well as the treatment of various diseases (Dhiman et al., 2020). The content of phenolic compounds, especially flavonoids and anthocyanins, causes *Morus alba* to have strong antioxidant activity, thereby potentially eliminating free radical production and cellular damage caused by free radicals (Hussain et al., 2017).

To complement the function of mulberry leaf extract (*Morus alba*), as a growth and molting stimulant, as well as an herb to maintain crab health. Added extracts of temulawak

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(*Curcuma xanthorrhiza*) and temu kunci (*Boesenbergia rotunda*), which are known to have various physiological functions, including appetite stimulant, hepatoprotector, antimicrobial, anti-inflammatory, analgesic, antipyretic, chloretic, and others. the latter is used as a "herbal" to improve intestinal health (Eng-Chong et al., 2012), and medicinal plants containing curcumin, xanthorrhizol, and pinostrobin (Qomaladewi & Cho, 2021)

This study examined the response of mud crabs to the various doses of mixed herbal extracts (combination of *Morus alba*, *Curcuma xanthorrhiza*, and *Boesenbergia rotunda*) as a functional feed additive on growth, molting, feed consumption, feed efficiency, condition factor and survival of mud crabs. The three medicinal plants are expected to synergize to improve the health and growth of crabs through the active compounds they contain. Furthermore, this combination of herbal extracts is expected to be one of the natural medicines that can be applied to juvenile crabs for growing out, fattening, and production of soft-shell crabs.

Materials and Methods

Experimental crab

The study was carried out at the Educational Farm of Hasanuddin University, Bojo Village, Mallusetasi District, Barru Regency. There were 240 mud crabs (*Scylla olivaceae*) with a bodyweight of 107.08 ± 11.93 g and carapace width of 80.91 ± 4.22 mm and were obtained from local fishermen's captures. Furthermore, weighing was carried out using an electric scale with an accuracy of 0.1 g and measuring the width of the carapace using a caliper with an accuracy of 0.1 mm as initial data. All crabs were then divided into 4 treatments and 6 replicates. There were four dosages of herbal extract, namely 0 mg kg⁻¹ of feed (A), 200 mg kg⁻¹ of feed (B), 400 mg kg⁻¹ of feed (C), and 600 mg kg⁻¹ of feed (D). Each dosage treatment consisted of 60 crabs, while each replicate consisted of 10 crabs.

The container used in this study was a crab box with a length, width, and height of 30 × 20 × 15 cm. The test animals were kept for 31 days individually in the crab box and given a label (tag) on each box as a marker of the treatment of the tested crab. Afterwards, each was placed on a bamboo raft with a float, to enhance floating on the surface of the pond water which had a depth of ± 80 cm.

Experimental Feed

The container used in this study was a crab box with a length, width, and height of 30 × 20 × 15 cm. The test animals were kept for 31 days individually in the crab box and given a label (tag) on each box as a marker of the treatment of the tested crab. Afterwards, each was placed on a bamboo raft with a float, to enhance floating on the surface of the pond water which had a depth of ± 80 cm.

The feed used in this study was fermented artificial feed in form of pellets, as recommended by Aslamyah et al. (2021). The feedstuff and results of feed proximate analysis are shown in **Table 1**.

Table 1 The composition (% dry matter) of feedstuff and results of feed proximate analysis

Composition	Percentage (%)
Fish flour	25
Fresh fish	20
Fish silage	10
Crab shell flour	5
Soy flour	8
Corn starch	10
Coconut meal flour	8
Pollard flour	10
Vitamin & mineral mix ¹	4
Amount	100
Water (%)	8.9
Ash (% dry weight)	13.49
Protein (% dry weight)	41.93
Fat (% dry weight)	7.43
Crude fiber (% dry weight)	7.82
NFE (% dry weight)	29.33
DE (kcal/kg) ²	2767.63
C/P (DE/g Protein)	6.76

¹ The composition of vitamins & mineral mix.

Every 10 kg contains 12,000,000 IU of Vitamin A; Vitamin D 2,000,000 IU; Vitamin E 8,000 IU; Vitamin K 2,000 mg; Vitamin B1 2,000 mg; Vitamin B 2 5,000; Vitamin B6 500 mg; Vitamin B12 12,000 g; Ascorbic acid 25,000 mg; Calcium-D-Phantothenate 6000 mg; Niacin 40,000 mg; Choline Chloride 10,000 mg; Methionine 30,000 mg; Lysine 30,000 mg; Manganese 120,000 mg; Iron 20,000 mg; Iodine 200 mg; Zinc 100,000 mg; Cobalt 200,000 mg; Copper 4,000 mg; Santoquin (antioxidant) 10,000 mg; Zinc bacitracin 21,000 m g.

² Calculation results based on the energy equation (NRC, 1988): 1 g carbohydrate = 2.5 kcal DE; 1 g protein = 3.5 kcal DE; 1 g fat = 8.1 kcal DE

The stages of making feed begin with preparing feedstuff, including dry ingredients, fish silage, and fresh fish. Dry feed ingredients were ground into flour, except for the vitamin and mineral mix. The ingredients were weighed and mixed starting from the smallest to the largest amount, and stirred until homogeneous. Afterwards, the ingredients were inoculated with 10 mL 100 g⁻¹ of feedstuff microorganism mix, which had previously been diluted with water in a ratio of 1: 3. The mixture was stirred evenly, stored in airtight plastic, and after 72 hours of incubation, opened and dried. Fish silage was prepared by removing fish scales, before washing and draining. The fish was grounded with a meat milling machine until smooth and placed in a closed bucket that was previously lined with a plastic bag. 1.5 L of formic acid was then added to 50 kg of fish grinder and stirred until evenly mixed. Stirring was performed 3 to 4 times daily until the 4th day, and on the 5th day, the water at the top was removed. Furthermore, the mixed microorganisms were inoculated with a dose of 10 mL 100 g⁻¹ of feedstuff and incubated for 7 days in a tightly closed and vacuum. Fresh fish was prepared by removing scales and washing thoroughly. The fish was then drained dried, and grounded until smooth.

Making feed was carried out by weighing all the ingredients used and mixing until homogeneous. The feed dough was printed using a pellet printer for crabs with a diameter of 1 cm and steamed for ± 40 minutes. Pellets were cut 2 cm long and dried in the sun. Furthermore, the dried feed was then cooled at room temperature or aerated, placed in a plastic bag and stored in a dry place, until use for the next test.

The artificial feed was coated with fermented herbal extracts that have been prepared previously. These fermented extracts were made up of three herbal extracts, including mulberry leaf extract (*Morus alba*), temulawak (*Curcuma xanthorrhiza*), and temu kunci (*Boesenbergia rotunda*), at equal concentrations, and fermented with *Lactobacillus casei* and *Saccharomyces cereviceae*. Approximately 1 mL of probiotic and 1 mL of molasses were added into distilled water containing 500 mg of herbal extracts of Stock FHE fermentation time: 1 month, and fermentation condition under room temperature. Furthermore, the dilution of stock FHE for each treatment was as follows: treatment A (100 mL of distilled water); treatment B (20 mL FHE stock + 80 mL distilled water); treatment C (60 mL FHE Stock + 40 mL distilled water); and treatment D (100 mL FHE stock; without distilled water). Feed was administered in the afternoon at a dose of 5% of body weight

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with a frequency of once every 2 days. Feed containing herbs were given at 1-day intervals compared to feed without herbs (control). The rest of the feed was then measured before the next feeding. The remaining feed was collected during the rearing period, dried, and weighed at the end of the study.

Visual observations were made daily to control the development of the test crabs. If a dead crab was found, it was recorded and weighed. If molting crabs were found, they were recorded and weighed, and placed back in the crab box for rearing until they reach the end of cultivation. Furthermore, observations of water quality including temperature, salinity, dissolved oxygen, and pH were carried out every day during cultivation, while ammonia was measured at the beginning and end of cultivation. The temperature was determined using a thermometer, salinity with a hand refractometer, dissolved oxygen with a DO meter, pH with a pH meter, and ammonia in the laboratory.

Growth performance

The growth involving absolute weight gain and specific growth rate. Absolute weight gain was measured using the formula:

$$WG = W_t - W_0$$

Where:

WG : absolute weight growth (g)

W_t : body weight (g)

W₀ : initial body weight (g)

Meanwhile, the specific growth rate was calculated using the formula:

$$SGR = \frac{\ln W_t - \ln W_0}{T} \times 100$$

Where:

SGR: Specific growth rate (%)

W_t : average weight of crabs at the end of the study (g)

W₀ : average weight of crabs at the beginning of the study (g)

T : maintenance time in days

Percentage of molting

The percentage of molting (MP) was measured by comparing the number of molting test crabs with live test crabs multiplied by 100.

Survival rate

The survival rate (SR) of mud crabs was calculated using the formula:

$$SR = \frac{N_t}{N_0} \times 100$$

Where:

SR : the survival rate (%)

N_t : the number of live crabs at the end of rearing (tails)

N₀ : the number of crabs at the beginning of rearing (tails)

Feed consumption

Feed consumption (FC) was calculated by determining the difference in the amount of feed given to the amount of feed remaining. This calculation was based on the dry weight of the feed.

Feed efficiency

Feed efficiency (FE) was calculated using the formula as proposed by Watanabe (1988):

$$FE = [(W_t + D) - W_0] / FC \times 100$$

where:

FE : feed efficiency

W_t : the average weight of crabs at the end of the study (g)

W₀ : the average weight of crabs at the beginning (g)

D : the weight of crabs that died during the study (g)

Condition Factor

The Condition factor was calculated using Fulton's condition factor formula, namely

$$CF = 100 \times W / L^3$$

where:

CF : the condition factor

W : the weight of the test crab

L : the width of the carapace of the test crab

To determine the level of change in the condition factor of the test crab at the beginning of the study compared to the end, it was calculated using the formula:

$$CoCF = (CF_b - CF_a) / CF_a \times 100$$

where:

CoCF : the rate of change in condition factor (%)

CF_a : the initial condition factor

CF_b : the final condition factor

Statistical analysis

Data were presented using tables and images. The effect of treatment on growth, feed consumption, feed efficiency, and condition factors were analyzed using Analysis of Variance (ANOVA). Further tests were carried out using the LSD test at a 90% confidence level. A difference of P<0.1 is stated to be statistically significant. Furthermore, to analyze the molting and non-molting crab data, an independent sample t-test was used. The analysis was performed using Excel software and SPSS version 16.0.

Results

Feed consumption, feed efficiency, growth, and changes in condition factors

The results showed that the tested crabs consumed feed well, which contained herbal extracts. Although the molting percentage was without significant difference between treatments after 31 days of rearing, there were differences in crab responses in terms of feed consumption, feed efficiency, growth, and changes in condition factors. Overall, crabs have a high survival rate above 90% in **Table 2** (below).

