Mealworm (*Tenebrio molitor*) as Calcium, Phosphor, and Chitosan Source

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ABSTRACT

The study were conducted in two experiments: the first experiment aimed to analyzed feed (cassava meal and pollard) and age (2 and 3 months larvae, beetle) effect on Ca and P content in larvae and beetle of *Tenebrio molitor* using 2x3 Factorial experiment with 3 replications. The second experiment aimed to analyzed *T. molitor* larvae age (2,3,4 months) effect on chitosan rendement and quality using Completely Randomized Design with 3 replications. The first experiment showed that age interacted with feed to affect moisture and ash contents. The highest water content was in 3 months larvae fed with cassava meal (62.28%), while the highest ash content was in 3 months larvae fed with pollard (2.01%). Age significantly affected Ca content which were highest in beetle (0.47%). The second experiment showed that age had significant effect on skin and chitin rendement, but not on chitosan rendement, moisture, ash, nitrogen, ash content, and deacetylation degree of chitosan. Three and four months larvae had the highest chitin rendement, but deacetylation degree of chitosan of 2 months larvae was far from the minimum standard value. Two months mealworm larvae is potential to be developed as chitin source and the beetle as Ca, and P source.

Key Words: Calcium, Phosphor, Chitosan, Deacetylation Degree, *Tenebrio Molitor*

INTRODUCTION

Calcium and phosphor are minerals needed for human and animal bone, while chitosan can be used as functional substance in food, cosmetic, and medicine.The three substances obtainable from mealworm (*T. molitor* L.). Mealworm is an insect that recently has been cultured and its meal is utilized as a feedstuff for poultry due to its high protein content. Beside protein, mealworm has high calcium (Ca) and phosphor (P) contents thus it is potentially source of Ca and P. Other livestock products are common source of only one of these two minerals, i.e. milk for Ca and egg for P. Mealworm is also potential as a source of chitin and chitosan for its external skeleton is built with these substances.

The study aimed to analyze effect of (1) feeds and age on larvae's and beetle's Ca and P content, (2) larval age on rendement and chitosan quality.

MATERIALS AND METHODS

The study was conducted in two experiments. The first experiment used 0.2 kg *T. molitor* larvae aged one month with 1.5 cm body length to be analyzed for its water, ash, Ca, and P content and 1.8 kg were divided into the 18 experiment units. Nine units were given 300 g mixed feeding 75% pollard and 25% concentrate, the other nine with 75% cassava meal and 25% concentrate. Table 1 showed the nutrient content of the feeds.

Table 1. Nutrient content of the feeds

Feed	Nutrient content (% dry matter)							
	Moisture	Crude lipid	Crude fiber	Crude protein	BETN	Ash	Ca	P
75% pollard+25% concentrate	11.36	5.23	5.64	18.73	54.47	4.57	0.33	0.59
75% cassava meal+25% concentrate	13.15	2.33	8.18	8.77	65.11	2.33	0.54	0.38

The feeds were replaced every ten days. Moisture, ash, Ca, and P body contents as observed variables were analyzed when the larvae reached the age of 2, 3 months and more than 3 months or beetle phase.

The 2x3 Factorial experiment was used with feed as A factor (pollard and cassava meal) and age as B factor (2 months, 3 months, and more than 3 months/beetle) with three replications. The data were analyzed by Least Square Means (LSM) for significant main effect and Least Significant Differences (LSD) for interaction at $\alpha = 0.05$ using SAS 6.12 program.

The second experiment used 3 kg *T. molitor* larvae, each kg aged 2, 3, and 4 months, which were steamed, pressed, sun dried, then grinded into meal. Chitin was extracted through deproteinase and demineralization, then chitosan from the chitin through deacetylation (Suptijah *et al.*, 1992). The observed variables included skin, chitin and chitosan rendement, chitosan color compared to shrimp chitosan, chitosan water, ash, and protein contents (AOAC, 1995), and deacetylation degree (Suptijah *et al.*, 1992).

The Completely Randomized Design was used in the second experiment with age as treatment (2, 3, and 4 months) and each treatment level had 3 replication. The data were analyzed by ANOVA and continued with Duncan's multiple range test at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Effect of Feed and Age on *T. molitor* Body Moisture, Ash, Ca, and P Content

Interaction of feed and age affected body moisture ($p<0.01$) and ash ($p<0.05$) content, while age affected Ca content (Table 2).

Moisture content increased with larval age but decreased at beetle phase in both feeds. Moisture content of 3 months larvae fed with cassava meal was the highest and vice versa in the beetle ($p<0.05$). Weight gain in 3 months larvae in both feed associated with substantial increased in body moisture content. When larvae reached the age of 3 months, it ready to enter pupa phase and did not eat anything. *T. molitor* pupa was not covered or naked, so moisture evaporation was high in this phase. To anticipate this, 3 months larvae, even though decreased its feed consumption to decrease metabolism, used the feeds more efficiently hence the increase body moisture content.

Moisture content in beetle was the lowest due to the measurement technique that was calculated by subtraction of fresh and dried larvae or beetle body weight. Larvae with thinner exoskeleton had lighter body weight than beetle with hard and thicker exoskeleton (Wigglesworth, 1972).

Ash content also increased with larval age and decreased at beetle phase in both feeds ($p<0.05$). Ash content in beetle was lower due to its lower need for metabolism (Wigglesworth,1972). Ash content of 2 and 3 months larvae fed with cassava meal were lower than pollard because of lower ash content in cassava meal (Table 1). The unsignificant effect of feeds on ash content in beetle phase indicated that larvae were more sensitive to feeds ash content.

