animals

MDPI

Article

Potential of insect meals as protein sources for meat-type ducks based on *in vitro* digestibility

Attawit Kovitvadhi [1,*], Pipatpong Chundang [1], Karun Thongprajukaew [2], Chanin Tirawattanawanich [3], Sunyanee Srikachar [4] and Banthari Chotimanothum [5]

[1] Department of Physiology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand; fvetawk@ku.ac.th, pichandang@gmail.com
[2] Department of Applied Science, Faculty of Science, Prince of Songkla University, Songkhla, Thailand; karun.t@psu.ac.th
[3] Innovation Cluster 2, Thailand Science Park, Ministry of Science and Technology, Pathum Thani, Thailand; chanint07@gmail.com
[4] Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand; sunyaneesrikachar@gmail.com
[5] The Queen Sirikit Department of Sericulture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand; tip3537@gmail.com
* Correspondence: fvetawk@ku.ac.th; Tel.: +66-89-2022-677

Received: date; Accepted: date; Published: date

Simple Summary: Over the past century, there has been a dramatic increase in duck meat consumption; therefore ducks should be the interesting alternative livestock. As we known, protein raw materials in diets affect on production cost. Animal-based proteins such as fishmeal and animal by-products, are valuable nutrients and high digestibility but cost fluctuation, pathogen contamination and environmental impacts are presented. Therefore, plant-based proteins such as soybean meals were used. However, inappropriate amino acids, anti-nutritional factors and mycotoxin contamination were reported. Insect meals have provided as protein sources in animals as they contain favorable nutrients, low production cost and environmental friendly. There is the large number of insect species around the world. Therefore, the purpose of this investigation is to screen the potential insects for using as protein sources in duck diets based on digestibility efficiency. From the results in this study, insect meals which comprised with the high proportion of low digestible components calling crude fiber and acid detergent fiber, influenced on lower digestibility. In conclusion, yellow mealworm larvae, giant mealworm larvae, lesser wax moth larvae, house fly larvae, mulberry silkworm pupae and American cockroach nymph contain potential to be alternative protein replacer for ducks comparing to other insect meals.

Abstract: Insect meals are widely studied and used in animal nutrition to replace common protein sources which have several disadvantages and to promote the sustainability on animal production. Two-step *in vitro* digestibility (Stomach and small intestine simulation) using crude enzyme extracts from digestive tracts of meat-type ducks (Cherry Valley) was performed in general protein sources (n=6) and insect meals (n=17) to compare the percentage of *in vitro* digestibility on organic matter (OMd) and crude protein (CPd) which was the objective of this study. The large variation of chemical component in insect meals was presented. The positive correlation was found between OMd and ether extracts composition in insect meals, whereas negative correlation was shown in crude fiber and acid detergent fiber. The contrast relationship was represented between CPd and crude fiber and acid detergent fiber in insect meals. In conclusion, yellow mealworm larvae (*Tenebrio molitor*), giant mealworm larvae (*Zophobas morio*), lesser wax moth larvae (*Achroia grisella*), house fly larvae (*Musca domestica*), mulberry silkworm pupae (*Bombyx mori*) and American cockroach nymph (*Periplaneta americana*) contain potential to be alternative protein sources for ducks based on OMd and CPd after the screening by *in vitro* digestibility technique.

Keywords: cherry valley; cricket; crude protein; fruit fly; *Hermetia illucens*; house fly; locust; mealworm; silkworm

1. Introduction

Broilers are the highest production yield for poultry sector, however the raise on duck production was observed year by year. In 2017, around 1,150 million ducks was produced which approximate increased at 6.89% from 2014. Around 63% of duck production in the world was observed in China following by Vietnam, Bangladesh, Indonesia, Russian Federation, Myanmar, France, India and Thailand [1]. Duck production plays an important role in the several Asian countries which is not only for large commercial scale production but also for poverty people. Great adaptation to fluctuating environments, high disease resistance, large variety of feed, higher selling price and symbiotic production system (Duck-cum-rice or Duck-cum-fish system) were advantage on ducks which promoted to be valuable alternative livestock with sustainability [2]. The major proportion of production cost is feed which around 60-80% especially on raw protein materials. Therefore, the reduction on feed cost by using alternative by-products or indigenous raw materials should be the solution to solve this problem, especially on protein-based sources.