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Table 2 Description of crabs used at the beginning of the study, molting percentage, and survival rate.

TC	N	W0 (g)	CL0 (mm)	CW0 (mm)	CF0	MP (%)	SR (%)
A	60	106.88±11.46	58±4.3	81.28±4.5	19.88±2.13	16.67	93.33
B	60	106.7±12.11	58.72±3.3	81.05±3.9	20.27±2.62	21.67	91.67
C	60	107.13±12.40	57.73±3.0	80.62±3.0	20.42±2.12	10.00	95.00
D	60	107.05±12.11	58.47±3.7	80.48±5.1	21.06±6.15	15.00	96.67

Treatment Code (TC); 0 mg kg⁻¹ of feed (A); 200 mg kg⁻¹ of feed (B); 400 mg kg⁻¹ of feed (C); and 600 mg kg⁻¹ of feed (D); Number of juvenile (N); Initial Weight (W0); Initial Carapace Length (CL0); Initial Carapace Width (CW0); Initial Condition Factor (CF0); **Molting Percentage** (MP) and Survival Rate (SR)

Based on the independent sample t-test analysis, it was observed that there was a very significant difference between molting and non-molting crabs, both in terms of growth, condition factors, feed consumption and feed efficiency (**Figure 1**). Therefore, molting and non-molting crab data were separated to reduce the bias in data analysis. Changes in unique condition factors due to molting caused crabs to enlarge, however, conditional factors were decreased (**Tables 3 and 4**).

Table 3 Growth, Feed consumption, and Feed Efficiency of molting crab

TC	WG (g)	CLG (mm)	CWG (mm)	SGR	CoCF (%)	FC (g)	FE (%)
A	43.73±13.30 ^a	8.7±3.6 ^a	12±5.4 ^a	12.9±1.25 ^a	-4.13±6.89 ^a	88.79±5.58 ^a	53.29±17.22 ^a
B	40.03±10.77 ^a	4.9±2.2 ^{ab}	8.6±2.2 ^{ab}	12.63±0.75 ^a	4.32±11.13 ^a	88.22±4.30 ^a	50.24±12.60 ^a
C	53.9±14.58 ^{ab}	9.4±2.2 ^{ac}	14.4±4.2 ^{ac}	13.51±0.73 ^a	-5.10±16.01 ^a	90.52±0.83 ^a	63.72±15.25 ^{ab}
D	60.73±11.41 ^{bc}	7.6±3.5 ^a	11.1±3.7 ^a	13.96±0.80 ^b	9.38±7.54 ^b	79.6±9.16 ^b	81.97±15.53 ^b

Different letters in the column are statistically different according to the LSD test ($p < 0.1$). Treatment Code (TC); 0 mg kg⁻¹ of feed (A); 200 mg kg⁻¹ of feed (B); 400 mg kg⁻¹ of feed (C); and 600 mg kg⁻¹ of feed (D); Weight gain (WG); Change of Carapace Length (CLG); Change of Carapace Width (CWG); Specific Growth Rate (SGR), Change of Condition Factor (CoCF); Feed Consumption (FC); Feed Efficiency (FE)

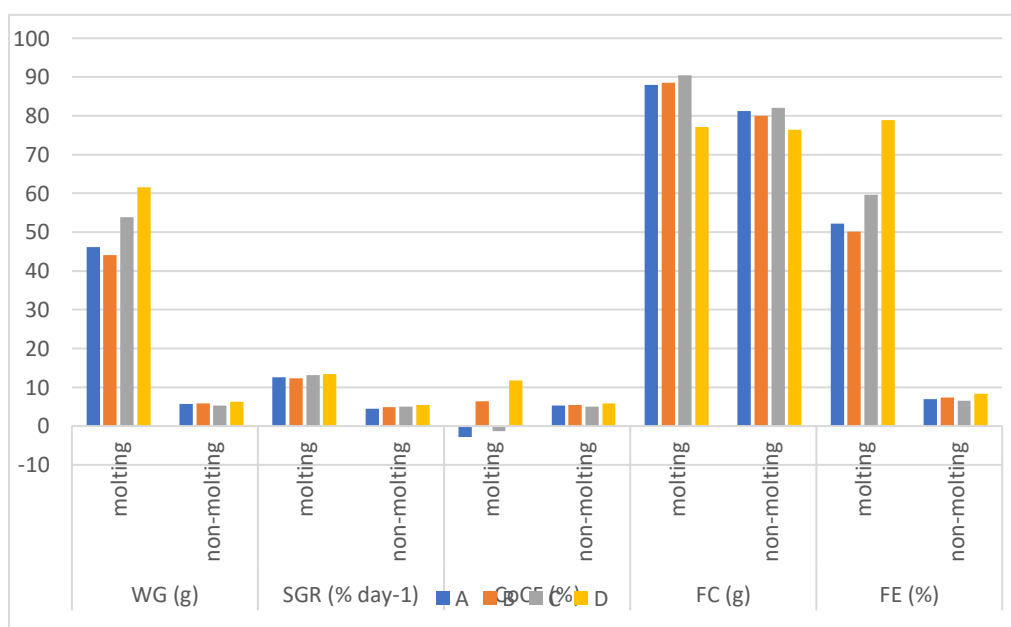


Figure 1 Comparison of growth, feed consumption, and feed efficiency of molting and non-molting crabs. $P < 0.05$ based on independent sample t-test; Treatment: 0 mg kg⁻¹ of feed (A), 200 mg kg⁻¹ of feed (B), 400 mg kg⁻¹ of feed (C), and 600 mg kg⁻¹ of feed (D); Weight gain (WG); Specific Growth Rate (SGR), Change of Condition Factor (CoCF); Feed Consumption (FC); Feed Efficiency (FE)

Table 4 Growth, Feed consumption and Feed Efficiency of non-molting crab

TC	WG (g)	SGR	CoCF (%)	FC (g)	FE (%)
A	5.44±2.11 ^a	4.3±1.32 ^a	5.03±2.09 ^a	81.57±2.80 ^a	6.61±2.74 ^a
B	5.93±1.28 ^a	4.95±0.56 ^a	5.22±1.76 ^a	88.87±20.95 ^{ab}	7.67±2.22 ^a
C	5.11±0.62 ^{ab}	4.8±0.51 ^a	4.81±0.69 ^a	81.95±2.04 ^a	6.26±0.94 ^{ab}
D	6.42±1.52 ^{ac}	5.52±0.65 ^b	5.96±1.37 ^a	76.71±7.12 ^{ac}	8.41±1.95 ^{ac}

Different letters in the column are statistically different according to the LSD test ($p < 0.1$). Treatment Code (TC); 0 mg kg⁻¹ of feed (A); 200 mg kg⁻¹ of feed (B); 400 mg kg⁻¹ of feed (C); and 600 mg kg⁻¹ of feed (D); Weight gain (WG); Specific Growth Rate (SGR), Change of Condition Factor (CoCF); Feed Consumption (FC); Feed Efficiency (FE)

Water quality

During the study, water quality was in fairly good condition, except for low dissolved oxygen in the morning. Temperatures ranged between 27–32 °C, salinity 28-35 ppt, pH 6.9–8.9, dissolved oxygen 2.1–5.6 ppm, and ammonia 0.002 ppm.

Discussion

In aquaculture, especially in crab farming, growth is very important, as it is related to time and maintenance. If growth is stimulated in a shorter period, farmers can harvest faster with lower maintenance costs, which enhances profitability. Functional feed additive treatments were derived from three herbal extracts, namely mulberry (*M. alba*) leaf extract, temulawak (*C. xanthorrhiza*) and temu kunci (*B. rotunda*) rhizome. These treatments obtained a positive effect in promoting growth and increasing feed efficiency. The results showed that the higher the dose of herbal extract was used, the higher the growth and feed efficiency. Furthermore, a dose of 600 mg of ethanol extract from a combination of three types of herbs per kg of feed obtained the highest growth and feed efficiency.

The results of this study also showed that the active compounds contained in the combination of extracts synergize to stimulate growth. Fujaya et al., (2018) stated that mulberry (*M. alba*) extract contains phytoecdysteroids, which are ecdysteroid mimic hormones naturally found in some plants (Klein, 2004; Dinan et al., 2009). Ecdysteroids in crustaceans and crabs are important hormones that control the moulting process. This compound plays a role in activating protein synthesis by increasing mRNA synthesis (Preston-mafham & Dinan, 2002). This activity is also strengthened by the role of extracts of temulawak (*Curcuma xanthorrhiza*) and temu kunci (*Boesenbergia rotunda*) which are known to contain curcumin and essential oils. Both compounds act as antioxidants and increase appetite. This enables the supply of raw materials for crab nutrition to be fulfilled.

The bioactive phytochemicals and secondary metabolites of the mulberry extract include steroids, saponins, alkaloids, glycosides, and phenolic compounds including terpenoids, flavonoids, anthocyanins, and tannins. These bioactive compounds of mulberry extract exhibit outstanding anti-oxidative, anti-diabetic, anti-stress, nephroprotective, antimicrobial, anti-mutagenic, anticancer, anxiolytic, hepatoprotective, anthelmintic, antimicrobial, immunomodulatory effects and has cholesterol-lowering effects (Hussain et al., 2017). The anti-stress effect was significantly demonstrated in experiments on rats subjected to restraint. Chronic stress restraint will result in cognitive dysfunction, altered behavioral parameters, increased leukocyte count, levels of superoxide dismutase (SOD), lipid peroxidation (LPO), glucose and corticosterone, with concomitant decreases in catalase (CAT) activity, and glutathione reductase (GSH). Furthermore, gastric ulcers, adrenal glands and spleen were also used as stress indexes. All these restraint-induced disturbances were attenuated by ethyl acetate soluble (EASF) from *Morus alba* (Nade & Yadav, 2010). This effect was certainly very necessary for crabs in the cage or crab box.

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The response of crabs to temulawak extract appears to be similar to that of other animals. According to [Hosseini et al. \(2016\)](#), *C. xanthorrhiza* (CX), known as temulawak, contains essential oils rich in phenols. The antioxidant activity of CXEO mainly refers to xanthorrhizol, arcurcumene, and curcumene. It has been widely documented that these phenolic compounds can enhance gut health resulting in better food absorption activities.

Similarly, temu kunci (*B. rotunda*) which is one of the main components of the herbal extract used in this study also contains essential oils. [Chahyadi et al. \(2014\)](#) also emphasized that the rhizome of *B. pandurata* contains essential oils and many flavonoid compounds which showed many interesting pharmacological characteristics, such as antifungal, antibacterial, and antioxidant. [Eng-Chong et al. \(2012\)](#) stated that *B. rotunda* has great medicinal potential, as nearly a hundred compounds were isolated from this plant which is also known as "Thai Ginseng". These compounds have many roles, including anti-microbial, anti-parasitic, antioxidant, and also inhibit the formation of biofilms by intestinal pathogens.

