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Nutritional studies on the utilisation of shrimp by-products as a potential protein source for chicken feed

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ABM Mustanur Rahman

Nutritional studies on the utilisation of shrimp by-products as a potential protein source for chicken feed

(エビ加工副産物を養鶏用飼料のタンパク質源として利用するための栄養学的研究)

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Chapter I

General Introduction

The poultry industry for domestic consumption and export in South and South-East Asian countries have been expanded rapidly over the last three decades, especially in Bangladesh, Thailand, India and Indonesia. In Bangladesh, according to FAO reports (2014), the poultry meat production was estimated at about 3.62 million tons and number of egg production was about 7.62 billion. This level of production leads to a great demand for feed resources, and hence, the industry has encountered the problem of obtaining feasible and adequate feed ingredients, especially protein sources which are import dependent and expensive. Unfortunately, it is extremely difficult to increase the production of such feed ingredients, because of limited land area. So, how do they overcome this difficulty? Development of alternative feed ingredients from domestic unused food by-products which are cheaply and safely available is one of the good solutions.

As a consequence, poultry nutritionists have made several attempts to partially or totally substitute by-products for conventional protein source (*i.e*. fish meal or soybean meal). Taking example of insect-derived materials, waste silkworm pupae, a by-product of the silk industry, was reported to be included successfully in both broiler and laying hen diets at 6% (Khatun *et al*., 2003; Khatun *et al*., 2005) and maggot meal in laying hen diets at 50% (Agunbiade *et al*., 2007) and 30% in broiler diets (Khan *et al*., 2016). Taking example of industry wastes, tannery wastes (Alam *et al.,* 2002) and poultry offal (Ologhobo *et al.*, 2012) were examined and the former can be included in broiler diets at 10% and the latter 100% without any adverse effect. In addition,

crustacean wastes, such as squilla (*Oratosquilla nepa*) waste, has been studied and this can be included in broiler diets at 12.5% (Reddy *et al.*, 1997): there are several studied on using shrimp waste as a protein source for chicken diet and the results were described later.

Furthermore, there is considerable emphasis on a shift toward more environmentally conscious farming techniques. Accordingly, their use would result in waste reduction and low additional nitrogen load in the environment. Shrimp waste, a waste product from shrimp processing industry, may be a good candidate, because of the great shrimp production in the South and South-East Asian countries, which account for more than 3/4 of the world shrimp production (Fig.1). As these shrimps are processed for export, about 40-50% shrimp waste (heads and hulls) are generated (Balogun and Samsons, 1992; Ngoan *et al.*, 2000; Rahman and Koh, 2014). These wastes constitute a serious environmental hazard through their decomposition when they are not properly disposed. Instead of allowing these wastes to decompose, they can be processed into a product of high nutritional quality if their collection is organised. Therefore, development of the shrimp meal (SM), dried shrimp waste, should be preferable to promoting poultry production and reducing environmental impact.

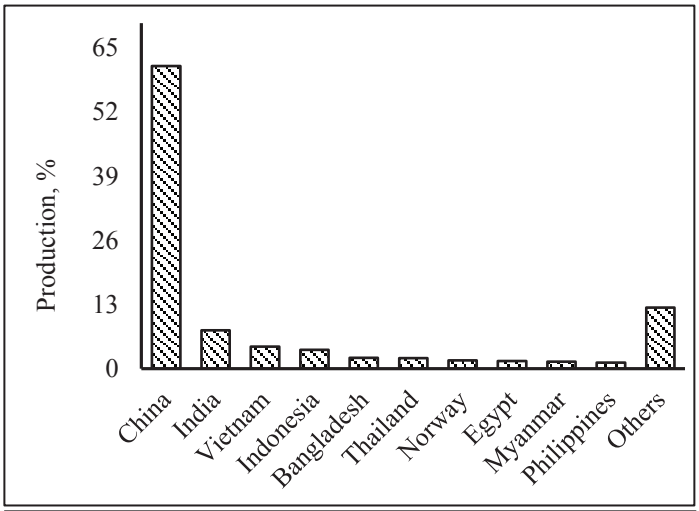

Fig. 1 World shrimp production (FAO, 2010)

In Bangladesh, the author home country, black tiger shrimp (*Penaeus monodon*), is raised in fresh and brackish water as the main species. Approximately 1,308 kilotons of shrimp was harvested annually in Bangladesh, which accounts for 5th position

(2.45%) of the world shrimp aquaculture production (FAO, 2012). As shrimp production increases, so does the great volume (approximately 523 kilotons, fresh basis) of waste products generated from processing industries. This huge amount of waste products might be an efficient substitute for imported soybean meal, and hopefully leads to save around 174 kilotons of soybean meal out of grossly 330 kilotons used annually as chicken feed in Bangladesh. It has been reported that SM can be the alternative source of protein in chicken diets: although several studies have been conducted to use SM as a protein source for chicken diets, the results did not necessarily show the similar trend (Islam *et al*., 1994; Rosenfeld *et al*., 1997; Gernat, 2001; Khempaka *et al.*, 2006a; Khempaka *et al.,* 2011; Rahman and Koh, 2016). The reason of these inconsistencies can be explained, in part, by the difference in species and body portion of shrimp used (Meyers, 1986; Ngoan *et al*., 2000; Heu *et al*., 2003; Rahman and Koh, 2014). Similar results have been reported in earlier studies (Fanimo *et al*., 1996; Gernat, 2001; Oduguwa *et al*., 2004; Khempaka *et al*., 2006a) where performances were decreased when chicken received diets containing more SM. Therefore, whatever the reason, there is still room for improvement in nutritional quality of SM.

In this connection, some treatments, such as physical, chemical or enzymatic treatments, may be needed to improve their nutritional quality as well as increase the safety margin of SM in chicken diets. So far, limited numbers of studies have been conducted to improve the nutritional quality of SM. According to studies made by Fox *et al*. (1994), who reported that formic acid treatment have an important impact on the physical and biochemical properties of SM, and resulted an improvement in growth and survival rate of juvenile *Penaeus monodon*, Oduguwa *et al*. (1998) showed that crude ash (CA) in SM decreased with HCl treatment, and Watkins *et al*. (1982) showed that chemical extraction can reduce chitin and Ca content and increase CP content in shrimp

and crab-processing waste. These findings believe that chemical treatments may be effective to improve the nutritional values of SM. However, there is no enough information about treatments to generate SM of improved quality as a potential protein source for chicken diets.

In addition, SM appears to provide a good source of calcium (Ca) because of its high CA level (Ngoan *et al*., 2000; Khempaka *et al*., 2006a). Therefore, it is possible that SM can improve the eggshell quality of laying hens as well as the skeletal quality of modern fast-growing chickens. In this context, it has been reported that mineral content did not have any adverse effect on broiler performance (Rosenfeld *et al*., 1997) and diets containing 1.8-3.0% Ca did not cause any detrimental effects on growth performance of broilers (Hussein *et al.*, 1986; Shafey and McDonald, 1991). On the contrary, high levels of dietary Ca depress the growth rate of broilers (Qian *et al*., 1997; Takasugi *et al*., 2005). In addition, it is well-known that SM contains astaxanthin, which is a natural carotenoid with red pigmenting properties which may have the potential to improve visual appearance of broiler meat (Chawan and Gerry, 1974; Khempaka *et al*., 2006b) and egg yolk colour (Gernat, 2001).

The present study deals primarily with the questions as to how improve the nutritional quality of SM, and what is the potential impact to utilise this SM as a potential protein source for chicken feed. Accordingly, this study was composed of the following experiments: in chapter II, improvement in nutritional quality of SM with autoclave and chemical treatments: an *in vitro* study was conducted. In chapter III, the effects of formic acid-treated SM on palatability of laying hens were investigated. In chapter IV, the effects of formic acid-treated SM on laying performance and egg quality of laying hens were examined. In chapter V, the effects of formic acid-treated SM on palatability of broilers were investigated. In chapter VI, the effects of formic acid-

treated SM on growth performance, nutrient digestibility and carcass quality of broilers were investigated. Finally, chapter VII discussed from the viewpoint of practical use of treated SM as a potential protein source for chicken feed.

Chapter II

Improvement in Nutritional Quality of Shrimp Meal with Autoclave and Chemical Treatments: an *in vitro* Study

Abstract

This study was conducted to improve the nutritional quality of SM comprising of heads and hulls of black tiger shrimp (*Penaeus monodon*) waste by autoclaving and chemical treatments. The sun-dried shrimp waste was divided into 5 treatment groups, such as 1) control (intact SM), 2) autoclaved group, 3) NaOH group, 4) HCl group, and 5) formic acid group. The autoclave treatment was done at 121°C with 2.09 kg/cm^2 for 10 min and then sun-dried. The chemical (NaOH, HCl, and formic acid) treatments were performed by approximately 100 g of sun-dried shrimp waste was suspended in 300 ml of 1, 3, and 5% of each solutions at room temperature for 20 minutes. After treatment, they were ground to pass through 1.0 mm mesh screen and then used for analyses of chemical composition and *in vitro* DM and CP digestibilities. There were no significant (P<0.05) difference in chemical composition and *in vitro* DM and CP digestibilities between control and autoclaved groups, suggesting that autoclaving affected the nutritional quality of the SM little. NaOH groups (1, 3, and 5% levels) exhibited significantly (P<0.05) decreased CP level and *in vitro* DM digestibility, increased CA level and unchanged *in vitro* CP digestibility. These results suggest that NaOH treatment affected the nutritional quality of SM adversely. In contrast, beneficial effects were obtained in acids treatment: significantly (P<0.05) increased CP level and *in vitro* digestibilities of DM and CP, and decreased CA level, showing that acid

treatment can improve nutritional quality of SM. In consequence, 3% level of formic acid treatment may be more effective because of the greater values in CP level and digestibilities and lesser values in crude fibre (CF), and chitin level than HCl group (P<0.05). In conclusion, it is suggested that acids, especially formic acid, treatment is promising to improve the nutritional quality of SM, and seemed to be used as a potential source of protein for chicken diets.