Fishmeal is the ideal protein sources for animals as appropriate amino acids respecting to requirements with high digestibility. However, the decrease on the usage of fishmeal as protein-based raw materials was presented because several disadvantages were discovered such as fluctuation on cost, pathogen contamination and environmental impacts [3]. Therefore, plant-based protein was replaced the fishmeal in several formulations on avian species [3,4]. Soybean meals, rapeseed meal, sesame meal, leucaena leaf meal and duck weed (*Lemna minor*) were introduced as raw materials for ducks [3,4]. The supplementation of synthetic amino acids (Methionine and/or lysine) is necessary because the deficiency of limiting amino acids in plant-based raw materials which is a great obstacle for organic farming [3]. Anti-nutritional factors are observed in the plants which deteriorate on productive performances, digestibility, health and products [3,4]. Moreover, the contamination of multiple mycotoxin (Aflatoxins, fumonisins and deoxynivalenol) was reported in soybean products [5] which was the serious problems as ducks is highly susceptibility to mycotoxin comparing to other avian species [6]. Therefore, the alternative protein sources which can solve these problems with sustainability should be studied.

Insects have been interested to be alternative protein sources for animal feed especially, in poultry nutrition [7-10], based on nutrient quality and environmentally friendly of production system [11-14]. Previous study was reported the increase on carcass weight in the Alabio ducks when fed by live black soldier fly larvae as supplementation [15], and black soldier fly defatted larva meal was successfully used in Muscovy duck [16]. However, chitin containing in the insects species may contribute to decrease protein digestibility by physically protecting the protein from enzyme hydrolysis [17]. Therefore, insect meals should be the interesting alternative protein materials to replace general protein sources. However, there are several potential insect species in tropical countries [11]. A screening technique should be used before performing in an experiment in animals.

The *in vitro* digestibility technique has not only potential as a promising tool in estimating the suitable of protein sources for animals but this technique probably a new definition of gastrointestinal functionality that can be used to establish a multidisciplinary approach to increase animal health, welfare and performance, since the digestion of feed is the main function of the gastrointestinal tract system [17-19], before use the potential insects in the *in vivo* experiments. Generally, commercial enzyme from swine was used to perform *in vitro* digestibility [18,19]. However, precaecal amino acid digestibility was diverse between broilers, turkeys and ducks [20]. Therefore, this study was designed to compare the percentage of *in vitro* digestibility on organic matter (OMd) and crude protein (CPd) between general protein sources and insect meals by using crude enzyme extract (CTX) from duck's digestive organs.

2. Materials and Methods

Twenty digestive tracts from healthy commercial meat-type ducks (Cherry Valley) were collected at a commercial slaughterhouse during evisceration process (Duck King Co., Ltd., Bangkok, Thailand). The digestive organs were immediately kept under the ice and transported to laboratory. Gastric mucosa, pancreas and duodenal mucosa were individually separated, pooled and homogenized with phosphate buffer solution (pH 7, 1:5 w/v). The homogenates were centrifuged at 18,000×g under 4°C for 30 minutes to obtain supernatant which was reserved under -80°C as CTX of each organ. This study was carried out following the standard guidelines being approved by Institutional Animal Care and Use Committee of Kasetsart University, Bangkok, Thailand (ACKU61-VET-035).

High protein fishmeal (FMh), low protein fishmeal (FMl), chicken by-product meal (CBM), pork by-product meal (PBM), dehulled-soybean meal (DSB) and hulled-soybean meal (HSB) were served as general protein sources which obtained from Bangkok Animal Research Center CO., LTD, (Bangkok, Thailand) and Protector Nutrition CO., LTD (Bangkok, Thailand). Seventeenth insect meals were collected from local company in Thailand (Dang Insect Distributor CO., LTD; Jae Tick CO., LTD; Jerry Pet Supplies CO., LTD; Jing Hleed CO., LTD; Orgafeed CO., LTD) and government sector (Department of Agriculture and the Queen Sirikit Department of Sericulture of Ministry of Agriculture and Cooperatives, Bangkok, Thailand) which were American cockroach (*Periplaneta americana*: PA, nymph), water scavenger beetle (*Hydrous cavistanum*: HC, adult), yellow mealworm (*Tenebrio molitor*: TM, larvae), giant mealworm (*Zophobas morio*: ZM, larvae), oriental fruit fly (*Bactrocera dorsalis*: BD, larvae), black soldier fly (*Hermetia illucens*: HI, prepupae), house fly (*Musca domestica*: MD, larvae), lesser wax moth (*Achroia grisella*: AG, larvae), mulberry silkworm (*Bombyx mori*: BMl, larvae), mulberry silkworm (*Bombyx mori*: BMp, pupae), eri silkworm (*Philosamia ricini*: PR, pupae), house cricket (*Acheta domesticus*: AD, adult), African mole cricket (*Gryllotalpa africana*: GA, adult), African cricket (*Gryllus bimaculatus*: GB, adult), ground cricket (*Gryllus testaceus*: GT, adult), oriental migratory locust (*Locusta migratoria*: LM, adult) and bombay locust (*Patanga succincta*: PS, adult). All insects were euthanized by immediately frozen at -20°C for five days. After that, insect samples were dried at 60°C for 48 h, grounded into 1 mm size and preserved at -20°C for using as substrates. All substrates were analyzed in triplicate for dry matter (DM), crude ash, crude protein (CP), ether extracts (EE) and crude fiber (CF) following the method from Association of Official Analytical Chemists [21], whereas acid detergent fiber (ADF) was determined in insect meals [22].