In this study, the condition factor was used to describe the level of obesity. There was a difference in the value of the condition factor in the test crabs. The initial condition factor of the crabs in this study varied between 14-54 based on the ratio of weight and width of the carapace. Furthermore, at the end of the study, the condition factor of crabs varied between 15-60, there were crabs whose condition factors increased while in some, this factor decreased. The decreasing condition factor occurred in crabs that have just moulted. The observations by [Moslen & Miebaka \(2018\)](#) showed that the variation in condition factors was caused by eating behaviour, biological factors, and responses to the environment. A low condition factor value means the crabs are light for their length, an indication of low feeding intensity and the presence of spawning activity. A high K value is the assumption of high feeding intensity and a gradual increase in fat accumulation, which also indicates preparation for a new reproductive period ([Suryandari et al., 2018](#)).

In this study, the dynamics of conditional factors were influenced by moulting activity. Crabs that had just moulted had a lower condition factor than other crabs before moulting. Therefore, crabs that had just moulted were classified as thin, while crabs before moulting were classified as fat. As the growth of crab weight was not always followed by the growth of the width or length of the carapace, the growth indicator using the condition factor value was significantly helpful. In portunid crabs, the moulting process is divided into four main stages, namely intermoult, premolt, ecdysis and postmolt ([Waiho et al., 2021](#)). The intermoult is the stage after cuticle formation, and the exoskeleton has hardened after mineralization has fully occurred. Meanwhile, the intermolt stage is the crab muscle growth stage or the fattening stage. The premolt is the preparation stage for moulting, consisting of the early, mid, and late premolt stages. In the premolt stage, a new exoskeleton begins to be formed under the old hard exoskeleton, while the new exoskeleton is retracted. This enables the old and new exoskeletons to separate with the help of enzymes. The process of releasing the crab from the old exoskeleton is called ecdysis, where the crab slowly pulls itself out of the old exoskeleton through a crack at the back of the carapace that borders the abdomen. The crabs that have just been released from the old exoskeleton have a soft shell which expands as more water is absorbed to stretch the new cuticle. At this time, the size of the crabs was enlarged but had a low condition factor resulting in the light weight and were classified thin.

There was a significantly different level of change in conditional factors between molting and non-moulting crabs (Table 3). The existence of a minus value illustrates that the crab decreased in the condition factor after moulting. This also showed that the crabs have moulted at the end of the rearing period, therefore, they have not had time to grow to fill their enlarged carapace. According to [Fujaya \(2011\)](#), moulting will naturally occur when body size increases due to growth. This phenomenon always occurs because the crab's body is wrapped by a rigid carapace and cannot develop following the development of body weight. Moulting is also influenced by external and internal factors. External factors include light, temperature, food availability, tides, while internal factors include body size and

hormones. Both of these factors influence the brain and stimulate the Y-organ to produce moulting hormones.

The results of this study showed the importance of moulting management in crab culture. Moulting is not only important for the production of soft-shell crabs, but also for the rearing and cultivation of fattening crabs. Herbal extracts can be used to help stimulate moulting, increase growth, and increase feed efficiency, thereby enables farmers to maximize profits in crab culture.

In conclusion, the best dose to stimulate growth, feed efficiency, and the level of fatness of mud crabs was 600 mg kg⁻¹ of feed, which was the highest dose applied. Therefore, further study is required to obtain optimal doses of herbal extracts (*M. alba*, *C. xanthorrhiza*, and *B. rotunda*) in stimulating the growth and molting of cultured mud crabs.

Acknowledgements

The authors are grateful to the Hasanuddin University Research and Community Service Institute for funding this study through the Unhas Applied Research Grant for the 2020 fiscal year with the contract number 1585/UN4.22/PT.OI.03/2020.

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Siti Aslmyah Feb 11, 2022 - 2:38 pm SST

Dear Dr. Shindo Hofstetter

I am sending you the necessary correction and missing information needed. As for correction that could be written in the red sentences, please find the attachment. Thank you so much for your direction.

Best Regards
Dr. Siti Aslmyah
Fisheries Department
Faculty of Marine and Fisheries
Hasanuddin University, Makassar City
Indonesia

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Utilization of Feed and Growth Performance of Mud Crabs: The Effect of Herbal Extracts as Functional Feed Additives

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Keywords: Crab, growth, herbal extracts, molting, feed utilization

Abstract

This study aimed to analyze the effect of herbal extracts as functional feed additives on the utilization of feed and growth performance of orange mud crabs (*Scylla olivacea*). The test animals used were 60 crabs for each treatment dose. The crabs are kept individually in a crab box which was placed on a floating bamboo raft. This was to enable floating on the surface of the pond water which had a depth of ± 80 cm. The treatments tested were four doses of a combination herbal extract from *Morus alba*, *Curcuma xanthorrhiza*, and *Boesenbergia rotunda*, namely 0, 200, 400, 600 mg kg⁻¹ of feed. Formulated feed with the protein content of 41.93%, fat 7.43%, Nitrogen Free Extract (NFE) 29.33%, and crude fiber 7.82% was given at a dose of 5% body weight every day. Based on one-way ANOVA, the results showed that the dose of herbal extracts influences the feed utilization and growth performance of mud crabs. The dose of herbal extract 600 mg kg⁻¹ of feed provided higher feed utilization and growth performance (LSD Test: $P < 0.1$). Significant differences were found between feed utilization and growth performance of mud crabs after molting and non-molting. In molting mud crabs, treatment with herbal extract 600 mg kg⁻¹ obtained an average weight gain of ± 60.73 from the initial weight, compared to control crabs (without herbal extracts) gaining an average weight gain of $\pm 43.73\%$. However, in non-molting crabs, ± 5.44 and ± 6.42 weight gain was observed, respectively. Significant differences also occurred between treatments for specific growth rates, feed consumption, feed efficiency, and condition factors. These results provided information that herbal extracts are potentially as feed additives to increase feed utilization and growth performance, as well as stimulate molting in crab cultivation.

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Introduction

Mud crab is one of the crab species with high economic value worldwide which are generally marketed alive, while some are sold as frozen or cooked products. There are four species of mud crab that are the focus of commercial fisheries and aquaculture production, namely *Scylla serrata*, *S. tranquebarica*, *S. paramamosain* and *S. olivacea*. These are in great demand in both export and domestic markets with prices ranging from 8 to 25 USD depending on the size and season (Lalramchhani et al., 2019). Prices are also influenced by the shape of the product such as soft-shell crab (Fujaya et al., 2020). Waiho et al. (2021) stated that soft-shell crab products are one of the crab culture products with world market demand that continues to increase at high prices. Currently, there are three different culture systems including crab fattening, grow-out, and soft-shell crab production in practice that target different tiers of the global crab market (Rahman et al. 2020). In Indonesia, grow out and fattening are the most widely practiced methods of crab cultivation, and both are generally carried out in earthen ponds. Often, there are no special innovations in the cultivation other than feeding crabs with trash fish, chicken intestines, and others. In recent years, crab farmers have also started producing soft-shell crabs, either using horizontal farming methods with bamboo and HDPE boxes, or vertical farming.

When it comes to rearing, fattening, and producing soft-shell crabs, growth is the main focus during the rearing period. If growth does not occur optimally, aquaculture as part of economic activity will suffer losses, as crab growth is affected by molting. Molting and growth in decapoda crustaceans such as crabs, lobsters, shrimps cannot be separated, and they influence each other. Molting is a precursor to growth and vice-versa. Therefore, various efforts have been made to stimulate growth and molting in crab culture, such as limb autotomy, eyestalk ablation, ecdysteroid and phytoecdysteroid application, control of physical parameters such as temperature and salinity, biogenic amines application, methyl farnesoate application, and molt-inhibiting hormone (MIH) inhibition (Waiho et al., 2021).

The use of herbs in the aquaculture industry is expected to be friendlier to the environment and cultivated animals. According to Bharati et al. (2019), increasing aquaculture growth cannot be separated from the development of quality feed, which requires appropriate and essential feed additives. Furthermore, the development of these additives shows promising results for aquaculture. These do not only improve fish growth performance but also their health performance. These functional feed additives originate from a variety of organic sources and are environmentally friendly for fish and the environment. Examples of these additives that have been developed include prebiotics, probiotics, seaweed, fungi, microalgae, enzymes, organic acids, mycotoxin binders, photogenic or Phyto biotic compounds, and yeast.

Herbal extracts are one of the promising natural medicines that are used as functional feed additives to stimulate growth and molting in crabs. Herbal extracts generally contain terpenoids, alkaloids, saponins, flavonoids which act as anti-oxidants, anti-bacterial, immune-nutrition (Singh et al., 2013). The phytoecdysteroids are present in several plants, and one of their roles is to stimulate protein synthesis (Klein, 2004). One of them is the mulberry plant (*Morus alba*). The ecdysteroids contained in *Morus alba* are of the 20-hydroxyecdysone group. At present, mulberry leaf extract has been successfully trialed as growth and molting stimulant for mud crabs with an optimal dose of **2.4 mg g⁻¹ feed or 240 mg kg⁻¹ feed** (Fujaya et al., 2018). In *Morus* spp. a wide range of phytochemicals are present in leaves, fruit, roots, and wood. Therefore, *Morus* spp. has various biological functions (antidiabetic, antiobesity, anti-inflammatory, anticancer, antibacterial, antioxidant, antiviral, cytoprotective and neuroprotective etc.). This plant is used traditionally for the development of different food products as well as the treatment of various diseases (Dhiman et al., 2020). The content of phenolic compounds, especially flavonoids and anthocyanins, causes *Morus alba* to have strong antioxidant activity, thereby potentially eliminating free radical production and cellular damage caused by free radicals (Hussain et al., 2017).

To complement the function of mulberry leaf extract (*Morus alba*), as a growth and molting stimulant, as well as an herb to maintain crab health. Added extracts of temulawak

(*Curcuma xanthorrhiza*) and temu kunci (*Boesenbergia rotunda*), which are known to have various physiological functions, including appetite stimulant, hepatoprotector, antimicrobial, anti-inflammatory, analgesic, antipyretic, chloretic, and others. The latter is used as a "herbal" to improve intestinal health (Eng-Chong et al., 2012), and medicinal plants containing curcumin, xanthorrhizol, and pinostrobin (Qomaladewi & Cho, 2021)

This study examined the response of mud crabs to the various doses of mixed herbal extracts (combination of *Morus alba*, *Curcuma xanthorrhiza*, and *Boesenbergia rotunda*) as a functional feed additive on growth, molting, feed consumption, feed efficiency, condition factor and survival of mud crabs. The three medicinal plants are expected to synergize to improve the health and growth of crabs through the active compounds they contain. Furthermore, this combination of herbal extracts is expected to be one of the natural medicines that can be applied to juvenile crabs for growing out, fattening, and production of soft-shell crabs.