Introduction

The nutritional quality of SM has been investigated in the previous study to use this for an alternative protein source for broilers, and clarified that its nutritional quality was not good enough to be included more than 10% in a diet (Rahman and Koh, 2016), although SM quality was somewhat different depending on the portion and species of shrimp (Rahman and Koh, 2014). Similar results have been reported in earlier studies (Fanimo *et al*., 1996; Gernat, 2001; Oduguwa *et al*., 2004; Khempaka *et al*., 2006a) where performances were decreased when chicken received diets containing more SM.

Several studies have been conducted to improve the nutritional quality of crustacean meals by means of physical and chemical treatments. For instance, autoclaving was applied to squilla (a stomatopod species) meal for broilers and failed to improve the nutritional quality, but assumed autoclaving temperature in their study is lower than usual one (Reddy *et al*., 1997). Alkali treatment, such as NaOH treatment, modified chemical composition of SM for broilers, so that CP and CA levels increased and CF level decreased (Septinova *et al*., 2010). Acid treatment, such as HCl and formic acid treatments, has been reported to improve the nutritional quality of SM, but these were conducted to develop a feed ingredient not for chicken but for rats (Oduguwa *et al*., 1998) or shrimps (Fox *et al*., 1994).

In the present study, the chemical composition and *in vitro* digestibilities of SM after receiving autoclaving or chemical treatments have been investigated, and determined the most suitable treatment to improve the nutritional quality of SM as a better CP source of chicken diets.

Materials and Methods

Preparation of Treated SM

The sun-dried shrimp waste made of heads and hulls of black tiger shrimp (*Penaeus monodon*) was obtained from a processing industry in Bangladesh. They were divided into five treatment groups: 1) control (intact SM), 2) autoclaved, 3) NaOH treated, 4) HCl treated and 5) formic acid treated SM. The autoclave treatment was conducted according to Reddy *et al*. (1997) with slight modifications. The sun-dried shrimp waste was autoclaved at 121°C with 2.09 kg/cm^2 for 10 minutes and then sun-dried until achieving the moisture content of approximately 10%. The chemical (NaOH, HCl and formic acid) treatments were performed according to Septinova *et al*. (2010), Oduguwa *et al*. (1998) and Fox *et al*. (1994) with slight modifications. Approximately 100 g of sun-dried shrimp waste was suspended in 300 ml of 1, 3, and 5% acids (HCl or formic acid) or alkali (NaOH) solutions at room temperature for 20 minutes. After that, they were filtered by using cheese cloth and washed with distilled water to adjust pH 7. After filtration, the solid portions were sun-dried and ground to pass through 1.0 mm mesh screen and then used as chemical treated SM. Each sample was divided into 4 aliquots for quadruplicate measurements.

Chemical Analysis

Proximate composition was analysed according to standard methods (AOAC, 1990). The chitin extraction process was done according to Ghanem *et al*. (2003), which was summarised as follows: about 1.0 g of sun-dried SM mixed with 12.5 ml of 2.5 N NaOH solutions, placed in an oven at 75°C for 6 hrs and then filtrate. The filtrate crude chitin residue was dried at 105°C in an oven for 1 hour. After drying, about 1.0 g of dried crude chitin was mixed with 10 ml of 1.7 N HCl and placed on a stir plate at room

temperature for 6 hours. After filtering, the residue was washed with 95% ethanol (20 ml/g of crude chitin) followed by a final washing with distilled water and then dried. The dried content was weighed as chitin.

Digestibility Measurements

The *in vitro* dry matter (DM) and CP digestibilities of SM were determined according to Saunders *et al*. (1973) with slight modifications: about 250 mg of dried ground SM sample was suspended in 15 ml of 0.1 N HCl containing 1.5 mg pepsin (10,000 U/mg protein) (Nacalai Tesque Inc., Kyoto, Japan), and gently shaken at 37°C for 3 hours in multi shaker (EYELA MMS-3010). After neutralisation with 0.5 N NaOH, the digesta was mixed with 7.5 ml of phosphate buffer at pH 8.0 containing pancreatin (amylase activity 3,220 U/g, protease activity 38,500 U/g and lipase activity 1,600 U/g) (Nacalai Tesque Inc., Kyoto, Japan) and shaken at 37°C for 24 hours. The solution was then centrifuged at 240 × *g* for 10 min, washed with distilled water, filtered and dried.

The DM and CP digestibilities of SM were determined as follows:

$$\text{DM digestibility (\%)} = \frac{\text{(Dried sample weight – Dried residue weight)}}{\text{Dried sample weight}} \times 100$$

$$\text{CP digestibility (\%)} = \frac{\text{N in sample – N in residue}}{\text{Total N in sample}} \times 100$$

Statistical Analysis

Results were presented as mean values ± standard error (SE). All the data obtained were statistically analysed by one-way ANOVA and differences among the treatment groups were evaluated by Tukey's multiple comparison test at a significance level of 5%.

(a) Black tiger shrimp (*Penaeus monodon*)

(b) Shrimp head (sun-dried)

(c) Shrimp meal

Fig. 2 (a) Black tiger shrimp (*Penaeus monodon*), **(b)** shrimp head (sun-dried), and **(c)** shrimp meal (milled)

Results

Chemical Composition of Treated SM (Table 1 & 2)

In control group, CP, CF, CA, ether extract (EE), and chitin accounted for 46, 14, 29, 4, and 17% of dry weight of SM, respectively. These values did not change significantly by autoclave treatment, except the lower EE level (P<0.05). Whereas SM treated by 1, 3, and 5% of NaOH showed decreased CP and EE levels, and increased CF and CA levels, comparing with the corresponding values in control group, but chitin level was affected only 3% NaOH group (P<0.05). Although SM was treated by acids (HCl or formic acid) at 1, 3, and 5% level resulted significantly increased CP and EE levels, comparing with the corresponding values of other treatment groups but the prominent effect was observed when SM was treated by 5% HCl and 3% formic acid. A noticeable effect was found in CA level which decreased by 3% HCl and formic acid treatment to 40% and 50%, respectively. Interestingly, CF and chitin levels were decreased by formic acid treatment but unexpectedly increased by HCl treatment, comparing with the corresponding values in other treatment group (P<0.05), though 5% formic acid-treated SM tended to decrease CP level and increase CF and chitin levels. However, the best effect was found for 3% formic acid-treated SM among the treatment groups.

Digestibility of Treated SM (Table 3 & 4)

DM and CP digestibilities in control group were about 44% and 74%, respectively, while autoclave treatment did not show any improve these values (P>0.05). However, DM and CP digestibility values were relatively deteriorated in all levels of NaOH concentration (P<0.05). In contrast, acids treatments improved DM and CP digestibilities, comparing with the corresponding values of other treatment groups

(P<0.05). The magnitudes of improvements were greater in formic acid treatment than HCl (P<0.05), and the maximum digestibility values were observed in 3% level of formic acid-treated SM. As a result, the 3% level of formic acid is best for significant improvement of SM quality.

Table 1. **Chemical composition of control (intact SM) and treated shrimp meal [1, 2]**

Treatments	Chemical composition, %				
	Crude protein	Crude fibre	Crude ash	Ether extract	Chitin
Control	45.5±0.1^{ab}	14.4±0.4^{a}	28.5±0.4^{a}	3.6±0.2^{ad}	17.3±0.2^{a}
Autoclaved SM	46.2±0.3^{a}	14.8±0.3^{ac}	29.6±0.4^{a}	2.4±0.1^{b}	16.6±0.2^{ade}
NaOH treated SM					
1%	43.7±0.6^{b}	16.3±0.2^{b}	32.3±0.5^{b}	2.3±0.2^{b}	17.7±0.3^{ab}
3%	37.8±0.2^{c}	16.2±0.5^{bc}	36.2±0.2^{c}	1.9±0.2^{bc}	17.8±0.2^{ab}
5%	38.6±0.3^{c}	15.4±0.1^{abc}	43.1±0.5^{d}	1.4±0.2^{c}	16.4±0.2 ^{de}
HCl treated SM					
1%	49.6±0.5^{d}	14.8±0.3^{ac}	20.1±0.4^{e}	3.3±0.1^{d}	17.8±0.6^{ab}
3%	54.3±0.1^{ef}	18.3±0.2^{c}	16.9±0.1^{f}	4.2±0.1^{a}	19.2±0.2^{bc}
5%	55.7±0.6^{eh}	19.2±0.1^{c}	13.2±0.6^{g}	5.3±0.2^{e}	20.4±0.3^{c}
Formic acid-treated SM					
1%	53.3±0.5^{f}	14.5±0.6^{a}	16.3±0.2^{f}	4.0±0.2^{ad}	16.8±0.3^{abd}
3%	58.1±0.4^{g}	12.7±0.2^{e}	13.6±0.2^{g}	5.4±0.1^{e}	15.3±0.4^{e}
5%	57.2±0.4^{gh}	13.1±0.5^{e}	12.2±0.5^{g}	6.1±0.4^{e}	15.7±0.3^{de}

[1]Values are expressed on air-dry matter basis.