A triplicate two-step *in vitro* digestibility with CTX was performed to simulate the condition in stomach (First step) and small intestine (Second step) of ducks. The procedures were adapted from previous research studies and followed the digestive physiology of ducks [17-19]. One hundred milligrams of each substrate were weighted for OMd analysis, whereas the amount of protein of each substrate was calibrated at 100 mg protein (CP = %Nitrogen × 6.25) for CPd analysis. The OMd was performed in the same time with the same procedure, equipment and researcher, whereas CPd was done in another set of experiment.

The first step, prepared substrates were incubated in conical tubes with 5 ml of stimulated gastric fluid (SGF containing; 0.0169 M NaCl, 0.0096 M KCl and 0.0100 M HCl) at pH 2, 200 μL of gastric mucosal CTX and 100 μL of 0.5% chloramphenicol. Each tube was covered with screw cap and placed in incubator shaker at 42 °C under constant shaking at 200 rpm for 4 h. The second step, 10 mL of stimulated pancreas-intestinal fluid (SPIF; 0.0851 M NaCl, 0.0148 M KCl, 0.0300 M NaH_2PO_4 and 0.1700 M Na_2HPO_4) at pH 8 was added to stop reaction of gastric enzymes and prepared the optimal condition for enzyme activity in the next step. Pancreatic and duodenal mucosal CTX were added at 100 μL each. The hydrolysis was continued for 8 h under the same conditions as first step. After hydrolysis, enzyme activity was terminated by adding 10% trichloroacetic acid. Undigested residues were collected by filtration using filtered crucible (pore diameter: 40 to 100 μm) and washed with distilled water, 95% ethanol and 96% acetone, respectively. The filtration procedure was performed in cold extraction unit of fiber analysis (Fibertec system 1021 cold extractor, TecatorTM, Denmark). DM of residuals was recorded after dry

overnight at 60°C. The crude ash or CP was determined in residuals by ignition at 600°C or Kjeldahl method [21] to obtain the information for OMd or CPd calculation, respectively. OMd and CPd were calculated based on the difference between the input and output of OM or CP, respectively. The blank tubes (n=6/experiment) were performed and placed in different positions of the incubator which contained all reagents but without substrate.

Statistical analysis in this study was calculated by R-statistic with Rcmdr Package in Rstudio. Pearson's correlation was performed between chemical composition of insect meals and their percentage on OMd and CPd. One-way analysis of variance was performed to evaluate the difference on OMd and CPd between different substrates (fixed factors) by using Tukey's honestly significant difference as post-hoc analysis. Hierarchical cluster analysis with Euclidean distance was used to establish a dendrogram between similarity between substrates on OMd and CPd. Statistical significance level was set at $P<0.05$.

3. Results

3.1. Chemical composition in substrates

The chemical composition of general protein sources and insect meals was illustrated in Table 1. The large variation of chemical component in general protein sources and insect meals was found in this study. The high proportion of crude ash was observed in general protein sources from animals (FMh, FMl, CBM and PBM) comparing to plant-based sources (DSB and HSB) and insect meals. The EE percentage on DM in most of insect meals was higher than animal-based protein (9.60-14.8%DM), except for PR (11.5%DM), PS (12.9%DM) and LM (3.59%DM). The large variation of CP content in animal-based protein (38.9-60.9%DM), plant-based protein (41.0-45.9%DM) and insect meals (37.6-64.5%DM) were presented. The low ADF proportion was identified in PA (5.53%DM), ZM (6.93%DM), BMp (7.63%DM) and TM (8.19%DM), whereas HC (20.6%DM) and GA (24.6%DM) contained high percentage of ADF. The ADF contents of most insect meals were between 10.1-15.8%DM.

Table 1. Chemical composition of general protein sources and insect meals.