Materials and Methods

Experimental crab

The study was carried out at the Educational Farm of Hasanuddin University, Bojo Village, Mallusetasi District, Barru Regency. There were 240 mud crabs (*Scylla olivaceae*) with a bodyweight of 107.08 ± 11.93 g and carapace width of 80.91 ± 4.22 mm and were obtained from local fishermen's captures. Furthermore, weighing was carried out using an electric scale with an accuracy of 0.1 g and measuring the width of the carapace using a caliper with an accuracy of 0.1 mm as initial data. All crabs were then divided into 4 treatments and 6 replicates. There were four dosages of herbal extract, namely 0 mg kg⁻¹ of feed (A), 200 mg kg⁻¹ of feed (B), 400 mg kg⁻¹ of feed (C), and 600 mg kg⁻¹ of feed (D). Each dosage treatment consisted of 60 crabs, while each replicate consisted of 10 crabs.

The container used in this study was a crab box with a length, width, and height of 30 × 20 × 15 cm. The test animals were kept for 31 days individually in the crab box and given a label (tag) on each box as a marker of the treatment of the tested crab. Afterwards, each was placed on a bamboo raft with a float, to enhance floating on the surface of the pond water which had a depth of ±80 cm.

Experimental Feed

The feed used in this study was fermented artificial feed in form of pellets, as recommended by Aslamyah et al. (2021). The feedstuff and results of feed proximate analysis are shown in **Table 1**.

The stages of making feed begin with preparing feedstuff, including dry ingredients, fish silage, and fresh fish. Dry feed ingredients were ground into flour, except for the vitamin and mineral mix. The ingredients were weighed and mixed starting from the smallest to the largest amount, and stirred until homogeneous. Afterwards, the ingredients were inoculated with 10 mL 100 g⁻¹ of feedstuff microorganism mix, which had previously been diluted with water in a ratio of 1:3. The mixture was stirred evenly, stored in airtight plastic, and after 72 hours of incubation, opened and dried. Fish silage was prepared by removing fish scales, before washing and draining. The fish was grounded with a meat milling machine until smooth and placed in a closed bucket that was previously lined with a plastic bag. 1.5 L of formic acid was then added to 50 kg of fish grinder and stirred until evenly mixed. Stirring was performed 3 to 4 times daily until the 4th day, and on the 5th day, the water at the top was removed. Furthermore, the mixed microorganisms were inoculated with a dose of 10 mL 100 g⁻¹ of feedstuff and incubated for 7 days in a tightly closed and vacuum. Fresh fish was prepared by removing scales and washing thoroughly. The fish was then drained dried, and grounded until smooth.

Table 1 The composition (% dry matter) of feedstuff and results of feed proximate analysis

Composition	Percentage (%)
Fish flour	25
Fresh fish	20
Fish silage	10
Crab shell flour	5
Soy flour	8
Corn starch	10
Coconut meal flour	8
Pollard flour	10
Vitamin & mineral mix ¹	4
Amount	100
Water (%)	8.9
Ash (% dry weight)	13.49
Protein (% dry weight)	41.93
Fat (% dry weight)	7.43
Crude fiber (% dry weight)	7.82
NFE (% dry weight)	29.33
DE (kcal/kg) ²	2767.63
C/P (DE/g Protein)	6.76

¹ The composition of vitamins & minerals mix.

Every 10 kg contains 12,000,000 IU of Vitamin A; Vitamin D 2,000,000 IU; Vitamin E 8,000 IU; Vitamin K 2,000 mg; Vitamin B1 2,000 mg; Vitamin B2 5,000; Vitamin B6 500 mg; Vitamin B12 12,000 g; Ascorbic acid 25,000 mg; Calcium-D-Phanthothenate 6000 mg; Niacin 40,000 mg; Choline Chloride 10,000 mg; Methionine 30,000 mg; Lysine 30,000 mg; Manganese 120,000 mg; Iron 20,000 mg; Iodine 200 mg; Zinc 100,000 mg; Cobalt 200,000 mg; Copper 4,000 mg; Santoquin (antioxidant) 10,000 mg; Zinc bacitracin 21,000 mg.

² Calculation results based on the energy equation (NRC, 1988): 1 g carbohydrate = 2.5 kcal DE; 1 g protein = 3.5 kcal DE; 1 g fat = 8.1 kcal DE

Making feed was carried out by weighing all the ingredients used and mixing until homogeneous. The feed dough was printed using a pellet printer for crabs with a diameter of 1 cm and steamed for ± 40 minutes. Pellets were cut 2 cm long and dried in the sun. Furthermore, the dried feed was then cooled at room temperature or aerated, placed in a plastic bag and stored in a dry place, until use for the next test.

The artificial feed was coated with fermented herbal extracts that have been prepared previously. These fermented extracts were made up of three herbal extracts, including mulberry leaf extract (*Morus alba*), temulawak (*Curcuma xanthorrhiza*), and temu kunci (*Boesenbergia rotunda*), at equal concentrations, and fermented with *Lactobacillus casei* and *Saccharomyces cereviceae*. Approximately 1 mL of probiotic and 1 mL of molasses were added into distilled water containing 500 mg of herbal extracts of Stock FHE fermentation time: 1 month, and fermentation condition under room temperature. Furthermore, the dilution of stock FHE for each treatment was as follows: treatment A (100 mL of distilled water); treatment B (20 mL FHE stock + 80 mL distilled water); treatment C (60 mL FHE Stock + 40 mL distilled water); and treatment D (100 mL FHE stock; without distilled water). Feed was administered in the afternoon at a dose of 5% of body weight with a frequency of once every 2 days. Feed containing herbs were given at 1-day intervals compared to feed without herbs (control). The rest of the feed was then measured before the next feeding. The remaining feed was collected during the rearing period, dried, and weighed at the end of the study.

Visual observations were made daily to control the development of the test crabs. If a dead crab was found, it was recorded and weighed. If molting crabs were found, they were recorded and weighed, and placed back in the crab box for rearing until they reach the end of cultivation. Furthermore, observations of water quality including temperature, salinity, dissolved oxygen, and pH were carried out every day during cultivation, while ammonia was measured at the beginning and end of cultivation. The temperature was determined using a thermometer, salinity with a hand refractometer, dissolved oxygen with a DO meter, pH with a pH meter, and ammonia in the laboratory.

Growth performance

The growth involving absolute weight gain and specific growth rate. Absolute weight gain was measured using the formula:

$$WG = W_t - W_0$$

Where:

WG : absolute weight growth (g)

W_t : body weight (g)

W₀ : initial body weight (g).

Meanwhile, the specific growth rate was calculated using the formula:

$$SGR = \frac{\ln W_t - \ln W_0}{T} \times 100$$

Where:

SGR: **specific growth rate** (%)

W_t : average weight of crabs at the end of the study (g)

W₀ : average weight of crabs at the beginning of the study (g)

T : maintenance time in days.

Percentage of molting

The percentage of molting (MP) was measured by comparing the number of molting test crabs with live test crabs multiplied by 100.

Survival rate

The survival rate (SR) of mud crabs was calculated using the formula:

$$SR = \frac{N_t}{N_0} \times 100$$

Where:

SR : the survival rate (%)

N_t : the number of live crabs at the end of rearing (tails)

N₀ : the number of crabs at the beginning of rearing (tails).

Feed consumption

Feed consumption (FC) was calculated by determining the difference in the amount of feed given to the amount of feed remaining. This calculation was based on the dry weight of the feed.

Feed efficiency

Feed efficiency (FE) was calculated using the formula as proposed by Watanabe (1988):

$$FE = [(W_t + D) - W_0] / F \times 100$$

where:

FE : feed efficiency

W_t : the average weight of crabs at the end of the study (g)

W₀ : the average weight of crabs at the beginning (g)

D : the weight of crabs that died during the study (g)

F : the amount of feed consumed (g).

Condition Factor

The Condition factor (CF) was calculated using Fulton's condition factor formula, namely:

$$CF = 100 \times W / L^3$$

where:

CF : the condition factor

W : the weight of the test crab,

L : the width of the carapace of the test crab.

To determine the level of change in the condition factor of the test crab at the beginning of the study compared to the end, it was calculated using the formula:

$$CoCF = (CF_b - CF_a) / CF_a \times 100$$

where:

CoCF : the rate of change in condition factor (%)

CF_a : the initial condition factor

CF_b : the final condition factor.

Statistical analysis

Data were presented using tables and images. The effect of treatment on growth, feed consumption, feed efficiency, and condition factors were analyzed using Analysis of Variance (ANOVA). Further tests were carried out using the LSD test at a 90% confidence level. A difference of $p < 0.1$ is stated to be statistically significant. Furthermore, to analyze the molting and non-molting crab data, an independent sample t-test was used. The analysis was performed using Excel software and SPSS version 16.0.

Results

Feed consumption, feed efficiency, growth, and changes in condition factors

The results showed that the tested crabs consumed feed well, which contained herbal extracts. Although the molting percentage was without significant difference between treatments after 31 days of rearing, there were differences in crab responses in terms of feed consumption, feed efficiency, growth, and changes in condition factors. Overall, crabs have a high survival rate above 90% in **Table 2**.

Table 2 Description of crabs used at the beginning of the study, molting %, and survival rate.

TC	N	W0 (g)	CL0 (mm)	CW0 (mm)	CF0	MP (%)	SR (%)
A	60	106.88±11.46	58±4.3	81.28±4.5	19.88±2.13	16.67	93.33
B	60	106.7±12.11	58.72±3.3	81.05±3.9	20.27±2.62	21.67	91.67
C	60	107.13±12.40	57.73±3.0	80.62±3.0	20.42±2.12	10.00	95.00
D	60	107.05±12.11	58.47±3.7	80.48±5.1	21.06±6.15	15.00	96.67

Treatment Code (TC); 0 mg kg⁻¹ of feed (A); 200 mg kg⁻¹ of feed (B); 400 mg kg⁻¹ of feed (C); and 600 mg kg⁻¹ of feed (D); Number of juvenile (N); Initial Weight (W0); Initial Carapace Length (CL0); Initial Carapace Width (CW0); Initial Condition Factor (CF0); Molting Percentage (MP) and Survival Rate (SR).

Based on the independent sample t-test analysis, it was observed that there was a very significant difference between molting and non-molting crabs, both in terms of growth, condition factors, feed consumption and feed efficiency (**Figure 1**). Therefore, molting and non-molting crab data were separated to reduce the bias in data analysis. Changes in unique condition factors due to molting caused crabs to enlarge, however, conditional factors were decreased (**Tables 3 and 4**).