[2]Values for each parameter represent mean ± SE values with 4 observations.

[a-h]Means within the same column with different superscripts are significantly different (P<0.05).

Table 2. **Average values of chemical composition of control (intact) and treated shrimp meal** [1]

Treatments	Chemical composition, %				
	Crude protein	Crude fibre	Crude ash	Ether extract	Chitin
Control	45.5±0.1[a]	14.4±0.4[a]	28.5±0.4[a]	3.6±0.2[a]	17.3±0.2[a]
Autoclaved SM	46.2±0.2[a]	14.8±0.3[a]	29.6±0.4[a]	2.4±0.1[a]	16.6±0.2[a]
NaOH treated SM	40.1±0.3[b]	16.0±0.2[b]	37.2±0.2[b]	1.9±0.2[b]	17.0±0.1[a]
HCl treated SM	53.2±0.3[c]	17.4±0.1[c]	16.7±0.2[c]	4.3±0.1[c]	19.1±0.3[b]
Formic acid-treated SM	56.2±0.2[d]	13.1±0.2[d]	14.1±0.1[d]	5.1±0.2[d]	15.9±0.2[c]

[1]Values for each parameter represent mean ± SE values with 4 observations.

[a-d]Means within the same column with different superscripts are significantly different (P<0.05).

Table 3. . ***In vitro*** **DM and CP digestibilities of control (intact SM) and treated shrimp meal[1, 2]**

Treatments	DM digestibility	CP digestibility
	%	
Control	44.3±0.3[a]	73.9±0.4[ac]
Autoclaved SM	43.9±0.3[a]	73.8±0.3[ac]
NaOH treated SM		
1%	39.4±0.4[b]	74.7±0.4[ad]
3%	39.6±0.4[b]	72.8±0.3[bc]
5%	36.6±0.5[c]	71.9±0.3[c]
HCl treated SM		
1%	44.7±0.4[a]	74.6±0.3[ad]
3%	48.1±0.5[d]	76.2±0.5[de]
5%	50.2±0.5[def]	76.9±0.3[ef]
Formic acid-treated SM		
1%	48.6±0.7[df]	79.4±0.4[g]
3%	51.6±0.3[e]	79.1±0.3[g]
5%	50.3±0.5[ef]	78.1±0.3[fg]

[1]Values are expressed on air-dry matter basis.

[2]Values for each parameter represent mean ± SE values with 4 observations.

[a-g]Means within the same column with different superscripts are significantly different (P<0.05).

Table 4. **Average values of *in vitro* DM and CP digestibilities of control (intact) and treated shrimp meal**[1]

Treatments	DM digestibility	CP digestibility
	%	
Control	44.3±0.3[a]	73.9±0.4[ab]
Autoclaved SM	43.9±0.3[a]	73.8±0.3[a]
NaOH treated SM	38.6±0.1[b]	73.2±0.1[b]
HCl treated SM	47.6±0.2[c]	75.9±0.2[c]
Formic acid-treated SM	50.2±0.2[d]	78.8±0.2[d]

[1]Values for each parameter represent mean ± SE values with 4 observations.

[a-d]Means within the same column with different superscripts are significantly different (P<0.05).

Discussion

The obtained results revealed that autoclaving failed to improve the nutritional quality of SM. Similar observation was found in squilla meal reported by Reddy *et al*. (1997). In this study, autoclaving condition (at 121°C for 10 min) seemed to be more severe than their condition (at 1.09 kg/cm^2 for 5 min), because according to Antoine equation (Thomson, 1946) of temperature in an autoclave increased until 102.05°C at a pressure of 1.09 kg/cm^2. This may suggest that this level of autoclaving is not effective to alter the chemical composition of crustacean meals. In this connection, autoclaving can improve pepsin digestibility of feather meal significantly, even though there was no significant impact on the chemical composition (Kim and Patterson, 2000). This makes to expect an improved *in vitro* digestibility of autoclaved SM in the present study, but the fact was different: not only DM but also CP digestibilities did not show improved values. Consequently, autoclaving may not be suitable to improve the nutritional quality of SM.

On the other hand, treating of SM with 1, 3, and 5% NaOH had lower CP level and higher CA level than other treatment groups (P<0.05), which was contrary to the results of Septinova *et al*. (2010) who found increased CP, CA levels and decreased CF level in 3% NaOH treated SM. Thus, it is quite difficult to discuss this inconsistency, because there was no difference in treatment condition between them. The results obtained in here exhibited decreased DM and CP digestibilities and it may assume that CP retention should be low in chickens, although Septinova *et al*. (2010) found increased protein retention in broilers given diet containing 3% NaOH treated SM. In this connection, it has been reported that NaOH treated feather meal showed decreased protein and increased ash levels, although *in vitro* protein digestibility increased

(Papadopoulos *et al*., 1985) and no significant difference in CP level (Steiner *et al*., 1983). Therefore, further investigation is necessary to confirm the effect of NaOH treatment on nutritional quality of SM.

Beneficial effect was obtained in two acid treatment experiments: significantly higher CP and lower CA levels were observed in both acid-treated SM comparing with the corresponding values of other treatment groups ($P<0.05$). This may be results of leaching the minerals, such as calcium, in exoskeleton (No *et al*., 1989; Fox *et al*., 1994; Oduguwa *et al*., 1998), and accordingly, relative content of CP increased. In this context, increased CF and chitin levels in HCl treated SM can also be explained, because these two components increased whatever the HCl concentrations (1, 3, and 5%). However, it is very interesting that formic acid treatment decreased CF and chitin levels, suggesting that chitin, main source of CF and possible factor to decrease digestibility (Austin *et al*., 1981; Fanimo *et al*., 2006; Khempaka *et al*., 2006b), was leached from SM by formic acid treatment. In this regard, Win and Stevens (2001) reported that formic acid treatment results in a weakening of the crystal structure and able to dissolve the shrimp chitin. Meanwhile, the value of CF and chitin appeared to decrease when SM treated with 3% formic acid ($P<0.05$).

Treating the SM with acids solution resulted in increased DM and CP digestibilities relative to other treatments ($P<0.05$). Similarly, it has been reported that apparent protein digestibility increased in rats (Oduguwa *et al*., 1998) and protein retention increased in broilers (Septinova *et al*., 2010) when HCl treated SM was included in diet. Moreover, significantly greater digestibilities observed in 3% formic acid-treated SM may be because of the higher CP level and lower CF, CA and chitin levels in this SM. The positive response of formic acid-treated SM was also observed by Fox *et al*. (1994) who reported an improvement in growth and survival rate in marine shrimps (*Penaeus monodon*) fed diet containing formic acid-treated SM.

Conclusion

The obtained results revealed that there were no significant differences in chemical composition and *in vitro* DM and CP digestibilities between control and autoclaved SM. NaOH groups exhibited significantly decreased CP level, increased CA level and unchanged CP digestibility. HCl and formic acids groups showed significantly increased CP level and *in vitro* DM and CP digestibilities, and decrease CA level, where formic acid may be more effective because of the greater values in CP level and digestibilities and decreased CF level in SM. In conclusion, formic acid treatment is promising to improve the nutritional quality of SM but autoclaving and NaOH treatment, and this SM can be used as a potential source of protein in chicken diets.

Chapter III

Effects of Formic Acid-Treated Shrimp Meal on Palatability of Laying Hens

Abstract

Palatability (choice-feeding) has been used to compare likes or dislikes of feedstuff for animals. The influences of different dietary SM or treated shrimp meal (TSM) on the free-choice feeding behavior of White Leghorn hens were investigated. The sun-dried shrimp waste was treated with 3% formic acid (w/v) at room temperature for 20 minutes, sun-dried, ground to pass through 1.0 mm mesh screen and then ready to use as TSM. Firstly, sixteen laying hens were distributed in four cages (4 birds each) and freely fed, a control diet, diets containing SM (5, 10, and 15%) and TSM (5, 10, and 15%) for 5 d experimental period. Feed intake (FI) was determined on a hen-day basis. The results indicated that hens fed the diets containing SM, FI decreased significantly and the poorest value observed in 15% SM group, which recommend that diet containing SM reduced acceptability as compared to that of the control and TSM groups (P<0.05). In contrast, diets containing TSM resulted in a no significant effect on acceptability as compared to control group (P>0.05) even though it was included up to the level of 15% TSM. These results may believe that the factor(s) which decreased FI can be removed or decomposed by formic acid treatment. Similar results were also perceived for feed preference, where SM groups showed poor preference than that of the corresponding value of the control and TSM groups (P<0.05). Thus, it was concluded that the inclusion level of TSM in the diets appeared to improve their palatability when compared with the SM-based diets.