Substrates	**Chemical composition (%DM)**					
	DM[1]	Ash	CP	EE	CF	ADF
General protein sources (Animal-based protein)						
Fishmeal: high protein (FMh)	91.1	20.7	58.6	9.60	0.57	-
Fishmeal: low protein (FMl)	92.2	24.6	38.9	14.8	0.59	-
Chicken by-product meal (CBM)	95.5	16.3	60.9	10.2	2.46	-
Pork by-product meal (PBM)	95.3	33.4	46.2	10.4	0.33	-
General protein sources (Plant-based protein)						
Dehulled-soybean meal (DSB)	87.7	6.18	45.9	0	4.31	-
Hulled-soybean meal (HSB)	88.4	6.96	41.0	0	7.55	-
Insect meals						
Order: Blattodea						
Periplaneta americana (PA:nymph)	94.6	3.98	64.4	23.6	4.36	5.53
Order: Coleoptera						
Hydrous cavistanum (HC:adult)	86.3	1.88	41.9	38.3	14.7	20.6
Tenebrio molitor (TM:larvae)	97.1	5.95	53.0	31.0	8.47	8.19
Zophobas morio (ZM:larvae)	96.8	5.53	42.0	41.7	6.28	6.93
Order: Diptera						

Bactrocera dorsalis (BD:larvae)	95.1	9.41	45.2	31.3	5.94	13.6
Hermetia illucens (HI:prepupae)	91.8	9.54	37.9	30.1	12.3	11.2
Musca domestica (MD:larvae)	93.8	6.78	54.8	21.7	9.65	14.9
Order: Lepidoptera						
Achroia grisella (AG:larvae)	97.2	6.02	37.6	48.6	3.02	12.7
Bombyx mori (BMl:larvae)	96.7	10.1	61.2	17.6	5.39	13.6
Bombyx mori (BMp:pupae)	95.2	4.51	50.4	35.0	4.61	7.63
Philosamia ricini (PR:pupae)	92.6	7.15	64.5	11.5	8.53	10.1
Order: Orthoptera						
Acheta domesticus (AD:adult)	95.8	4.66	52.8	24.5	10.0	12.3
Gryllotalpa africana (GA:adult)	94.9	4.12	54.3	17.7	17.8	24.6
Gryllus bimaculatus (GB:adult)	92.2	5.05	53.3	22.6	8.98	13.5
Gryllus testaceus (GT:adult)	95.7	4.54	40.2	24.7	8.35	13.2
Locusta migratoria (LM:adult)	91.9	4.56	58.5	3.59	12.7	15.8
Patanga succincta (PS:adult)	92.0	4.29	63.3	12.9	15.1	12.8

DM = Dry matter, CP = Crude protein, EE = Ether extract, CF = Crude fiber, ADF = Acid detergent fiber
[1] Expressed as fresh matter.

3.2. Correlation between chemical composition and in vitro digestibility of insect meals

The results of the correlation analysis between chemical composition and in vitro digestibility of insect meals were summarised in Table 2. The positive correlation between CF and ADF (r = 0.71; P<0.01) were found, whereas negative correlation was presented between CP and CF (r = -0.77; P<0.01) of insect meals. The great relationship with statistical significance was observed between OMd and CPd (r = 0.89; P<0.01). OMd was influenced by EE, CF and ADF in insect meals. The higher proportion of EE in insect meals correlated to the higher value of OMd (r = 0.77; P<0.01), whereas CF (r = -0.56; P<0.05) and ADF (r = -0.59; P<0.05) consequenced on the lower of OMd. The lower of CPd percentage was affected by the higher proportion of CF and/or ADF in insect meals (r = -0.54 and -0.68; P<0.05 and <0.01, respectively).

Table 2. Correlation coefficients between chemical components of insect meals and *in vitro* digestibility on organic matter (OMd) and crude protein (CPd).

	CP	**EE**	**CF**	**ADF**	**OMd**	**CPd**
Ash	-0.05	-0.03	-0.35	-0.21	-0.05	0.11
CP		-0.77**	0.11	-0.10	-0.39	-0.03
EE			-0.45	-0.23	0.77**	0.47
CF				0.71**	-0.56*	-0.54*
ADF					-0.59*	-0.68**
OMd						0.89**

CP = Crude protein, EE = Ether extract, CF = Crude fiber, ADF = Acid detergent fiber
* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

3.3. In vitro digestibility on organic matter and crude protein

The comparing and grouping on OMd and CPd of general protein sources and insect meals was represented in Figure 1. The substrates were grouped into three major groups based on the results from the cluster analysis on OMd. The high OMd group contained only insect meals which were AG, ZM, TM, PA and BMp. General protein-based raw materials (FMh, CBM, PBM and FMl) were

concluded in moderate OMd group with some of insect meals (MD, BD, HC, HI, AD and PS). GT, GB, LM, BMl, PR and GA were clustered in the low OMd group with plant-based protein (DSB and HSB). The results of the CPd between the substrates were also summarized in Figure 1.