Table 3 Growth, feed consumption, and feed efficiency of molting crab

TC	WG (g)	CLG (mm)	CWG (mm)	SGR	CoCF (%)	FC (g)	FE (%)
A	43.73±13.30 ^a	8.7±3.6 ^a	12±5.4 ^a	12.9±1.25 ^a	-4.13±6.89 ^a	88.79±5.58 ^a	53.29±17.22 ^a
B	40.03±10.77 ^a	4.9±2.2 ^{ab}	8.6±2.2 ^{ab}	12.63±0.75 ^a	4.32±11.13 ^a	88.22±4.30 ^a	50.24±12.60 ^a
C	53.9±14.58 ^{ab}	9.4±2.2 ^{ac}	14.4±4.2 ^{ac}	13.51±0.73 ^a	-5.10±16.01 ^a	90.52±0.83 ^a	63.72±15.25 ^{ab}
D	60.73±11.41 ^{bc}	7.6±3.5 ^a	11.1±3.7 ^a	13.96±0.80 ^b	9.38±7.54 ^b	79.6±9.16 ^b	81.97±15.53 ^b

Different letters in the column are statistically different according to the LSD test ($p < 0.1$). Treatment Code (TC); 0 mg kg⁻¹ of feed (A); 200 mg kg⁻¹ of feed (B); 400 mg kg⁻¹ of feed (C); and 600 mg kg⁻¹ of feed (D); Weight gain (WG); Change of Carapace Length (CLG); Change of Carapace Width (CWG); Specific Growth Rate (SGR); Change of Condition Factor (CoCF); Feed Consumption (FC); Feed Efficiency (FE).

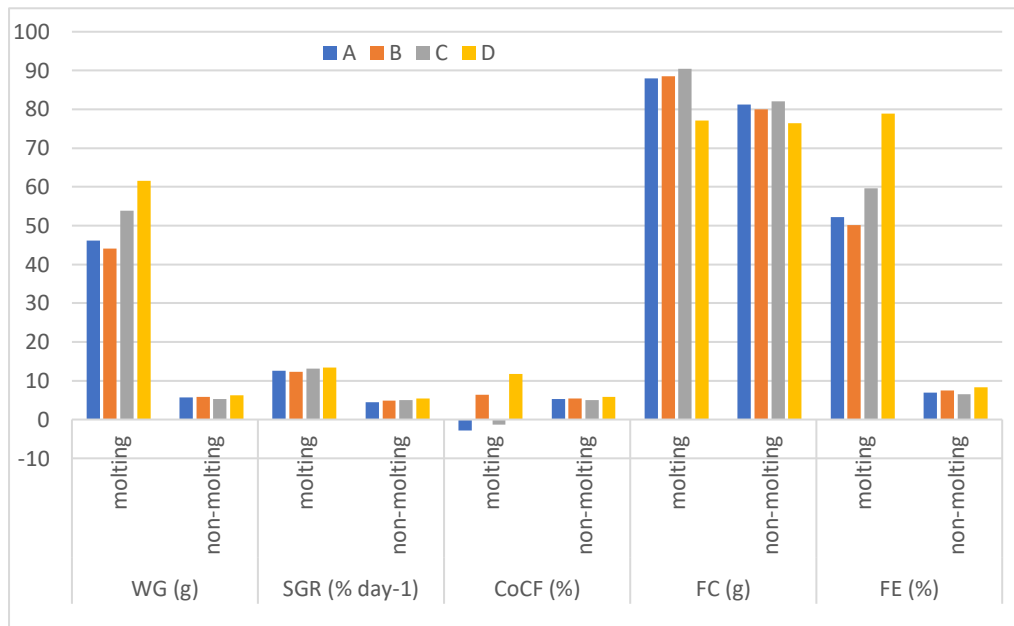


Figure 1 Comparison of growth, feed consumption, and feed efficiency of molting and non-molting crabs. $P < 0.05$ based on independent sample t-test; Treatment: 0 mg kg⁻¹ of feed (A), 200 mg kg⁻¹ of feed (B), 400 mg kg⁻¹ of feed (C), and 600 mg kg⁻¹ (D); Weight gain (WG); Specific Growth Rate (SGR), Change of Condition Factor (CoCF); Feed Consumption (FC); Feed Efficiency (FE).

Table 4 Growth, feed consumption and feed efficiency of non-molting crab

TC	WG (g)	SGR	CoCF (%)	FC (g)	FE (%)
A	5.44±2.11 ^a	4.3±1.32 ^a	5.03±2.09 ^a	81.57±2.80 ^a	6.61±2.74 ^a
B	5.93±1.28 ^a	4.95±0.56 ^a	5.22±1.76 ^a	88.87±20.95 ^{ab}	7.67±2.22 ^a
C	5.11±0.62 ^{ab}	4.8±0.51 ^a	4.81±0.69 ^a	81.95±2.04 ^a	6.26±0.94 ^{ab}
D	6.42±1.52 ^{ac}	5.52±0.65 ^b	5.96±1.37 ^a	76.71±7.12 ^{ac}	8.41±1.95 ^{ac}

Different letters in the column are statistically different according to the LSD test ($p < 0.1$). Treatment Code (TC); 0 mg kg⁻¹ of feed (A); 200 mg kg⁻¹ of feed (B); 400 mg kg⁻¹ of feed (C); and 600 mg kg⁻¹ of feed (D); Weight gain (WG); Specific Growth Rate (SGR); Change of Condition Factor (CoCF); Feed Consumption (FC); Feed Efficiency (FE).

Water quality

During the study, water quality was in fairly good condition, except for low dissolved oxygen in the morning. Temperatures ranged between 27–32 °C, salinity 28-35 ppt, pH 6.9–8.9, dissolved oxygen 2.1–5.6 ppm, and ammonia 0.002 ppm.

Discussion

In aquaculture, especially in crab farming, growth is very important, as it is related to time and maintenance. If growth is stimulated in a shorter period, farmers can harvest faster with lower maintenance costs, which enhances profitability. Functional feed additive treatments were derived from three herbal extracts, namely mulberry (*M. alba*) leaf extract, temulawak (*C. xanthorrhiza*) and temu kunci (*B. rotunda*) rhizome. These treatments obtained a positive effect in promoting growth and increasing feed efficiency. The results showed that the higher the dose of herbal extract was used, the higher the growth and feed efficiency. Furthermore, a dose of 600 mg of ethanol extract from a combination of three types of herbs per kg of feed obtained the highest growth and feed efficiency.

The results of this study also showed that the active compounds contained in the combination of extracts synergize to stimulate growth. Fujaya et al. (2018) stated that mulberry (*M. alba*) extract contains phytoecdysteroids, which are ecdysteroid mimic hormones naturally found in some plants (Klein, 2004; Dinan et al., 2009). Ecdysteroids in crustaceans and crabs are important hormones that control the molting process. This compound plays a role in activating protein synthesis by increasing mRNA synthesis (Preston-mafham & Dinan, 2002). This activity is also strengthened by the role of extracts of temulawak (*Curcuma xanthorrhiza*) and temu kunci (*Boesenbergia rotunda*) which are known to contain curcumin and essential oils. Both compounds act as antioxidants and increase appetite. This enables the supply of raw materials for crab nutrition to be fulfilled.

The bioactive phytochemicals and secondary metabolites of the mulberry extract include steroids, saponins, alkaloids, glycosides, and phenolic compounds including terpenoids, flavonoids, anthocyanins, and tannins. These bioactive compounds of mulberry extract exhibit outstanding anti-oxidative, anti-diabetic, anti-stress, nephroprotective, antimicrobial, anti-mutagenic, anticancer, anxiolytic, hepatoprotective, anthelmintic, antimicrobial, immunomodulatory effects and has cholesterol-lowering effects (Hussain et al., 2017). The anti-stress effect was significantly demonstrated in experiments on rats subjected to restraint. Chronic stress restraint will result in cognitive dysfunction, altered behavioral parameters, increased leukocyte count, levels of superoxide dismutase (SOD), lipid peroxidation (LPO), glucose and corticosterone, with concomitant decreases in catalase (CAT) activity, and glutathione reductase (GSH). Furthermore, gastric ulcers, adrenal glands and spleen were also used as stress indexes. All these restraint-induced disturbances were attenuated by ethyl acetate soluble (EASF) from *Morus alba* (Nade & Yadav, 2010). This effect was certainly very necessary for crabs in the cage or crab box.

The response of crabs to temulawak extract appears to be similar to that of other animals. According to Hosseini et al. (2016), *C. xanthorrhiza* (CX), known as temulawak, contains essential oils rich in phenols. The antioxidant activity of CXEO mainly refers to xanthorrhizol, arcurcumene, and curcumene. It has been widely documented that these phenolic compounds can enhance gut health resulting in better food absorption activities.

Similarly, temu kunci (*B. rotunda*) which is one of the main components of the herbal extract used in this study also contains essential oils. Chahyadi et al. (2014) also emphasized that the rhizome of *B. pandurata* contains essential oils and many flavonoid compounds which showed many interesting pharmacological characteristics, such as antifungal, antibacterial, and antioxidant. Eng-Chong et al. (2012) stated that *B. rotunda* has great medicinal potential, as nearly a hundred compounds were isolated from this plant which is also known as "Thai Ginseng". These compounds have many roles, including anti-microbial, anti-parasitic, antioxidant, and also inhibit the formation of biofilms by intestinal pathogens.

In this study, the condition factor was used to describe the level of obesity. There was a difference in the value of the condition factor in the test crabs. The initial condition factor of the crabs in this study varied between 14-54 based on the ratio of weight and width of the carapace. Furthermore, at the end of the study, the condition factor of crabs varied between 15-60, there were crabs whose condition factors increased while in some, this factor decreased. The decreasing condition factor occurred in crabs that have just molted.

The observations by Moslen & Miebaka (2018) showed that the variation in condition factors was caused by eating behavior, biological factors, and responses to the environment. A low condition factor value means the crabs are light for their length, an indication of low feeding intensity and the presence of spawning activity. A high K value is the assumption of high feeding intensity and a gradual increase in fat accumulation, which also indicates preparation for a new reproductive period (Suryandari et al., 2018).

In this study, the dynamics of conditional factors were influenced by molting activity. Crabs that had just molted had a lower condition factor than other crabs before molting. Therefore, crabs that had just molted were classified as thin, while crabs before molting were classified as fat. As the growth of crab weight was not always followed by the growth of the width or length of the carapace, the growth indicator using the condition factor value was significantly helpful. In portunid crabs, the molting process is divided into four main stages, namely intermolt, premolt, ecdysis and postmolt (Waiho et al., 2021). The intermolt is the stage after cuticle formation, and the exoskeleton has hardened after mineralization has fully occurred. Meanwhile, the intermolt stage is the crab muscle growth stage or the fattening stage. The premolt is the preparation stage for molting, consisting of the early, mid, and late premolt stages. In the premolt stage, a new exoskeleton begins to be formed under the old hard exoskeleton, while the new exoskeleton is retracted. This enables the old and new exoskeletons to separate with the help of enzymes. The process of releasing the crab from the old exoskeleton is called ecdysis, where the crab slowly pulls itself out of the old exoskeleton through a crack at the back of the carapace that borders the abdomen. The crabs that have just been released from the old exoskeleton have a soft shell which expands as more water is absorbed to stretch the new cuticle. At this time, the size of the crabs was enlarged but had a low condition factor resulting in the light weight and were classified thin.