Introduction

There is evidence that both wild and domesticated fowl are able to adjust their nutrient intake by selecting from a range of feedstuffs a diet that matches their nutrient requirements for maintenance, growth and production (Hughes, 1984). It is necessary to know which factors might influence the selection of the diet by the birds under a free-choice situation. In this regards, Yo *et al*. (1997) suggested that FI in a choice situation depends on the nutrient requirements of the animal and feed composition. In addition, the nutritional balance of diets, other dietary factors such as colour, smell, taste, texture, temperature and so on also affect FI by changing feed palatability. In order to measure palatability, animals are given a choice of two or more diets so that they can express a preference. In addition, Kare and Scott (1962) showed that chicks would select some practical feedstuffs in preference to others (e.g., corn was preferred over barley or rye) when those feedstuffs were offered in a free-choice situation. Furthermore, Oduguwa *et al*. (2005) reported that SM is unpalatable when fed to bird, due to the high proportion of exoskeleton in the diets, and resulted in decreased FI. Although there has been controversy (Fox *et al*., 1994), who reported that SM in fish diets appeared to improve their palatability when compared with the fish meal based diet. These results believe that TSM seemed to improve their palatability. In fact, there is no report, so far, which examined about palatability of SM in chickens. Accordingly, this study was conducted to investigate the palatability of dietary SM and TSM in laying hens (choice-feeding), and discussed their practical use in the poultry industry.

Materials and Methods

This research was conducted in accordance with guidelines for regulation of animal experimentation of Shinshu University, Japan.

Preparation of Treated SM

The sun-dried shrimp waste, composed of heads and hulls of black tiger shrimp (*Penaeus monodon*), was treated with formic acid. Approximately 100 g of shrimp waste was suspended with 300 ml of 3% formic acid at room temperature for 20 minutes. The shrimp waste was then sun-dried and ground through a 1.0 mm mesh screen, and was then ready to use as the TSM. Proximate components, Ca, phosphorus, chitin and astaxanthin content were analysed according to the methods of AOAC (1990), Ghanem *et al*. (2003) and HPLC, respectively (Table 5).

Birds, Diets and Feeding

Sixteen White Leghorn hens were randomly distributed in four cages based on similar body weight (1.6±0.02 kg). Each cage provided septuplicate identical feeders mounted in juxtaposition at the front of the cage. Seven experimental diets (1 control diet, diets containing 5, 10, and 15% SM, and diets containing 5, 10, and 15% TSM) were allocated randomly to each feeder and were provided therein *ad libitum* for the 5 d experimental period. Diets (approximately 173 g of CP and approximately 2935 kcal/kg of ME) were formulated to meet or exceed the nutrient requirements for laying hen defined by Japanese feeding standard for poultry (2011) (Table 6). The feeding trial was conducted on the basis of free-choice system. Feed was exchanged between feeders in each cage daily to avoid potential bias due to feeder or feeder position. Each cage was covered by a blue sheet to protect the birds to see next to the cage diets.

Data Recording

The amount of feed consumed from each feeder was determined every day and results were expressed on the basis of the average intake per birds. Feed preference was defined as the amount of test diet consumed expressed as the percentage of total feed consumption.

Statistical Analysis

Data were initially analysed with ANOVA using JMP version 10.0 (SAS Institute, 2012) and significant differences among the dietary groups were evaluated with Tukey's multiple comparison tests. Statements of statistical significance are based on $P<0.05$.

Table 5. **Chemical composition of untreated and treated shrimp meal and soybean meal (air dry matter basis)**

Components	SM[1]	TSM[2]	Soybean meal[3]
	g/kg		
Crude protein	445	549	450
Crude fiber	164	145	53
Ether extract	32	57	1.9
Ash	289	172	64
Chitin	183	155	-
Calcium	103	75	0.37
Available phosphorus	18	10	0.72
Astaxanthin, mg/kg	7.5	4.7	-
ME, kcal/kg	1230[3]	1230[3]	2400

[1]SM=untreated shrimp meal; [2]TSM=treated shrimp meal

[3]Standard Tables of Feed Composition in Japan (NARO, 2009).

Table 6. **Ingredients and chemical composition of experimental diets (g/kg)**

Items	Control	SM[1] (%)			TSM[2] (%)		
		5	10	15	5	10	15
Ingredients							
Commercial diet[3]	350	350	350	350	350	350	350
Soybean meal	190	144	98	52	128.5	69.5	10
Corn	335	343	347	354	356	374	394
Shrimp meal	0	50	100	150	50	100	150
Corn oil	46	47	51	53	47	49	50
$CaCO_3$	66	55	45	33	56.5	47.5	37
Tri-calcium phosphate	5	3	1	0	4	2	1
Salt	3	3	3	3	3	3	3
Vitamin-mineral premix[4]	5	5	5	5	5	5	5
Total amount	1000	1000	1000	1000	1000	1000	1000
Calculated composition (g/kg, as fed)							
ME, kcal/kg	2935	2932	2937	2938	2932	2935	2934
Calcium, Ca	36.5	36.6	36.9	37.1	36.6	37.0	37.1
Available, P	4.5	4.6	5.0	5.3	4.6	4.7	4.8
Astaxanthin, mg/kg	0	0.4	0.8	1.2	0.2	0.5	0.7
Analysed composition (g/kg, as-fed)							
Crude protein	173	173	173	174	174	175	176
Crude fibre	31	47	49	53	38	42	46
Ash	136	137	145	150	135	140	145
Chitin	0	8.7	16.2	25.6	7.3	14.9	22.8

[1]SM=untreated shrimp meal; [2]TSM=treated shrimp meal

[3]Layer diet (CP≥17.0%, ME≥2850 kcal/kg, Nippon Formula Feed Mfg. Kanagawa, Japan)

[4]Vitamin-mineral premix (units/ kg): vitamin A, 5,00,000 IU; vitamin D_3, 1,00,000 IU; vitamin E, 50 IU; vitamin K_3, 100 mg; vitamin B_1, 800 mg; vitamin B_2, 600 mg; vitamin B_6, 600 mg; vitamin B_{12}, 5.4 mg; pantothenic acid, 800 mg; nicotinic acid, 800; choline chloride, 20,000 mg; foliate, 104 mg; phosphorus, 106 g; iron, 2 mg; copper, 362 mg; zinc, 3368 mg; manganese, 2,560 mg; iodine, 45 mg.

Results

Palatability (Table 7)

The results showed that when hens were given the choice between the control diet and the diet containing SM and TSM, they consumed the control diet exclusively during the entire experimental period. However, FI was decreased with increasing level of the SM in the diets ($P<0.05$). On the other hand, this value tended to show no significant difference in the TSM groups when compared with the control. In addition, the preference of control diet 16%, which was compatible to the TSM-based diet ($P>0.05$). However, this value was decreased with increasing of dietary SM in the diets ($P<0.05$). According to the results of FI and preference, the acceptability of diets was better in TSM groups than SM groups and comparable to the control group.

Table 7. **The effects of untreated and treated shrimp meal on palatability of broilers**[1]

Treatments	Feed intake, g/b/d	Preference[2], %
Control	22.3±0.27[a]	16.3±0.07[a]
5% SM[3]	19.1±0.32[b]	13.9±0.25[b]
10% SM	16.9±0.37[b]	12.3±0.20[b]
15% SM	14.5±0.38[c]	10.6±0.26[c]
5% TSM[4]	22.4±0.44[a]	16.3±0.22[a]
10% TSM	21.1±0.25[a]	15.4±0.09[a]
15% TSM	20.9±0.11[a]	15.5±0.13[a]

[1]Values for each parameter represent mean ± SE values with 4 observations.

[2]Preference= (g of experimental diet consumed/g of total diet consumed) ×100

[3]SM=untreated shrimp meal; [4]TSM=treated shrimp meal.

[a-c]Means within the same column with different superscripts are significantly different (P<0.05).

Discussion

Palatability

Palatability for animal is the measure of intake of a feed that indicates acceptance or the measure of preference of one feed over another. In this regards, the results obtained here revealed that FI was reduced markedly in SM-based diets probably because of the high proportion of the fibre or other components in SM than TSM. If considered from a physiological viewpoint, bulk, roughage, texture and smell of SM may be factors related to the decrease in FI in SM groups. This could have caused discomfort for the birds. Similar observation was made by Oduguwa *et al*. (2005) who indicated that in spite of the good aroma and palatability, the acceptability of SM was slightly affected when high a level of SM was employed. In contrast, FI did not decrease even in 15% level of TSM in the diets, which suggest that formic acid treatment can remove or degrade a FI decreasing factor(s) in the SM. Correspondingly, Fox *et al*. (1994) reported that incorporation of formic acid-treated SM in the diets appeared to improve significantly their palatability when compared with the fishmeal-based diet. Thus, improved performance may result from the greater rates of consumption of TSM based-diets when hens are fed free-choice, and in consequence increased FI due to improved feed palatability conferred by TSM. While these tests answer some questions about the birds' perception of the feed, but also opportunities for future research in developing methods that would help understand palatability-related issues or provide better models to predict bird feed selection.

Conclusion

The results obtained here revealed that FI and preference of feed were decreased significantly with increasing levels of the dietary SM when compared with the control and TSM groups. On the other hand, the TSM groups did not exhibit such detrimental effects observed in the SM groups and comparable to the control group, even though it was included up to the level of 15% in the diets. As a result, it may conclude that the formic acid treatment is promising to remove or degrade a FI decreasing factor(s) in SM and subsequently increased the palatability which suggests that TSM can be used as potential source of protein for layer hen diets.