General protein sources and insect meals for ducks based on CPd can be separated into five groups and arranged in ascending order as followed: Group 1 contained GT and GA; Group 2 contained only HC; Group 3 contained LM, BMl, PR, GB, FMl and HI; Group 4 contained AD, HSB, PBM, BD, PS, FMh and SMd; Group 5 contained PA, BMp, MD, AG, CBM, ZM and TM. The statistical differences on OMd and CPd between general protein sources and insect meals were illustrated in Figure 1.

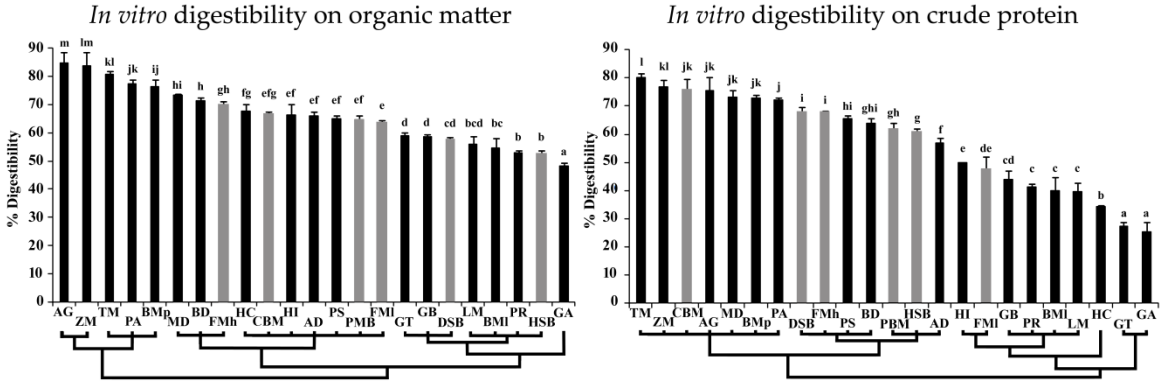

Figure 1. Dendrogram of the *in vitro* digestibility on organic matter and crude protein between general protein sources (grey column) and insect meals (black column). The different superscripts on the right of column represent statistically significant difference at $P<0.05$. Standard deviation is presented by error bar.

4. Discussion

The chemical composition in insect meals in this study was in line comparing to other experiments with the large variation between insect species and developmental stages [13,14,23,24]. The differences on mineral composition should be the cause of the contrast outcomes on ash contents between general protein sources from animals, plant-based sources and insect meals [13,24]. Bones and intestinal organs are rich in mineral contents which comprised in fishmeal and animal by-products. Therefore, higher amount of ash was observed in animals-based sources than others.

The EE in insects was influenced by developmental stage, season, sex, environment, diet for insect and postharvest methods [13,23-26]. The lower of fat content was commonly observed in order Orthoptera [13] which correlated to the results in this study on LM and PS. In contrast, other insect species in this order (AD, GA, GB and GT) were high level of EE contents because commercial starter broiler diets were served as their feed. The higher fat content in commercial diets promotes a higher energy and lipid to storage in insect body comparing to plant-based diets. Cassava leaves (*Manihot esculenta*) were used for PR diet, therefore the low fat content was presented. Generally, larval stage contained higher fat composition than fully developmental stage due to the energy preservation before metamorphosis, so that highest fat content was observed in AG larvae that fed by honey.

The ADF content correlated to chitin content in insects [17]. From this reason, ADF was used to estimate on OMd and CPd in this study. The ADF proportion was changed depending on developmental stage of insects which adult stage with exoskeleton was higher on ADF than larval stage. The CP content of insect meals exhibited a large variation which diet is the major factor [13,14,24]. Most of insects in this study were produced in the commercial farms which fed starter or finisher diets of broilers, commercial insect diets and/or organic waste, except for HC, LM, PS and GA that were harvested from wild. Unfortunately, there have been no precise details on diets for insects in this study. Therefore, this hypothesis need further study to confirm.