There was a significantly different level of change in conditional factors between molting and non-molting crabs (**Table 3**). The existence of a minus value illustrates that the crab decreased in the condition factor after molting. This also showed that the crabs have molted at the end of the rearing period, therefore, they have not had time to grow to fill their enlarged carapace. According to Fujaya (2011), molting will naturally occur when body size increases due to growth. This phenomenon always occurs because the crab's body is wrapped by a rigid carapace and cannot develop following the development of body weight. Molting is also influenced by external and internal factors. External factors include light, temperature, food availability, tides, while internal factors include body size and hormones. Both of these factors influence the brain and stimulate the Y-organ to produce molting hormones.

The results of this study showed the importance of molting management in crab culture. Molting is not only important for the production of soft-shell crabs, but also for the rearing and cultivation of fattening crabs. Herbal extracts can be used to help stimulate molting, increase growth, and increase feed efficiency, thereby enables farmers to maximize profits in crab culture.

In conclusion, the best dose to stimulate growth, feed efficiency, and the level of fatness of mud crabs was 600 mg kg⁻¹ of feed, which was the highest dose applied. Therefore, further study is required to obtain optimal doses of herbal extracts (*M. alba*, *C. xanthorrhiza*, and *B. rotunda*) in stimulating the growth and molting of cultured mud crabs.

Acknowledgements

The authors are grateful to the Hasanuddin University Research and Community Service Institute for funding this study through the Unhas Applied Research Grant for the 2020 fiscal year with the contract number 1585/UN4.22/PT.OI.03/2020.

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Best Regards
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Utilization of Feed and Growth Performance of Mud Crabs: The Effect of Herbal Extracts as Functional Feed Additives

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Keywords: Crab, growth, herbal extracts, molting, feed utilization

Abstract

This study aimed to analyze the effect of herbal extracts as functional feed additives on the utilization of feed and growth performance of orange mud crabs (*Scylla olivacea*). The test animals used were 60 crabs for each treatment dose. The crabs are kept individually in a crab box which was placed on a floating bamboo raft. This was to enable floating on the surface of the pond water which had a depth of ± 80 cm. The treatments tested were four doses of a combination herbal extract from *Morus alba*, *Curcuma xanthorrhiza*, and *Boesenbergia rotunda*, namely 0, 200, 400, 600 mg kg⁻¹ of feed. Formulated feed with the protein content of 41.93%, fat 7.43%, Nitrogen Free Extract (NFE) 29.33%, and crude fiber 7.82% was given at a dose of 5% body weight every day. Based on one-way ANOVA, the results showed that the dose of herbal extracts influences the feed utilization and growth performance of mud crabs. The dose of herbal extract 600 mg kg⁻¹ of feed provided higher feed utilization and growth performance (LSD Test: $P < 0.1$). Significant differences were found between feed utilization and growth performance of mud crabs after molting and non-molting. In molting mud crabs, treatment with herbal extract 600 mg kg⁻¹ obtained an average weight gain of ± 60.73 from the initial weight, compared to control crabs (without herbal extracts) gaining an average weight gain of $\pm 43.73\%$. However, in non-molting crabs, ± 5.44 and ± 6.42 weight gain was observed, respectively. Significant differences also occurred between treatments for specific growth rates, feed consumption, feed efficiency, and condition factors. These results provided information that herbal extracts are potentially as feed additives to increase feed utilization and growth performance, as well as stimulate molting in crab cultivation.

Introduction

Mud crab is one of the crab species with high economic value worldwide which are generally marketed alive, while some are sold as frozen or cooked products. There are four species of mud crab that are the focus of commercial fisheries and aquaculture production, namely *Scylla serrata*, *S. tranquebarica*, *S. paramamosain* and *S. olivacea*. These are in great demand in both export and domestic markets with prices ranging from 8 to 25 USD depending on the size and season (Lalramchhani et al., 2019). Prices are also influenced by the shape of the product such as soft-shell crab (Fujaya et al., 2020). Waiho et al. (2021) stated that soft-shell crab products are one of the crab culture products with world market demand that continues to increase at high prices. Currently, there are three different culture systems including crab fattening, grow-out, and soft-shell crab production in practice that target different tiers of the global crab market (Rahman et al. 2020). In Indonesia, grow out and fattening are the most widely practiced methods of crab cultivation, and both are generally carried out in earthen ponds. Often, there are no special innovations in the cultivation other than feeding crabs with trash fish, chicken intestines, and others. In recent years, crab farmers have also started producing soft-shell crabs, either using horizontal farming methods with bamboo and HDPE boxes, or vertical farming.

When it comes to rearing, fattening, and producing soft-shell crabs, growth is the main focus during the rearing period. If growth does not occur optimally, aquaculture as part of economic activity will suffer losses, as crab growth is affected by molting. Molting and growth in decapoda crustaceans such as crabs, lobsters, shrimps cannot be separated, and they influence each other. Molting is a precursor to growth and vice-versa. Therefore, various efforts have been made to stimulate growth and molting in crab culture, such as limb autotomy, eyestalk ablation, ecdysteroid and phytoecdysteroid application, control of physical parameters such as temperature and salinity, biogenic amines application, methyl farnesoate application, and molt-inhibiting hormone (MIH) inhibition (Waiho et al., 2021).

The use of herbs in the aquaculture industry is expected to be friendlier to the environment and cultivated animals. According to Bharati et al. (2019), increasing aquaculture growth cannot be separated from the development of quality feed, which requires appropriate and essential feed additives. Furthermore, the development of these additives shows promising results for aquaculture. These do not only improve fish growth performance but also their health performance. These functional feed additives originate from a variety of organic sources and are environmentally friendly for fish and the environment. Examples of these additives that have been developed include prebiotics, probiotics, seaweed, fungi, microalgae, enzymes, organic acids, mycotoxin binders, photogenic or Phyto biotic compounds, and yeast.

Herbal extracts are one of the promising natural medicines that are used as functional feed additives to stimulate growth and molting in crabs. Herbal extracts generally contain terpenoids, alkaloids, saponins, flavonoids which act as anti-oxidants, anti-bacterial, immune-nutrition (Singh et al., 2013). The phytoecdysteroids are present in several plants, and one of their roles is to stimulate protein synthesis (Klein, 2004). One of them is the mulberry plant (*Morus alba*). The ecdysteroids contained in *Morus alba* are of the 20-hydroxyecdysone group. At present, mulberry leaf extract has been successfully tried as growth and molting stimulant for mud crabs with an optimal dose of 2.4 mg g⁻¹ feed or 240 mg kg⁻¹ feed (Fujaya et al., 2018). In *Morus* spp. a wide range of phytochemicals are present in leaves, fruit, roots, and wood. Therefore, *Morus* spp. has various biological functions (antidiabetic, antiobesity, anti-inflammatory, anticancer, antibacterial, antioxidant, antiviral, cytoprotective and neuroprotective etc.). This plant is used traditionally for the development of different food products as well as the treatment of various diseases (Dhiman et al., 2020). The content of phenolic compounds, especially flavonoids and anthocyanins, causes *Morus alba* to have strong antioxidant activity, thereby potentially eliminating free radical production and cellular damage caused by free radicals (Hussain et al., 2017).

To complement the function of mulberry leaf extract (*Morus alba*), as a growth and molting stimulant, as well as an herb to maintain crab health. Added extracts of temulawak

(*Curcuma xanthorrhiza*) and temu kunci (*Boesenbergia rotunda*), which are known to have various physiological functions, including appetite stimulant, hepatoprotector, antimicrobial, anti-inflammatory, analgesic, antipyretic, chloretic, and others. The latter is used as a "herbal" to improve intestinal health (Eng-Chong et al., 2012), and medicinal plants containing curcumin, xanthorrhizol, and pinostrobin (Qomaladewi & Cho, 2021)

This study examined the response of mud crabs to the various doses of mixed herbal extracts (combination of *Morus alba*, *Curcuma xanthorrhiza*, and *Boesenbergia rotunda*) as a functional feed additive on growth, molting, feed consumption, feed efficiency, condition factor and survival of mud crabs. The three medicinal plants are expected to synergize to improve the health and growth of crabs through the active compounds they contain. Furthermore, this combination of herbal extracts is expected to be one of the natural medicines that can be applied to juvenile crabs for growing out, fattening, and production of soft-shell crabs.

Materials and Methods

Experimental crab

The study was carried out at the Educational Farm of Hasanuddin University, Bojo Village, Mallusetasi District, Barru Regency. There were 240 mud crabs (*Scylla olivaceae*) with a bodyweight of 107.08 ± 11.93 g and carapace width of 80.91 ± 4.22 mm and were obtained from local fishermen's captures. Furthermore, weighing was carried out using an electric scale with an accuracy of 0.1 g and measuring the width of the carapace using a caliper with an accuracy of 0.1 mm as initial data. All crabs were then divided into 4 treatments and 6 replicates. There were four dosages of herbal extract, namely 0 mg kg⁻¹ of feed (A), 200 mg kg⁻¹ of feed (B), 400 mg kg⁻¹ of feed (C), and 600 mg kg⁻¹ of feed (D). Each dosage treatment consisted of 60 crabs, while each replicate consisted of 10 crabs.

The container used in this study was a crab box with a length, width, and height of 30 × 20 × 15 cm. The test animals were kept for 31 days individually in the crab box and given a label (tag) on each box as a marker of the treatment of the tested crab. Afterwards, each was placed on a bamboo raft with a float, to enhance floating on the surface of the pond water which had a depth of ±80 cm.

Experimental Feed

The feed used in this study was fermented artificial feed in form of pellets, as recommended by Aslamyah et al. (2021). The feedstuff and results of feed proximate analysis are shown in **Table 1**.