Chapter IV

Effects of Formic Acid-Treated Shrimp Meal on Laying Performance and Egg Quality of Laying Hens

Abstract

This study was conducted to measure the effects of TSM as a protein source on laying performance and egg quality in laying hens. Fifty-six White Leghorn hens (85-week-old) were placed in individual cages and assigned randomly into 7 dietary groups (8 hens each) having similar body weight (1.6±0.02 kg). The experimental diets were prepared as follows: a control diet, diets containing 5, 10, and 15% of SM and diets containing 5, 10, and 15% of TSM. During the 30 d feeding trial period, birds had free access to feed and water. Egg production performance and egg quality parameters were measured during the experimental period. The results indicated that hen-day egg production, egg mass and FI decreased with increasing levels of the SM (P<0.05). FCR was deteriorated with increasing levels of the SM (P<0.05). However, all TSM groups did not show such detrimental effects. Egg weight, shell thickness and specific gravity did not differ significantly (P>0.05) among the dietary groups. The eggshell strength improved significantly (P<0.05) with increasing level of the SM and TSM in comparison to the control group. Moreover, egg yolk colour was significantly (P<0.05) increased with increasing levels of the SM and TSM.

In conclusion, hens received diets containing TSM showed better laying performance along with improved shell strength and yolk colour, suggest that formic acid-treated SM is promising as a potential protein in laying hen diets.

Introduction

In chapter II discussed the improvement of nutritional quality of SM: a chemical treatment had been applied and found that formic acid-treated SM can be more digestible protein source than SM. Accordingly, chapter III confirmed that TSM had the beneficial effects on acceptability of the diets which showed the better FI in laying hens compared to SM-based diet and comparable to control diet, and suggest that this SM can be included in laying hen diets without showing any detrimental effects. Consequently, Fox *et al*. (1994) reported that formic acid treatment have an important impact on the physical and biochemical properties of SM, and resulted an improvement in growth and survival of juvenile *Penaeus monodon*. In addition, it is well-known that SM contains high level of Ca (Ngoan *et al*., 2000; Khampaka *et al*., 2006a; Rahman and Koh, 2014) and carotenoid pigment (astaxanthin) (Chawan and Gerry, 1974; Gernat, 2001; Khempaka *et al*., 2006b): the former can improve egg shell quality and the latter yolk colour. Therefore, these two components may be remaining unchanged even after treatment and hence it is interesting to use this SM as a protein source for laying hen diets.

The purpose of the present study was to evaluate the effects of dietary TSM on laying performance, and egg quality in laying hens, and discussed the suitability of TSM as a good protein source of laying hen diets.

Materials and Methods

This research was conducted in accordance with guidelines for regulation of animal experimentation of Shinshu University, Japan.

Preparation of Treated SM

The TSM was prepared from heads and hulls of black tiger shrimp (*Penaeus monodon*), as explained detail in chapter II, and the data on proximate components, Ca, phosphorus, chitin, and astaxanthin content of the SM and TSM were quoted from the chapter III, and used to formulate the laying hen diets (Table 5).

Experimental Birds, Diets and Feeding

A total of 56 Single Comb White Leghorn hens (85 weeks of age) were obtained from a commercial laying facility. The birds were selected on the basis of having similar rates of egg production and body weight. After that, they were randomly distributed into seven dietary groups (8 birds each), and placed in individual cages. The birds were given a 7 d adaptation period prior to the initiation of the experiment. Seven diets (1 control diet, 3 SM diets, 3 TSM diets) containing a commercial layer diet (CP≥170 g, ME≥2850 kcal/kg, Nippon Formula Feed Mfg. Kanagawa, Japan) as a basal diet, were prepared (Table 6). In experimental diets, SM and TSM were added at 5, 10, and 15% of the diet at the expense of soybean meal. Levels of corn and corn oil in experimental diets were also changed to adjust the chemical composition. All diets (approximately 175 g of CP and approximately 2935 kcal/kg of ME) were formulated to meet or slightly exceed the nutrient requirements of laying hens defined by Japanese feeding standard for poultry (2011), and fed for 30 d experimental periods. Lighting was provided for 16 h/d, and had free access to feed and water throughout the experimental period.

Laying Performance Measurements

Body weight was obtained by weighing hens at the beginning and end of the experiment. FI and egg weight were recorded daily. Egg production was recorded daily and egg mass was calculated from collected data of egg production and egg weight, using the following equation: egg mass= (egg production × egg weight)/day. FCR was also calculated.

Egg Quality Measurements

The eggs were analysed to determine the eggshell quality (shell breaking strength, shell weight and shell thickness) of all collected eggs produced during the 10 day of experimental period. Eggshell breaking strength (kgf/cm^2) was measured using force gauge (IMADA ZT series, IMADA Co. Ltd., Japan). Eggs were then broken and then eggshell, albumen and yolk were separated. Eggshells were rinsed in running water and dried at 100°C for 2 hrs and shell thickness (excluding the inner shell membranes) was measured at three different points on the eggs (one point on the air cell, and two randomised points on the equator) using a micrometer (Mitutoyo 700-119-20, Mitutoyo Corporation, Japan). Eggshells were weighed using an electric balance. Specific gravity was measured by the floating method using NaCl solutions (10, 11, 12, 13, 14, and 15% of NaCl) ranging in specific gravity from 1.056 to 1.084. Pigmentation of yolk colour was measured visually by the Roche yolk colour fan (F. Hoffman-La Roche Ltd., Basel, Switzerland) having 15 sample colours of ranging from 1 (the lightest) to 15 (darkest), and was scored by means of a colour fan.

Statistical Analysis

Results were presented as means ± SE. All the data were statistically analysed with ANOVA using JMP software version 12. 0 (SAS Institute, 2012) and significant differences among the dietary groups were evaluated by Tukey's multiple comparison tests. Statements of statistical significance were based on $P<0.05$.

Results

Laying Performance (Table 8)

None of the laying hens died, all appeared to remain healthy throughout the entire experimental period. Hen-day egg production and egg mass were decreased with increasing levels of the SM ($P<0.05$), but not in the TSM group. Consequently, FI was significantly ($P<0.05$) decreased in layers fed diets containing SM compared to the corresponding value of control and TSM groups. As the results, FCR was found to be significantly ($P<0.05$) deteriorated in SM group, and markedly lowest value was observed in 15% SM group. On the other hand, in comparison to control group, TSM group exhibited non-significant effects on FCR, even though the diets contained at least up to the level of 15% TSM. All layers irrespective of treatment exhibited non-significant weight losses ($P>0.05$). Taking into account, the safer level of SM in laying hen diets seems to be 15% of the TSM.

Egg Quality (Table 9)

In all experimental groups, egg weight, shell thickness and specific gravity traits were statistically comparable ($P>0.05$). However, egg shell strength and shell weight were increased dependent on increasing levels of dietary SM and TSM ($P<0.05$). The yolk colour was significantly ($P<0.05$) increased with the increasing levels of dietary SM and TSM, where dietary levels of 15% SM and TSM groups showed the highest score.

Table 8. **The effect of dietary untreated and treated shrimp meal on laying performance of laying hens**[1]

Parameters	Control	SM[2] (%)			TSM[3] (%)		
		5	10	15	5	10	15
Hen-day egg production, %	87.5±0.91[a]	85.8±3.77[ab]	77.2±2.05[bc]	68.5±2.35[c]	87.9±1.72[a]	88.4±0.63[a]	84.5±2.35[ab]
Egg mass, g/hen/day	61.4±1.15[ab]	60.3±3.16[ab]	53.8±1.78[bc]	50.0±1.20[c]	62.8±1.56[a]	62.3±0.87[a]	60.1±2.52[ab]
Feed intake, g/hen/day	109.8±0.76[a]	109.1±0.40[ab]	106.5±0.25[bc]	104.7±0.27[c]	109.9±0.56[a]	111.1±0.42[a]	111.7±1.13[a]
FCR, g feed/g egg	1.87±0.05[a]	1.93±0.06[a]	2.03±0.05[a]	2.45±0.05[b]	1.87±0.08[a]	1.84±0.06[a]	1.98±0.11[a]
Body weight change, g/hen	-80±0.04	-58±0.05	-113±0.17	-57±0.08	-34±0.04	-51±0.06	-35±0.05

[1]Values for each parameter represent mean ± SE values with 8 observations.

[2]SM=untreated shrimp meal; [3]TSM=treated shrimp meal.

[a-c]Means in a row with different superscripts are significantly different ($P<0.05$).

Table 9. **The effect of dietary untreated and treated shrimp meal on egg quality of laying hens**[1]

Parameters	Control	SM[2] (%)			TSM[3] (%)		
		5	10	15	5	10	15
Egg weight, g	70.1±1.36	70.3±1.98	69.7±1.52	70.2±1.57	71.4±0.81	70.6±0.93	70.8±1.35
Shell strength, kgf/cm	4.5±0.08^{a}	4.7±0.07^{abc}	5.1±0.02^{d}	5.2±0.11^{d}	4.7±0.12^{ac}	4.9±0.06^{bcd}	5.0±0.11^{bd}
Shell thickness, mm	0.54±0.01	0.55±0.02	0.54±0.03	0.58±0.01	0.56±0.01	0.56±0.01	0.57±0.01
Shell weight, g	5.5±0.12^{a}	5.9±0.17^{ab}	6.3±0.15^{b}	6.3±0.11^{b}	5.9±0.16^{ab}	6.1±0.11^{b}	6.2±0.10^{b}
Yolk colour[4]	9.1±0.35^{a}	11.2±0.13^{b}	12.1±0.42^{c}	12.3±0.16^{c}	11.2±0.21^{b}	11.7±0.16^{bc}	12.0±0.68^{c}
Specific gravity	1.069±0.001	1.071±0.001	1.069±0.001	1.070±0.001	1.069±0.001	1.069±0.001	1.069±0.001

[1]Values for each parameter represent mean ± SE values with 8 observations.