CF content of insects could be used to estimate or replace the ADF because the statistically positive correlation was observed in this study. Moreover, CF may use for prediction on chitin

content for insects [17]. However, further study with the larger number of insect species should be done to confirm this hypothesis. OMd may be used to represent the CPd which the OMd method is easier and lower cost than CPd because the great correlation between OMd and CPd was found in current study. However, the difference between OMd and CPd on each insect species was presented which revealed the inaccuracy of this parameter. Therefore, CPd is still recommended for representation on CP digestibility efficiency. Base on the result in this current study, EE, CF and ADF contents in insect meals influenced on OMd. Fat can be easily digested by animal enzymes. Therefore, EE content was significantly positively correlated to OMd. CF and ADF are considered as low digestible materials by animal enzymes. On the one hand, the negatively correlation between fiber proportion (CF and ADF) and OMd was observed in this study, as well as the other [17].

Kjeldahl method for CP determination was calculated base on nitrogen molecules which cannot distinguish between protein nitrogen and non-protein nitrogen compounds [21]. None of correlation between CPd and CP content in insect meals was found in this study. However, Marono et al. [17] found the positive correlation between CP content in insect meal (HI) and CPd. The difference on *in vitro* digestibility techniques should be one of the different outcomes. Moreover, the correlation in the study of Marono et al. [17] was calculated only in black soldier fly pre-pupae (HI). Therefore, CP was not suggested to use as predicted parameters for CPd in other insect meals but should only use in HI.

The amount of ADF and/or CF in insect meals contrasted to CPd. ADF was the better predictors than CF based on the statistical results in this study. This negative correlation was also reported in another study between these chemical compositions [17]. The cause of consequences should be the poor digestible efficiency by animal enzymes on CF and ADF [15,17,20]. As the complicate technique to evaluate chitin content, the ADF was suggested to be estimated parameters for chitin [27]. The larger variation of insect meals in term of species and chemical composition should be performed to create the estimation equation in the further study.

AG, ZM, TM, PA and BMp were higher on OMd than general protein sources and other insect meals. The high proportion of EE should be the cause of this consequence as described above. As the screening or selection on digestibility efficiency in protein raw materials is objective of the study, CPd was suggested to use rather than OMd. However, OMd of defatted substrates may correlate to the CPd. Therefore, further study should be studied to confirm this hypothesis. MD, BD, HC, HI, AD and PS were similar in OMd with general protein-based raw materials, whereas GT, GB, LM, BMl, PR and GA were grouped with general plant-based raw materials. There was none of study about the screening insect meals for ducks by *in vitro* digestibility techniques; however the study in broilers which comparing OMd between cricket meals and general protein sources by using *in vitro* digestibility techniques can be discussed. The OMd of AD was comparable to fishmeal, whereas GB and de-hulled soybean meal were lower. The results in this study are similar in trend as observed in broilers [19]. However, lower percentage of OMd was reported in broilers comparing to this study which performed in ducks. The difference on enzyme activities between species and *in vitro* digestibility protocols should be the cause of diverse consequences [20]. Therefore, the specific experimental design should be studied to compare digestibility efficiency between species by using *in vitro* technique.

GT, GA, HC, LM, BMl, PR, GB, FMl and HI were low on CPd. Therefore, the using of these insects for duck feed formulation should be considered on the adverse effects on low digestibility coefficient. Based on the CPd results in this study, AD, BD and PS have potential to replace FMh, DSB, PBM and HSB. Moreover, PA, BMp, MD, AG, ZM and TM have a great opportunity to be alternative protein sources for ducks as high CPd value which similar to CBM. Marono et al. [17] reported the higher CPd in HI at pre-pupal stage than in TM by *in vitro* digestibility techniques, whereas TM had the highest CPd comparing to other insect meals in the current study. The difference on chemical composition of insect meals and *in vitro* digestibility procedures using commercial enzyme from swine should be the cause of contrast outcomes. The improvement on carcass percentage was observed in Alabio ducks fed by live the black soldier fly larvae [15]. Based on results from this study, the appropriate supplementation level is important following their

digestibility coefficient. Therefore, the using HI in high percentage in duck feed formulation should be avoided as the low digestibility coefficient comparing to general protein sources.

In conclusion, TM, ZM, AG, MD, BMp and PA were suggested to be potential insect meals which using for alternative protein sources with sustainability in ducks based on *in vitro* digestibility technique. Therefore, the *in vivo* study in several aspects (palatability, amino acid profiles, productive performances, health, carcass characteristics, meat quality and cost efficiency) is necessary to confirm the screened results obtained from the current study.