The stages of making feed begin with preparing feedstuff, including dry ingredients, fish silage, and fresh fish. Dry feed ingredients were ground into flour, except for the vitamin and mineral mix. The ingredients were weighed and mixed starting from the smallest to the largest amount, and stirred until homogeneous. Afterwards, the ingredients were inoculated with 10 mL 100 g⁻¹ of feedstuff microorganism mix, which had previously been diluted with water in a ratio of 1:3. The mixture was stirred evenly, stored in airtight plastic, and after 72 hours of incubation, opened and dried. Fish silage was prepared by removing fish scales, before washing and draining. The fish was grounded with a meat milling machine until smooth and placed in a closed bucket that was previously lined with a plastic bag. 1.5 L of formic acid was then added to 50 kg of fish grinder and stirred until evenly mixed. Stirring was performed 3 to 4 times daily until the 4th day, and on the 5th day, the water at the top was removed. Furthermore, the mixed microorganisms were inoculated with a dose of 10 mL 100 g⁻¹ of feedstuff and incubated for 7 days in a tightly closed and vacuum. Fresh fish was prepared by removing scales and washing thoroughly. The fish was then drained dried, and grounded until smooth.

Table 1 The composition (% dry matter) of feedstuff and results of feed proximate analysis

Composition	Percentage (%)
Fish flour	25
Fresh fish	20
Fish silage	10
Crab shell flour	5
Soy flour	8
Corn starch	10
Coconut meal flour	8
Pollard flour	10
Vitamin & mineral mix ¹	4
Amount	100
Water (%)	8.9
Ash (% dry weight)	13.49
Protein (% dry weight)	41.93
Fat (% dry weight)	7.43
Crude fiber (% dry weight)	7.82
NFE (% dry weight)	29.33
DE (kcal/kg) ²	2767.63
C/P (DE/g Protein)	6.76

¹ The composition of vitamins & minerals mix.

Every 10 kg contains 12,000,000 IU of Vitamin A; Vitamin D 2,000,000 IU; Vitamin E 8,000 IU; Vitamin K 2,000 mg; Vitamin B1 2,000 mg; Vitamin B2 5,000; Vitamin B6 500 mg; Vitamin B12 12,000 g; Ascorbic acid 25,000 mg; Calcium-D-Phantothenate 6000 mg; Niacin 40,000 mg; Choline Chloride 10,000 mg; Methionine 30,000 mg; Lysine 30,000 mg; Manganese 120,000 mg; Iron 20,000 mg; Iodine 200 mg; Zinc 100,000 mg; Cobalt 200,000 mg; Copper 4,000 mg; Santoquin (antioxidant) 10,000 mg; Zinc bacitracin 21,000 mg.

² Calculation results based on the energy equation (NRC, 1988): 1 g carbohydrate = 2.5 kcal DE; 1 g protein = 3.5 kcal DE; 1 g fat = 8.1 kcal DE

Making feed was carried out by weighing all the ingredients used and mixing until homogeneous. The feed dough was printed using a pellet printer for crabs with a diameter of 1 cm and steamed for ± 40 minutes. Pellets were cut 2 cm long and dried in the sun. Furthermore, the dried feed was then cooled at room temperature or aerated, placed in a plastic bag and stored in a dry place, until use for the next test.

The artificial feed was coated with fermented herbal extracts that have been prepared previously. These fermented extracts were made up of three herbal extracts, including mulberry leaf extract (*Morus alba*), temulawak (*Curcuma xanthorrhiza*), and temu kunci (*Boesenbergia rotunda*), at equal concentrations, and fermented with *Lactobacillus casei* and *Saccharomyces cereviceae*. Approximately 1 mL of probiotic and 1 mL of molasses were added into distilled water containing 500 mg of herbal extracts of Stock FHE fermentation time: 1 month, and fermentation condition under room temperature. Furthermore, the dilution of stock FHE for each treatment was as follows: treatment A (100 mL of distilled water); treatment B (20 mL FHE stock + 80 mL distilled water); treatment C (60 mL FHE Stock + 40 mL distilled water); and treatment D (100 mL FHE stock; without distilled water). Feed was administered in the afternoon at a dose of 5% of body weight with a frequency of once every 2 days. Feed containing herbs were given at 1-day intervals compared to feed without herbs (control). The rest of the feed was then measured before the next feeding. The remaining feed was collected during the rearing period, dried, and weighed at the end of the study.

Visual observations were made daily to control the development of the test crabs. If a dead crab was found, it was recorded and weighed. If molting crabs were found, they were recorded and weighed, and placed back in the crab box for rearing until they reach the end of cultivation. Furthermore, observations of water quality including temperature, salinity, dissolved oxygen, and pH were carried out every day during cultivation, while ammonia was measured at the beginning and end of cultivation. The temperature was determined using a thermometer, salinity with a hand refractometer, dissolved oxygen with a DO meter, pH with a pH meter, and ammonia in the laboratory.

Growth performance

The growth involving absolute weight gain and specific growth rate. Absolute weight gain was measured using the formula:

$$WG = W_t - W_0$$

Where:

WG : absolute weight growth (g)

W_t : body weight (g)

W₀ : initial body weight (g).

Meanwhile, the specific growth rate was calculated using the formula:

$$SGR = \frac{\ln W_t - \ln W_0}{T} \times 100$$

Where:

SGR: specific growth rate (%)

W_t : average weight of crabs at the end of the study (g)

W₀ : average weight of crabs at the beginning of the study (g)

T : maintenance time in days.

Percentage of molting

The percentage of molting (MP) was measured by comparing the number of molting test crabs with live test crabs multiplied by 100.

Survival rate

The survival rate (SR) of mud crabs was calculated using the formula:

$$SR = \frac{N_t}{N_0} \times 100$$

Where:

SR : the survival rate (%)

N_t : the number of live crabs at the end of rearing (tails)

N₀ : the number of crabs at the beginning of rearing (tails).

Feed consumption

Feed consumption (FC) was calculated by determining the difference in the amount of feed given to the amount of feed remaining. This calculation was based on the dry weight of the feed.

Feed efficiency

Feed efficiency (FE) was calculated using the formula as proposed by Watanabe (1988):

$$FE = [(W_t + D) - W_0] / F \times 100$$

where:

FE : feed efficiency

W_t : the average weight of crabs at the end of the study (g)

W₀ : the average weight of crabs at the beginning (g)

D : the weight of crabs that died during the study (g)

F : the amount of feed consumed (g).

Condition Factor

The Condition factor (CF) was calculated using Fulton's condition factor formula, namely:

$$CF = 100 \times W / L^3$$

where:

CF : the condition factor

W : the weight of the test crab,

L : the width of the carapace of the test crab.

To determine the level of change in the condition factor of the test crab at the beginning of the study compared to the end, it was calculated using the formula:

$$CoCF = (CF_b - CF_a) / CF_a \times 100$$

where:

CoCF : the rate of change in condition factor (%)

CF_a : the initial condition factor

CF_b : the final condition factor.

Statistical analysis

Data were presented using tables and images. The effect of treatment on growth, feed consumption, feed efficiency, and condition factors were analyzed using Analysis of Variance (ANOVA). Further tests were carried out using the LSD test at a 90% confidence level. A difference of $p < 0.1$ is stated to be statistically significant. Furthermore, to analyze the molting and non-molting crab data, an independent sample t-test was used. The analysis was performed using Excel software and SPSS version 16.0.

Results

Feed consumption, feed efficiency, growth, and changes in condition factors

The results showed that the tested crabs consumed feed well, which contained herbal extracts. Although the molting percentage was without significant difference between treatments after 31 days of rearing, there were differences in crab responses in terms of feed consumption, feed efficiency, growth, and changes in condition factors. Overall, crabs have a high survival rate above 90% in **Table 2**.

Table 2 Description of crabs used at the beginning of the study, molting %, and survival rate.

TC	N	W0 (g)	CL0 (mm)	CW0 (mm)	CF0	MP (%)	SR (%)
A	60	106.88±11.46	58±4.3	81.28±4.5	19.88±2.13	16.67	93.33
B	60	106.7±12.11	58.72±3.3	81.05±3.9	20.27±2.62	21.67	91.67
C	60	107.13±12.40	57.73±3.0	80.62±3.0	20.42±2.12	10.00	95.00
D	60	107.05±12.11	58.47±3.7	80.48±5.1	21.06±6.15	15.00	96.67

Treatment Code (TC); 0 mg kg⁻¹ of feed (A); 200 mg kg⁻¹ of feed (B); 400 mg kg⁻¹ of feed (C); and 600 mg kg⁻¹ of feed (D); Number of juvenile (N); Initial Weight (W0); Initial Carapace Length (CL0); Initial Carapace Width (CW0); Initial Condition Factor (CF0); Molting Percentage (MP) and Survival Rate (SR).

Based on the independent sample t-test analysis, it was observed that there was a very significant difference between molting and non-molting crabs, both in terms of growth, condition factors, feed consumption and feed efficiency (**Figure 1**). Therefore, molting and non-molting crab data were separated to reduce the bias in data analysis. Changes in unique condition factors due to molting caused crabs to enlarge, however, conditional factors were decreased (**Tables 3 and 4**).

Table 3 Growth, feed consumption, and feed efficiency of molting crab

TC	WG (g)	CLG (mm)	CWG (mm)	SGR	CoCF (%)	FC (g)	FE (%)
A	43.73±13.30 ^a	8.7±3.6 ^a	12±5.4 ^a	12.9±1.25 ^a	-4.13±6.89 ^a	88.79±5.58 ^a	53.29±17.22 ^a
B	40.03±10.77 ^a	4.9±2.2 ^{ab}	8.6±2.2 ^{ab}	12.63±0.75 ^a	4.32±11.13 ^a	88.22±4.30 ^a	50.24±12.60 ^a
C	53.9±14.58 ^{ab}	9.4±2.2 ^{ac}	14.4±4.2 ^{ac}	13.51±0.73 ^a	-5.10±16.01 ^a	90.52±0.83 ^a	63.72±15.25 ^{ab}
D	60.73±11.41 ^{bc}	7.6±3.5 ^a	11.1±3.7 ^a	13.96±0.80 ^b	9.38±7.54 ^b	79.6±9.16 ^b	81.97±15.53 ^b

Different letters in the column are statistically different according to the LSD test ($p < 0.1$). Treatment Code (TC); 0 mg kg⁻¹ of feed (A); 200 mg kg⁻¹ of feed (B); 400 mg kg⁻¹ of feed (C); and 600 mg kg⁻¹ of feed (D); Weight gain (WG); Change of Carapace Length (CLG); Change of Carapace Width (CWG); Specific Growth Rate (SGR); Change of Condition Factor (CoCF); Feed Consumption (FC); Feed Efficiency (FE).