[2]SM=untreated shrimp meal; [3]TSM=treated shrimp meal; [4]Roche yolk colour fan, yolk colour ranges from 1 to 15 units.

[a-d]Means in a row with different superscripts are significantly different (P<0.05).

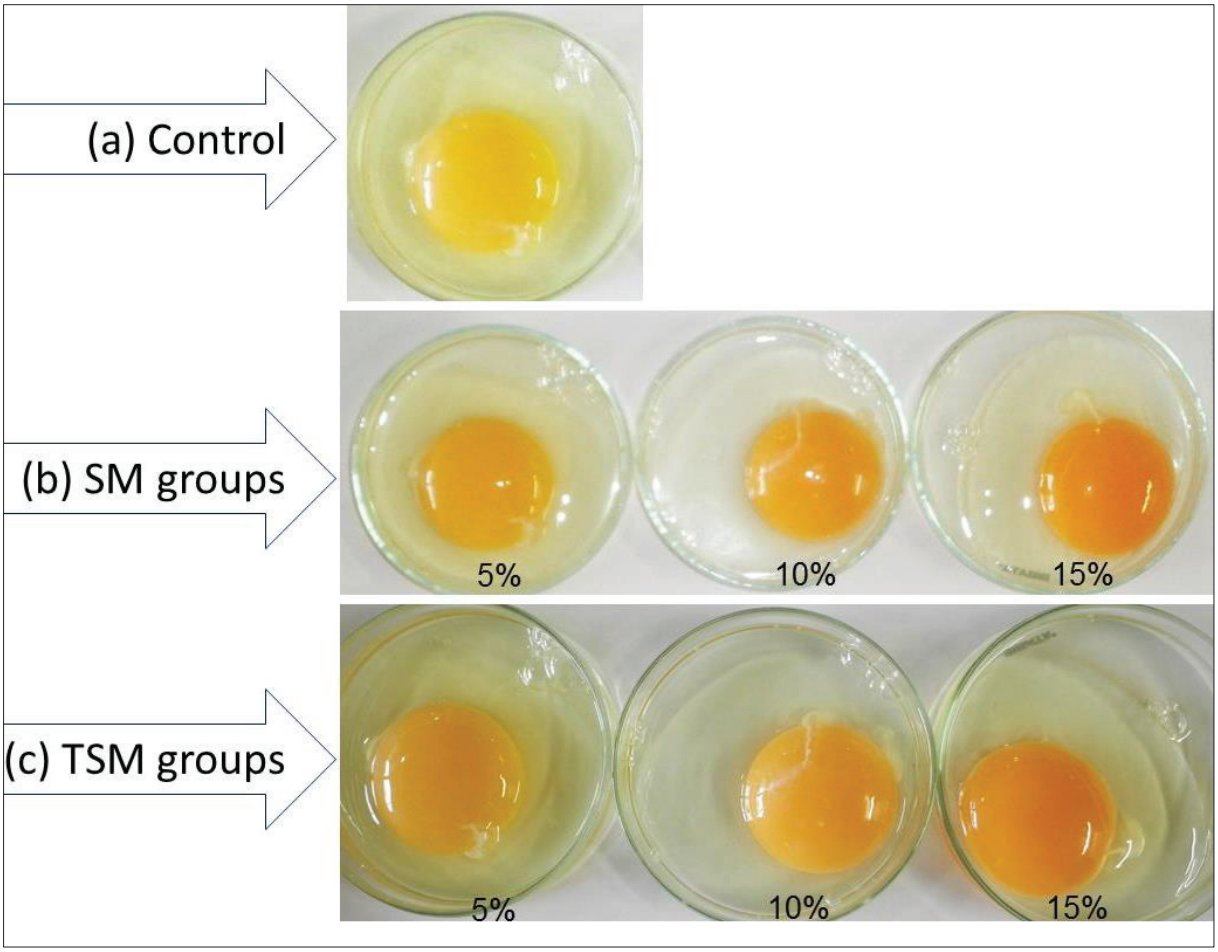

Fig. 3 Egg yolk colour: (a) control, (b) SM, and (c) TSM.

Discussion

Laying Performance

Hen-day egg production, egg mass FI decreased and FCR increased significantly with increasing levels of the dietary SM (Table 8). Similar observation was reported by Oduguwa *et al*. (2005): laying performance was impaired when hens received a diet containing high (20%) levels of the SM. There may be two reasons why laying performance decreased in the SM group: one is decreased digestibility, and another decreased FI. The former may result, in part, from chitin in the SM, which has been reported to decrease DM digestibility in broilers by means of its own low digestibility (Khempaka *el al*., 2006a). This idea is compatible with the result of FCR which was impaired with increasing level of the SM. The latter may not be due to chitin, because chitin affected little to FI in broilers (Khempaka *et al*., 2006b). Although there are some reports showing that the SM led to decreased FI in chickens (Oduguwa *et al*., 2004; Khempaka *et al*., 2006a), and also confirmed the results made by chapter III.

In contrast, all TSM groups did not show such detrimental effects observed in the SM group (Table 8). This can be explained, in part, by the decreased level of chitin, a factor to decrease digestibility, in the TSM. Interestingly, FI did not decrease even in 15% TSM group, which suggests that formic acid treatment can remove or degraded not only chitin but also a FI decreasing factor in the SM. The similar results were observed in chapter III. Moreover, all birds irrespective of treatment exhibited non-significant weight losses (P>0.05). Taking these findings into account, it seems that dietary TSM can improves the laying performance of layers by reducing the effects of the anorectic and anti-digestive factors contained in

the SM, and this SM can be included at least up to the level of 15% in laying hen diets.

Egg Quality

There were no significant effects ($P<0.05$) of the diets containing the SM and TSM over the control diet on egg weight, shell thickness, and specific gravity (Table 9). According to Adams and Bell (1998), the larger size of the eggs produced by aged laying hens also results in lower eggshell quality, but the fact was partially different in this study: along with the larger size of eggs, the eggshell strength was increased significantly ($P<0.05$) with increasing the inclusion levels of the dietary SM and TSM (Table 9). Similarly, dietary SM and TSM groups exhibited increased ($P<0.05$) eggshell weight over the control. Since Ca was contained at the same level in all dietary groups, improved eggshell strength in the SM and TSM groups may be explained, in part, by the difference in Ca availability between Ca in SM and reagent $CaCO_3$: Scott *et al*. (1971) attributed the improved eggshell strength obtained from feeding oyster shell than pulverised $CaCO_3$ source, because oyster shell remained longer time (24 hrs.) in the intestinal tract than the ground Ca source (14-16 hrs.). Furthermore, increasing dietary Ca from 24-25 to 36-40 g/kg improved egg production, shell weight and shell thickness in aged laying hens (Bar *et al*., 2002) and similar results obtained in the present study where Ca level in the diet was within the range (Table 6). Considerable point is that the eggshell quality was remained unchanged when diets contained the SM and TSM in aged laying hens.

In addition, yolk colour increased significantly ($P<0.05$) with increasing levels of the SM and TSM, which is compatible with the finding of Gernat (2001) for laying hens given dietary SM. Increase of yolk colour is a secondary effect of the SM as a protein source, and taking practical use of the TSM as an ingredient of

laying hen diet, this nature gives an additional value to the TSM. Such increased yolk colour may be due to the increased level of astaxanthin in the diets (Table 6), because it is well known that this pigment can increase the yolk colour (Anderson *et al*., 2008). However, astaxanthin level decreased by formic acid treatment, but about 60% of that was retained after the treatment. Similar observation has been reported by Torrissen *et al.* (1981) and Fox *et al.* (1994).

Conclusion

The results obtained here confirmed that laying hens received high levels of dietary SM, such as at and above 5%, exhibited adverse effects on hen-day egg production and FI when compared to the corresponding values of the control group. On the contrary, all TSM groups did not show such detrimental effects observed in the SM groups and compared to the control group. Interestingly, the eggshell strength and yolk colour were significantly increased with increasing levels of the dietary SM and TSM over the control group. In conclusion, it is suggested that TSM can be used as a potential protein source in laying hen diets without showing any negative effects on laying performance and egg quality, even though it was included up to the level of 15% in the diets.

Chapter V

Effects of Formic Acid-Treated Shrimp Meal on Palatability of Broilers

Abstract

The purpose of this study was to investigate the effects of SM and TSM on palatability of diets using a choice feed preference test. A total of 20 male broiler chicks (15 d old, Ross 308) were distributed in five pens (4 chicks each) and freely fed, a control diet, diets containing SM (5, 10, and 15%) and TSM (5, 10, and 15%) for 7 d experimental period. The intake of each diet was measured daily, and intake of each as a proportion of total diet intake calculated. The results indicated that chicks fed the diets containing SM, FI decreased significantly and the poorest value observed in 15% SM group, which praised that diet containing SM reduced acceptability as compared to that of the control (P<0.05). In contrast, diets containing TSM resulted in a no significant effect on acceptability as compared to control group (P>0.05) even though it was included up to the level of 15% TSM. Similar results were also perceived for feed preference, where SM groups showed poor preference than that of the corresponding value of the control and TSM groups (P<0.05). These results suggest that TSM-based diets appeared to improve their palatability when compared with the SM-based diets in broilers.