Ca contents was significantly affected only by age. Ca content in 2 and 3 months larvae were not different, but lower than beetle ($p<0.0.5$). Ca ratio to ash in larvae and beetles were 13%,

17% and 30%. The beetles needed higher Ca for amino acid formation to support sperm and egg production (Wigglesworth, 1972), hence its higher Ca content. P content was not significantly affected by feed or age, but its ratio to ash was higher (36% -51%) than Ca (<30%). Formation of chitin as exoskeleton produced plenty of phosphate (Bernard *et al.,* 1997), thus larvae and beetle exoskeleton riched in P.

Tenebrio molitor had much higher Ca and P contents (Table 2) than livestock products such as meat (0.01% and 0.15%-0.26%), milk (0.12% and 0.095%), and egg (0.054% and 0.205) (Anggorodi, 1979). Even its Ca: P ratio (1:1.71 to 1: 2.8) were closer to human needs (2:1) than livestock products, i. e. meat (1:15.2), chicken meat (1:24.1) and egg (1:3.79), except for milk (1.3:1) (Anggorodi, 1979) This makes *T. molitor,* especially its beetle, potentially source of Ca and P.

Table 2. Body moisture, ash, Ca, P content of *T. molitor* given pollard or cassava meal, rendement and chitosan quality of larvae skin at various age, and supporting data

	Variables	Feed	Age/Phase				Average
			2 months	3 months	4 months	Beetle	
Experiment 1	Moisture (%)	Pollard	59.22^{C}	60.89^{D}	-	57.41^{B}	-
		Cassava meal	60.45^{CD}	62.28^{E}	-	53.42^{A}	-
	Ash (%)	Pollard	1.68^{BC}	2.01^{C}	-	1.51^{B}	-
		Cassava meal	1.20^{A}	1.57^{B}	-	1.53^{B}	-
	Ca (%)	Pollard	0.22	0.24	-	0.42	0.29
		Cassava meal	0.25	0.23	-	0.52	0.33
		Average	0.24^{P}	0.24^{P}	-	0.47^{Q}	-
	P (%)	Pollard	0.80	0.66	-	0.81	0.76
		Cassava meal	0.56	0.64	-	0.74	0.65
		Average	0.68	0.65	-	0.78	0.71
	Variables						
	Rendement (%)	Skin	14.89^{Q}	10.46^{P}	11.93^{P}	-	-
		Chitin	10.35^{P}	13.77^{Q}	13.60^{Q}	-	-
		Chitosan	7.46	8.70	9.73	-	8.63
Experiment 2	Chitosan color		Brown	light brown	beige	-	-
	Chitosan composition (%)	Moisture	13.71	13.59	16.20	-	14.50
		Ash	0.24	0.16	0.11	-	0.17
		N	5.72	6.33	6.21	-	6.09
		Deacetylation percentage	82.54	73.31	65.75	-	73.87
Supporting Data in Experiment 1							
Mass weight/container (g)		Pollard	132.30	148.00	-	147.10	
		Cassava meal	145.40	154.00	-	153.20	
Body weight gain (g/container/10 days)		Pollard	0.32	0.48	-	0.47	
		Cassava meal	0.45	0.54	-	0.53	
Consumption (g/container/10 days)		Pollard	0.57	0.36	-	0.49	
		Cassava meal	0.63	0.51	-	0.49	
Feed conversion		Pollard	1.78	0.75	-	1.04	
		Cassava meal	1.40	0.94	-	0.92	

A, B, …, E : different superscript in the different row and column of moisture or ash content meant significantly different value ($p<0.05$)
P. Q : different superscript in same row meant significantly different value ($p<0.05$)

Effect of *T. molitor* Larval Age on Rendement and Chitosan Quality

Larval age significantly affected rendement ($p<0.05$) and chitosan color, but not on chitosan quality (Table 2). Skin rendement was higher in 2 months larvae than 3 and 4 months, but the reverse showed in chitin rendement, while chitosan rendement was not significantly differ in all age. Two months larvae had higher skin rendement due to high larval number in the same mass weight, but lower chitin rendement because of thinner skin/exoskeleton which contained chitin. Regression analysis showed significant correlation between age and chitin

rendement (r=0.740), but no significant correlation between age and chitosan rendement. Chitin and chitosan rendement in this study were higher than cricket's (7.1% and 5.2%, Adiwati, 2005) but lower than shrimp's (13% to 15%, Faozan, 2001).

Bright color chitosan was in accordance with larval age. Darker color indicated that NaOH was not able to release protein and pigment completely from the skin in the deproteinase process. The lower the chitosan protein content, the lighter the chitosan color (Suptijah *et al.,* 1992). Chitosan of *T. molitor* were darker than shrimp's but lighter than cricket's.

Chitosan compositions were not different in all larval age were (Table 2). Moisture and nitrogen contents did not meet the chitosan standard (≤10% and <3%) (Protan Laboratories, 1987). High water content in larval chitosan caused low solubility (Rinaudo, 2006) and needed longer time to dissolve it. To improve solubility, it is better to use other drying method than sun drying, such as oven drying. Chitosan should be stored in vacuum container due to its hygroscopic characteristic. High nitrogen content in chitosan caused by pH of water used in the deproteinase process (around 6), when it should be 7 (Suptijah *et al.,* 1992).

Ash content in the second experiment was lower than the first because it was only from the skin and later was immersed in water to release the minerals. Ash content in this experiment met the chitosan standard. Nevertheless, mineral content of *T. molitor* larvae and beetle were much lower than shrimp (30% to 40%; Faozan, 2001) so that immersion time and HCl concentration in the demineralization process could be reduced to save time and cost.

Deacetylation degree of *T. molitor* larval chitosan was near the minimum standard value (>70%), however the chitosan diluted perfectly in acetic acid. Two months larvae had deacetylation degree that far from the minimum standard value, so it is potential to be developed as chitosan source although its chitosan rendement was not different from the older larvae.

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