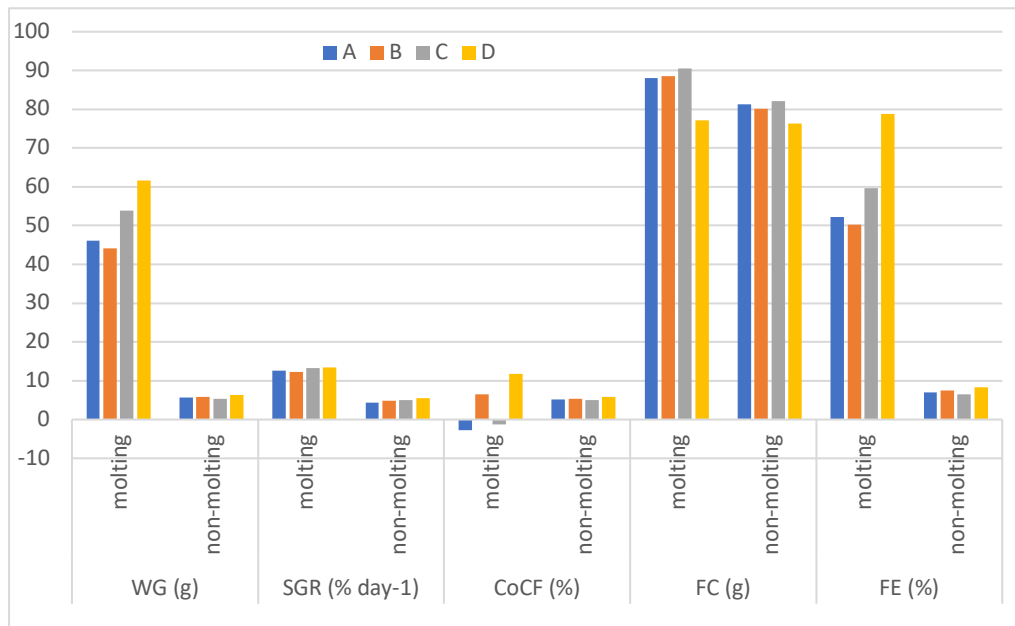


Figure 1 Comparison of growth, feed consumption, and feed efficiency of molting and non-molting crabs. $P < 0.05$ based on independent sample t-test; Treatment: 0 mg kg⁻¹ of feed (A), 200 mg kg⁻¹ of feed (B), 400 mg kg⁻¹ of feed (C), and 600 mg kg⁻¹ of feed (D); Weight gain (WG); Specific Growth Rate (SGR), Change of Condition Factor (CoCF); Feed Consumption (FC); Feed Efficiency (FE).

Table 4 Growth, feed consumption and feed efficiency of non-molting crab

TC	WG (g)	SGR	CoCF (%)	FC (g)	FE (%)
A	5.44±2.11 ^a	4.3±1.32 ^a	5.03±2.09 ^a	81.57±2.80 ^a	6.61±2.74 ^a
B	5.93±1.28 ^a	4.95±0.56 ^a	5.22±1.76 ^a	88.87±20.95 ^{ab}	7.67±2.22 ^a
C	5.11±0.62 ^{ab}	4.8±0.51 ^a	4.81±0.69 ^a	81.95±2.04 ^a	6.26±0.94 ^{ab}
D	6.42±1.52 ^{ac}	5.52±0.65 ^b	5.96±1.37 ^a	76.71±7.12 ^{ac}	8.41±1.95 ^{ac}

Different letters in the column are statistically different according to the LSD test ($p < 0.1$). Treatment Code (TC); 0 mg kg⁻¹ of feed (A); 200 mg kg⁻¹ of feed (B); 400 mg kg⁻¹ of feed (C); and 600 mg kg⁻¹ of feed (D); Weight gain (WG); Specific Growth Rate (SGR); Change of Condition Factor (CoCF); Feed Consumption (FC); Feed Efficiency (FE).

Water quality

During the study, water quality was in fairly good condition, except for low dissolved oxygen in the morning. Temperatures ranged between 27–32 °C, salinity 28-35 ppt, pH 6.9–8.9, dissolved oxygen 2.1–5.6 ppm, and ammonia 0.002 ppm.

Discussion

In aquaculture, especially in crab farming, growth is very important, as it is related to time and maintenance. If growth is stimulated in a shorter period, farmers can harvest faster with lower maintenance costs, which enhances profitability. Functional feed additive treatments were derived from three herbal extracts, namely mulberry (*M. alba*) leaf extract, temulawak (*C. xanthorrhiza*) and temu kunci (*B. rotunda*) rhizome. These treatments obtained a positive effect in promoting growth and increasing feed efficiency. The results showed that the higher the dose of herbal extract was used, the higher the growth and feed efficiency. Furthermore, a dose of 600 mg of ethanol extract from a combination of three types of herbs per kg of feed obtained the highest growth and feed efficiency.

The results of this study also showed that the active compounds contained in the combination of extracts synergize to stimulate growth. Fujaya et al. (2018) stated that mulberry (*M. alba*) extract contains phytoecdysteroids, which are ecdysteroid mimic hormones naturally found in some plants (Klein, 2004; Dinan et al., 2009). Ecdysteroids in crustaceans and crabs are important hormones that control the molting process. This compound plays a role in activating protein synthesis by increasing mRNA synthesis (Preston-mafham & Dinan, 2002). This activity is also strengthened by the role of extracts of temulawak (*Curcuma xanthorrhiza*) and temu kunci (*Boesenbergia rotunda*) which are known to contain curcumin and essential oils. Both compounds act as antioxidants and increase appetite. This enables the supply of raw materials for crab nutrition to be fulfilled.

The bioactive phytochemicals and secondary metabolites of the mulberry extract include steroids, saponins, alkaloids, glycosides, and phenolic compounds including terpenoids, flavonoids, anthocyanins, and tannins. These bioactive compounds of mulberry extract exhibit outstanding anti-oxidative, anti-diabetic, anti-stress, nephroprotective, antimicrobial, anti-mutagenic, anticancer, anxiolytic, hepatoprotective, anthelmintic, antimicrobial, immunomodulatory effects and has cholesterol-lowering effects (Hussain et al., 2017). The anti-stress effect was significantly demonstrated in experiments on rats subjected to restraint. Chronic stress restraint will result in cognitive dysfunction, altered behavioral parameters, increased leukocyte count, levels of superoxide dismutase (SOD), lipid peroxidation (LPO), glucose and corticosterone, with concomitant decreases in catalase (CAT) activity, and glutathione reductase (GSH). Furthermore, gastric ulcers, adrenal glands and spleen were also used as stress indexes. All these restraint-induced disturbances were attenuated by ethyl acetate soluble (EASF) from *Morus alba* (Nade & Yadav, 2010). This effect was certainly very necessary for crabs in the cage or crab box.

The response of crabs to temulawak extract appears to be similar to that of other animals. According to Hosseini et al. (2016), *C. xanthorrhiza* (CX), known as temulawak, contains essential oils rich in phenols. The antioxidant activity of CXEO mainly refers to xanthorrhizol, arcurcumene, and curcumene. It has been widely documented that these phenolic compounds can enhance gut health resulting in better food absorption activities.

Similarly, temu kunci (*B. rotunda*) which is one of the main components of the herbal extract used in this study also contains essential oils. Chahyadi et al. (2014) also emphasized that the rhizome of *B. pandurata* contains essential oils and many flavonoid compounds which showed many interesting pharmacological characteristics, such as antifungal, antibacterial, and antioxidant. Eng-Chong et al. (2012) stated that *B. rotunda* has great medicinal potential, as nearly a hundred compounds were isolated from this plant which is also known as "Thai Ginseng". These compounds have many roles, including anti-microbial, anti-parasitic, antioxidant, and also inhibit the formation of biofilms by intestinal pathogens.

In this study, the condition factor was used to describe the level of obesity. There was a difference in the value of the condition factor in the test crabs. The initial condition factor of the crabs in this study varied between 14-54 based on the ratio of weight and width of the carapace. Furthermore, at the end of the study, the condition factor of crabs varied between 15-60, there were crabs whose condition factors increased while in some, this factor decreased. The decreasing condition factor occurred in crabs that have just molted.

The observations by Moslen & Miebaka (2018) showed that the variation in condition factors was caused by eating behavior, biological factors, and responses to the environment. A low condition factor value means the crabs are light for their length, an indication of low feeding intensity and the presence of spawning activity. A high K value is the assumption of high feeding intensity and a gradual increase in fat accumulation, which also indicates preparation for a new reproductive period (Suryandari et al., 2018).

In this study, the dynamics of conditional factors were influenced by molting activity. Crabs that had just molted had a lower condition factor than other crabs before molting. Therefore, crabs that had just molted were classified as thin, while crabs before molting were classified as fat. As the growth of crab weight was not always followed by the growth of the width or length of the carapace, the growth indicator using the condition factor value was significantly helpful. In portunid crabs, the molting process is divided into four main stages, namely intermolt, premolt, ecdysis and postmolt (Waiho et al., 2021). The intermolt is the stage after cuticle formation, and the exoskeleton has hardened after mineralization has fully occurred. Meanwhile, the intermolt stage is the crab muscle growth stage or the fattening stage. The premolt is the preparation stage for molting, consisting of the early, mid, and late premolt stages. In the premolt stage, a new exoskeleton begins to be formed under the old hard exoskeleton, while the new exoskeleton is retracted. This enables the old and new exoskeletons to separate with the help of enzymes. The process of releasing the crab from the old exoskeleton is called ecdysis, where the crab slowly pulls itself out of the old exoskeleton through a crack at the back of the carapace that borders the abdomen. The crabs that have just been released from the old exoskeleton have a soft shell which expands as more water is absorbed to stretch the new cuticle. At this time, the size of the crabs was enlarged but had a low condition factor resulting in the light weight and were classified thin.

There was a significantly different level of change in conditional factors between molting and non-molting crabs (Table 3). The existence of a minus value illustrates that the crab decreased in the condition factor after molting. This also showed that the crabs have molted at the end of the rearing period, therefore, they have not had time to grow to fill their enlarged carapace. According to Fujaya (2011), molting will naturally occur when body size increases due to growth. This phenomenon always occurs because the crab's body is wrapped by a rigid carapace and cannot develop following the development of body weight. Molting is also influenced by external and internal factors. External factors include light, temperature, food availability, tides, while internal factors include body size and hormones. Both of these factors influence the brain and stimulate the Y-organ to produce molting hormones.

The results of this study showed the importance of molting management in crab culture. Molting is not only important for the production of soft-shell crabs, but also for the rearing and cultivation of fattening crabs. Herbal extracts can be used to help stimulate molting, increase growth, and increase feed efficiency, thereby enables farmers to maximize profits in crab culture.

In conclusion, the best dose to stimulate growth, feed efficiency, and the level of fatness of mud crabs was 600 mg kg⁻¹ of feed, which was the highest dose applied. Therefore, further study is required to obtain optimal doses of herbal extracts (*M. alba*, *C. xanthorrhiza*, and *B. rotunda*) in stimulating the growth and molting of cultured mud crabs.

Acknowledgements

The authors are grateful to the Hasanuddin University Research and Community Service Institute for funding this study through the Unhas Applied Research Grant for the 2020 fiscal year with the contract number 1585/UN4.22/PT.OI.03/2020.

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