Introduction

The voluntary intake of feed is an extremely important factor, which often determines the quality of nutrients that the birds obtain from their diets when fed *ad libitum*. The nutritive value of each feedstuff, palatability is an important factor affecting the utilisation of diet. However, in some circumstances the birds are not able to select feeds effectively either due to palatability or composition effects. The recent development in using by-products and waste products may affect palatability of the ration. This should be kept in mind because a reduction in feed consumption will lead to a lower growth rate of broilers or a decreased egg production of layers. Similarly, Oduguwa *et al*. (2005) reported that SM contained high proportion of exoskeleton which showed markedly reduced feed intake in layers. Although there has been controversy (Fox *et al*., 1994), who reported that SM in fish diets appeared to improve their palatability when compared with the fish meal based diet. Furthermore, chapter III studied the palatability of dietary TSM for laying hens, and found that FI and preference of dietary TSM was better than dietary SM. Therefore, studies conducted on feeding SM based diet to the animal have resulted in conflicting conclusions. Dietary self-selection or choice feeding has been suggested as a means of estimating nutrients needs of broilers (Hughes, 1984; Rose and Kyriazakis, 1991). In this regards, choice feeding is applied to determine the likes and dislikes of feedstuff for animals, which believe that TSM seemed to be more acceptable in broilers. Accordingly, this study was conducted to investigate the palatability of dietary SM and TSM in broilers (choice-feeding), and discussed their practical use in chicken diets.

Materials and Methods

This research was conducted in accordance with guidelines for regulation of animal experimentation of Shinshu University, Japan.

Preparation of Treated SM

The TSM was prepared from heads and hulls of black tiger shrimp (*Penaeus monodon*), as explained detail in chapter II. Proximate components, Ca, phosphorus, and chitin content of the SM and TSM were analysed according to AOAC (1990) and Ghanem *et al*. (2003) methods, respectively (Table 10).

Birds, Diets and Sampling

A total of 20 male broiler chicks (15 d old, Ross 308) were randomly distributed in five pens based on their similar body weight. Each pen provided septuplicate identical feeders placed inside the pen. Seven experimental diets (1 control diet, diets containing 5, 10, and 15% SM, and diets containing 5, 10, and 15% TSM) were allocated randomly to each feeder and were provided therein *ad libitum* for the 7 d experimental period. Diets (approximately 3180 kcal/kg of energy and approximately 235 g/kg of CP) were formulated to meet or exceed the nutrient requirements for broilers (Japanese feeding standard for poultry, 2011) (Table 11).The feeding trial was conducted on the basis of free-choice system. Feed was exchanged between feeders in each pen daily to avoid potential bias due to feeder or feeder position. Each pen was separated by using a wood board to defend the birds to see next to the pen diets.

Data Recording

The amount of feed consumed from each feeder was determined every day and results were expressed on the basis of the average intake per birds. Feed

preference was defined as the amount of test diet consumed expressed as the percentage of total feed consumption.

Statistical Analysis

Data were initially analysed with ANOVA using JMP version 10.0 (SAS Institute, 2012) and significant differences among the dietary groups were evaluated with Tukey's multiple comparison tests. Statements of statistical significance are based on $P<0.05$.

Table 10. **Chemical composition of untreated and treated SM and soybean meal (air dry matter basis)**

Components	SM[1]	TSM[2]	Soybean meal[3]
	g/kg		
Crude protein	454	533	450
Crude fibre	159	145	53
Ether extract	36	42	19
Ash	285	163	64
Chitin	173	153	-
Calcium	89	68	3.7
Phosphorus	19	11	7.2
ME, kcal/kg	1230[3]	1230[3]	2400

[1]SM=untreated shrimp meal; [2]TSM=treated shrimp meal.

[3]Standard Tables of Feed Composition in Japan (NARO, 2009).

Table 11. **Ingredients and chemical composition of experimental diets (g/kg)**

Items	Control	SM[1] (%)			TSM[2] (%)		
		5	10	15	5	10	15
Ingredients							
Commercial diet[3]	550	550	550	550	550	550	550
Soybean meal	185	135	88	42	130	78	25
Corn	239	225	210	193	234	222	214
Shrimp meal	0	50	100	150	50	100	150
Corn oil	10.5	24.5	36.5	49.5	20.5	34.5	45.5
Premix[4]	15.5	15.5	15.5	15.5	15.5	15.5	15.5
Calculated composition (g/kg, as fed basis)							
ME, kcal/kg	3180	3180	3174	3173	3182	3180	3175
Calcium	10.8	9.5	13.7	17.9	8.5	11.5	14.7
Available P	4.8	5.3	6.3	6.7	4.8	5.1	5.5
Analysed composition (g/kg, as fed basis)							
Crude protein	236	235	234	234	235	236	235
Crude fibre	39.7	44.7	45.9	55.1	43.5	47.4	51.4
Ash	49.5	60.9	72.4	83.9	54.8	60.2	65.6
Chitin	0	9.2	17.9	26.6	8.1	15.7	23.6

[1]SM=untreated shrimp meal; [2]TSM=treated shrimp meal.

[3]Broiler starter diet (CP≥23.5%, ME≥3050 kcal/kg, Nippon Formula Feed Mfg. Kanagawa, Japan).

[4]Premix (units/ kg): vitamin A, 5,00,000 IU; vitamin D_3, 1,00,000 IU; vitamin E, 50 IU; vitamin K_3, 100 mg; vitamin B_1, 800 mg; vitamin B_2, 600 mg; vitamin B_6, 600 mg; vitamin B_{12}, 5.4 mg; pantothenic acid, 800 mg; nicotinic acid, 800; choline chloride, 20,000 mg; foliate, 104 mg; phosphorus, 106 g; iron, 2 mg; copper, 362 mg; zinc, 3368 mg; manganese, 2,560 mg; iodine, 45 mg.

Results

Palatability (Table 12)

The results showed that when chicks were given the choice between the control diet and the diet containing SM and TSM, they consumed the control and TSM-based diets exclusively during the entire experimental period. However, FI was decreased with increasing level of the SM in the diets ($P<0.05$). On the other hand, this value tended to show no significant difference in the TSM groups when compared with the control. Moreover, the preference of control diet, which was compatible to the diets contained TSM ($P>0.05$). But, these values were decreased with increasing of SM in the diets ($P<0.05$). Overall, the acceptability of TSM-based diets was better than SM-based diets and comparable to the control group.

Table 12. **The effects of untreated and treated shrimp meal on palatability of broilers**[1]

Treatments	Feed intake, g/b/d	Preference[2], %
Control	13.85±0.17[a]	14.75±0.09[a]
5% SM[3]	13.54±0.32[ab]	14.45±0.28[ab]
10% SM	12.73±0.11[bc]	13.58±0.13[bc]
15% SM	12.31±0.40[c]	13.16±0.39[c]
5% TSM[4]	14.01±0.21[a]	14.94±0.26[a]
10% TSM	13.78±0.11[ab]	14.69±0.12[a]
15% STM	13.52±0.16[ab]	14.42±0.22[ab]

[1]Values for each parameter represent mean ± SE values with 5 observations.

[2]Preference= (g of experimental diet consumed/g of total diet consumed) ×100

[3]SM=untreated shrimp meal; [4]TSM=treated shrimp meal.

[a-c]Means within the same column with different superscripts are significantly different (P<0.05).

Discussion

Palatability

The results obtained here revealed that FI and feed preference were decreased with increasing level of the dietary SM probably because of the high proportion of ash components in SM than TSM (Table 10). This could have caused discomfort for the birds. In this regards, it has been reported that high levels (1.3-1.5%) of Ca decreased FI in chickens (Smith and Taylor, 1961; Watkins *et al*., 1989). Considering the results of them, Ca in SM and TSM diets described in chapters II and III may be reasonable for a decrease in FI since the Ca levels of 5, 10, and 15% SM and TSM diets were 0.95, 1.37, 1.79, 0.85, 1.15, and 1.47%, respectively. In addition, a palatability test was performed with laying hens in chapter III and confirmed that dietary TSM was more palatable than SM. Consequently, laying performance and egg quality of laying hens fed diets containing SM and TSM were examined in chapter IV and found that FI was greater in TSM groups than SM groups. Similarly, Fox *et al*. (1994) reported that cultured fishes accepted SM diet and their intake increased when SM was treated with formic acid than fishmeal-based diet. These results may believe that formic acid treatment can remove or degrade a FI decreasing factor(s) in the SM, which enhance the acceptability to the chickens. Thus, improved performance may result from the greater rates of consumption of TSM based-diets when birds are fed free-choice, and in consequence increased FI due to improved feed palatability conferred by TSM.

Conclusion

The results obtained here revealed that FI and preference of feed were decreased significantly with increasing levels of the dietary SM when compared with the control and TSM groups. In contrast, the TSM groups did not exhibit such detrimental effects observed in the SM groups and comparable to the control group, even though it was included up to the level of 15% in the diets. Therefore, it may conclude that the formic acid treatment is promising to remove or degrade a FI decreasing factor(s) in SM and subsequently increased the palatability which suggests that TSM can be used as potential source of protein for broiler diets.