Author Contributions: Conceptualization, A.K.; Methodology, A.K. and P.C.; Investigation and laboratory analysis, A.K. and P.C.; Resources, A.K., S.S. and B.C.; Data curation, A.K.; Writing—original draft preparation, A.K.; Writing—review and editing, A.K., P.C., K.T., C.T., S.S. and B.C.; Project administration, A.K.; Funding acquisition, A.K.

Funding: This research was financially supported by Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand.

Acknowledgments: The authors would like to humbly thank Duck King Co., Ltd. (Bangkok, Thailand) for providing digestive organ samples and all companies for providing the general protein sources and insect samples (Dang Insect Distributor CO., LTD; Jae Tick CO., LTD; Jerry Pet Supplies CO., LTD; Jing Hleed CO., LTD; Orgafeed CO., LTD; Department of Agriculture and the Queen Sirikit Department of Sericulture of Ministry of Agriculture and Cooperatives, Bangkok, Thailand).

Conflicts of Interest: All authors declare that we have no conflict of interest in this study.

References

1. FAOSTAT. Available online: http://www.fao.org/faostat/en/#data (accessed on 15 February 2019).
2. Adzitey, F.; Adzitey, S.P. Duck production: has a potential to reduce poverty among rural households in Asian communities – a review. *J World's Poult Res* **2011**, *1*, 7-10.
3. Thongwittaya, N. Substitution of plant protein for fish meal in the diet of laying ducks. *Animal Sci J* **2007**, *78*, 351-355.
4. Fazhi, X.; Lvmu, L.; Jiaping, X.; Kun, Q,; Zhide, Z.; Zhangyi, L. Effects of fermented rapeseed meal on growth performance and serum parameters in ducks. *Asian-Aust J Anim Sci* **2011**, *24*, 678-684.
5. Gutleb, A.C.; Caloni, F.; Giraud, F.; Cortinovis, C.; Pizzo, F.; Hoffmann, L.; Bohn, T.; Pasquali, M. Detection of multiple mycotoxin occurrences in soy animal feed by traditional mycological identification combined with molecular species identification. *Toxicol Rep* **2015**, *2*, 275-279.
6. Han, X.Y.; Huang, Q.C.; Li, W.F.; Jiang, J.F.; Xu, Z.R. Changes in growth performance, digestive enzyme activities and nutrient digestibility of cherry valley ducks in response to aflatoxin B1 levels. *Livest Sci* **2008**, *119*, 216-220.
7. Biasato, I.; Gasco, L.; De Marco, M.; Renna, M.; Rotolo, L.; Dabbou, S.; Capucchio, M.T.; Biasibetti, E.; Tarantola, M.; Bianchi, C.; Cavallarin, L.;, Gai, F.; Pozzo, L.; Dezzutto, D.; Bergagna, S.; Schiavone, A., Effects of yellow mealworm larvae (*Tenebrio molitor*) inclusion in diets for female broiler chickens: implications for animal health and gut histology. *Anim. Feed Sci. Technol*. **2017**, *234*, 253-263.
8. Schiavone, A.; De Marco, M.; Martínez, S.; Dabbou, S.; Renna, M.; Madrid, J.; Hernandez, F.; Rotolo, L.; Costa, P.; Gai, F.; Gasco, L., Nutritional value of a partially defatted and a highly defatted black soldier fly larvae (*Hermetia illucens* L.) meal for broiler chickens: Apparent nutrient digestibility, apparent metabolizable energy and apparent ileal amino acid digestibility. *J. Anim. Sci. Biotechnol.* **2017**, *51*, 1-9.
9. Biasato, I.; Gasco, L.; De Marco, M.; Renna, M.; Rotolo, L.; Dabbou, S.; Capucchio, M.T.; Biasibetti, E.; Tarantola, M.; Sterpone, L..; Cavallarin, L.;, Gai, F.; Pozzo, L.; Dezzutto, D.; Bergagna, S.; Schiavone, A., Yellow mealworm larvae (*Tenebrio molitor*) inclusion in diets for male broiler chickens: Effects on growth performance, gut morphology, and histological findings. *Poult. Sci.*, **2018**, *97*, 540-548.
10. Dabbou, S.; Gai, F.; Biasato, I.; Capucchio, M.T.; Biasibetti, E.; Dezzutto, D.; Meneguz, M.; Plachà, I.; Gasco, L.; Schiavone, A., Black soldier fly defatted meal as a dietary protein source for broiler chickens: Effects on growth performance, blood traits, gut morphology and histological features. *J. Anim. Sci. Biotechnol.* **2018**, *49*, 1-10.

11. Hanboonsong, Y.; Jamjanya, T.; Durst, B.P. Six-legged livestock: edible insect farming, collection and marketing in Thailand, 1st ed.; Food and Agriculture Organization of the United Nations, Rome, Italy, 2013.
12. Van Huis, A. Potential of insects as food and feed in assuring food security. *Annu Rev Entomol* **2013**, *58*, 563-583.
13. Makkar, P.S.H.; Tran, G.; Heuzé, V.; Ankersa, P., State-of-the-art on use of insects as animal feed. *Anim Feed Sci Technol* **2014**, *197*, 1-33.
14. Sánchez-Muros, M.; Fernando, G.B.; Manzano-Agugliaro, F., Insect meal as renewable source of food for animal feeding: a review. *J Clean Prod* **2014**, *65*, 16-27.
15. Gunawan, A.; Erlina, S.; Samudera, R.; Syarif D, M.; Noor, M.Y.; Lantu, A.X. Effect of supplement maggot black soldier fly live on the percentage of carcass and weight of carcass of male Alabio ducks. Proceeding of the 1st International Conference on Food and Agriculture, Nusa Dua, Bali, Indonesia, 20-21 October 2018.
16. Gariglio M; Dabbou S.; Biasato I.; Capucchio M.T.; Colombino E.; Hernandez F.; Madrid Sanchez J.;Martinez S.; Gai F.; Caimi C.; Bellezza Oddon S.; Meneguz M.; Trocino A.; Vincenzi R.; Gasco L.; Schiavone A., Nutritional effects of the dietary inclusion of partially defatted *Hermetia illucens* larva meal in Muscovy duck. . *J. Anim. Sci. Biotechnol. **in press***.
17. Marono, S.; Piccolo, G.; Loponte, R.; Di Meo, C.; Attia, Y.A.; Nizza, A.; Bovera, F., *In vitro* crude protein digestibility of *Tenebrio molitor* and *Hermetia illucens* insect meals and its correlation with chemical composition traits. *Ital J Anim Sci* **2015**, *14*, 338-343.
18. Zhao, F.; Zhang, L.; Mi, B.M.; Zhang, H.F.; Hou, S.S.; Zhang, Z.Y. Using a computer-controlled simulated digestion system to predict the energetic value of corn for ducks. *Poult Sci* **2014**, *93*, 1410-1420.
19. Kovitvadhi, A.; Luapan, J.; Amarapitak, P.; Sriyaphai, P.; Buahom, R.; Cham-iam, T.; Chandang, P.; Leelehapongsathon, K.; Tirawattanawanich, C.; Thongprajukaew, K. Screening three cricket species (*Gryllus bimaculatus*, *Acheta domestica* and *Modicogryllus confirmata*) for broiler diets by *in vitro* digestibility techniques. Proceeding of 6th Mediterranean Poultry Summit, Turin, Italy, 18-21 June 2018.
20. Kluth, H; Rodehutscord, M. Comparison of amino acid digestibility in broiler chickens, turkeys, and Pekin ducks. *Poult Sci* **2006**, *85*, 1953-1960.
21. Association of Official Analytical Chemists, Official Method of Analysis, 18th ed.; Association of Official Analytical Chemists, Virginia, USA, 2006.
22. Van Soest, P.J.; Robertson, J.B.; Lewis, B.A. Methods for dietary fiber, neutral detergent fibre, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci* **1991**, *74*, 3583-3591.
23. Finke, M.D. Complete nutrient composition of commercially raised invertebrates used as food for insectivores. *Zoo Biol* **2002**, *21*, 269-285.
24. Barroso, F.G.; Haro, C.; Sánchez-Muros, M.J.; Venegas, E.; Martínez-Sánchez, A.; Pérez-Bañón, C. The potential of various insect species for use as food for fish. *Aquacul* **2014**, *422*, 193-201.
25. Adámková, A.; Mlček, J.; Kouřimská, L.; Borkovcová, M.; Bušina, T.; Adámek, M.; Bednářová, M.; Krajsa, J. Nutritional potential of selected insect species reared on the Island of Sumatra. *Int J Environ Res Public Health* **2017**, *14*, 521-530.
26. Meneguz, M., Schiavone, A., Gai, F., Dama, A., Lussiana, C., Renna, M., Gasco, L., 2018. Effect of rearing substrate on growth performance, waste reduction efficiency and chemical composition of black soldier fly (*Hermetia illucens*) larvae. J. Sci. Food Agric. 98, 776-5784. https://doi.org/10.1002/jsfa.9127

Finke, M.D. Estimate of chitin in raw whole insects. *Zoo Biol* **2007**, *26*, 105-115.