Chapter VI

Effects of Formic Acid-Treated Shrimp Meal on Growth Performance, Nutrient Digestibility and Carcass Quality of Broilers

Abstract

This study was conducted to know the effect of formic acid-treated shrimp meal as a protein source on growth performance, digestibilities, N retention, and carcass quality for broilers. Forty-two male broiler chicks (8 d old, Ross 308) were randomly divided into 7 dietary groups (6 birds each), namely control diet, diets containing 5, 10, and 15% of SM, and diets containing 5, 10, and 15% of TSM and offered diets till 35 d old. Final body weight, body weight gain and feed intake decreased significantly with increasing levels of SM in diets. FCR also decreased with increasing levels of the SM ($P<0.05$). Similar trend was observed in the TSM group, but the adverse effects of the TSM were milder in comparison to the SM group ($P<0.05$). The DM digestibility tended to decrease ($P<0.05$) with increasing levels of the SM but unchanged with increasing level of the TSM. Availability of ash decreased with increasing levels of the SM and TSM in diets ($P<0.05$). Although N retention decreased ($P<0.05$) with increasing level of the SM and TSM in diets but the decreasing trend was milder in the TSM groups than the SM groups. Moreover, chitin digestibility was significantly greater in the TSM groups than the SM groups. In addition, there were no significant effects on carcass traits among the dietary treatment groups ($P>0.05$).

In conclusion, broilers received diets containing the TSM showed better growth performance along with improved nutrient digestibility and N retention, which suggests that formic acid-treated SM can be used as a potential protein source in broiler diets.

Introduction

In general, the nutritional quality of SM, as a protein source for chicken diets, is poor, although it depends on the species of shrimp and the body parts used (Meyers, 1986; Ngoan *et al*., 2000; Rahman and Koh, 2014). The maximum feed inclusion levels for shells and heads of black tiger shrimp (*Penaeus monodon*), and heads and white leg shrimp (*Litopenaeus vannamei*) were 4, 5, and 10%, respectively (Khempaka *et al*., 2006a; Rahman and Koh, 2016). Some researchers have reported that these limited inclusion levels could be explained, in part, by the presence of chitin, which can decrease digestibility in broilers (Khempaka *et al*., 2006a) and rats (Oduguwa *et al*., 1998). In this regard, *in vitro* study (chapter II) clarified that formic acid could successfully reduce the chitin level in SM, and increase the digestibilities for TSM than for SM. Moreover, the beneficiary effects of the dietary TSM on laying performance, along with improved eggshell strength, and yolk colour were observed in chapter IV. Therefore, it has been hypothesised that TSM might be a promising protein source for broiler diets.

The purpose of the present study was to measure growth performance, nutrient digestibilities, N retention, and carcass quality in broilers that received diets containing SM and TSM, and to discuss the suitability of this SM as a potential protein source for broilers.

Materials and Methods

This research was conducted in accordance with guidelines for regulation of animal experimentation of Shinshu University, Japan.

Preparation of Treated SM

The TSM was prepared from heads and hulls of black tiger shrimp (*Penaeus monodon*), as explained detail in chapter II, and the data on proximate components, Ca, phosphorus, chitin, and astaxanthin content of the SM and TSM were quoted from the chapter V, and used to formulate broiler diets (Table 10).

Birds, Diets and Sampling

Forty-two male broiler chicks (8 d old, Ross 308) were distributed into seven dietary groups based on similar body weight (BW). A control diet, diets containing 5, 10, and 15% of SM, and diets containing 5, 10, and 15% of TSM were prepared. In the SM and TSM diets, SM was included mainly as a substitute for soybean meal. Corn and corn oil were also used to adjust the nutrient requirements. Diets (approximately 3180 kcal/kg of energy and approximately 235 g/kg of CP) were formulated to meet or exceed the nutrient requirements for broilers (Japanese feeding standard for poultry, 2011) (Table 11). Diets and water were provided *ad libitum* for the 28 d experimental period (from 8 to 35 d old). BW and feed intake (FI) were recorded weekly and daily, respectively. Feed conversion ratio (FCR) was also calculated. Excreta were collected from 32 to 35 d of age and stored in a freezer (-20°C) until analysis.

Chemical Analysis

DM and ash in diets and excreta were measured to estimate their digestibilities according to standard methods (AOAC, 1990). N in diets and excreta

was measured using a CHNS/O analyser (PerkinElmer 2400 Series II), and chitin in excreta was analysed according to the method of Ghanem *et al*. (2003) to estimate their retention and digestibility, respectively.

Statistical Analysis

Data were initially analysed with ANOVA using JMP version 10.0 (SAS Institute, 2012) and significant differences among the dietary groups were evaluated with Tukey's multiple comparison tests. Statements of statistical significance are based on $P<0.05$. Further, regression analyses were performed to determine the relationships between dietary chitin levels, and digestibilities and N retention.

Results

Growth Performance (Table 13)

The results showed that final BW and body weight gain (BWG) in control group were 2136 g and 1945 g, respectively. There tend to be no significant (P>0.05) differences in the final BW and BWG of broilers fed on up to 10% of the TSM diets relative to the control. However, these parameters decreased with increasing levels of the SM and the lowest values were recorded on 15% SM group which grew almost 82.3% of the control (P<0.05). Therefore, FI decreased with increasing levels of the SM in the diets. This value tend to show no significant (P>0.05) difference in the TSM groups when compared with the control. Broilers fed 15% of the TSM had a significantly (P<0.05) inferior FCR relative to the control but compared statistically when diet containing 5% SM.

Digestibilities and N Retention (Table 14)

In control group, DM and ash digestibilities were 79% and 43%, respectively. These values tend to decrease significantly (P<0.05) with increasing levels of the SM in diets when compared with the control. In contrast, TSM groups did not show such tendency in relative to the control (P>0.05). Chitin digestibility was greater overall in the TSM (25.1% - 33.6%) than the SM (19.3% - 29.3%). Therefore, N retention decreased with increasing levels of the SM in diets and the lowest value (54%) was perceived in 15% SM group (P<0.05). Overall, the nutrient digestibility and retention of broiler fed diets containing TSM showed better results than that of the SM groups and comparable to the control.

Carcass Quality (Table 16)

Carcass weight decreased significantly ($P<0.05$) with increasing levels of the SM in the diet where the lowest value recorded in 15% SM group. However, this value tend to show no significant ($P>0.05$) difference in the TSM groups when compared with the control group. Accordingly, the percentage of carcass and giblets weight did not differ significantly ($P>0.05$) between the treatment groups by inclusion of the SM and TSM. On the other hand, abdominal fat percentage was numerically higher ($P>0.05$) in the SM and TSM fed groups compared to control group, where 15% SM had a highest value (1.5%). Relative weight percentage of head and shank were not affected by the addition of the SM and TSM in the diets. The intestinal length also did not differ significantly ($P>0.05$) among the treatment groups.

Table 13. **The effects of dietary untreated and treated shrimp meal on growth performance in broilers**[1]

Parameters	Control	SM[2] (%)			TSM[3] (%)		
		5	10	15	5	10	15
Final BW, g	2135.8±14.1[a]	2094.2±14.4[ab]	1923.3±33.8[cd]	1795.0±38.7[d]	2188.7±14.7[a]	2120.3±46.4[ab]	1995.9±21.9[bc]
BWG, g	1944.6±12.4[a]	1912.0±14.9[ab]	1738.6±32.8[c]	1601.3±37.9[d]	2001.3±15.0[a]	1915.5±51.1[ab]	1803.6±20.8[bc]
Feed intake, g/bird/d	111.5±0.8[a]	111.2±0.7[a]	105.9±1.7[bc]	104.4±1.4[c]	112.9±0.6[a]	110.7±1.6[ab]	108.5±0.8[abc]
FCR, g feed/g BW	1.61±0.01[ad]	1.63±0.01[ad]	1.71±0.01[b]	1.83±0.03[c]	1.58±0.02[a]	1.62±0.02[ad]	1.67±0.05[bd]

[1]Values for each parameter represent mean ± SE values with 6 observations.

[2]SM=untreated shrimp meal; [3]TSM=treated shrimp meal.

[a-d]Means in a row with different superscripts are significantly different (P<0.05).

Table 14. **The effects of dietary untreated and treated shrimp meal on nutrient digestibilities and N retention in broilers**[1]

Parameters	Control	SM[2] (%)			TSM[3] (%)		
		5	10	15	5	10	15
DM digestibility, %	78.5±0.53[a]	77.2±0.48[ab]	74.3±0.75[bc]	73.2±0.98[c]	77.7±0.76[a]	76.8±0.82[ab]	75.8±0.84[abc]
Ash digestibility, %	42.5±0.53[a]	41.4±0.39[ab]	35.7±0.55[c]	30.3±0.37[d]	42.1±0.43[ab]	40.4±0.40[b]	37.4±0.26[c]
Chitin digestibility, %	-	29.3±0.38[a]	25.5±0.66[c]	19.3±0.55[d]	33.6±0.77[b]	28.5±0.51[a]	25.1±0.61[c]
N retention, %	68.1±0.23[a]	65.4±0.31[b]	58.1±0.17[c]	53.7±0.39[d]	68.2±0.29[a]	66.8±0.49[ab]	66.0±0.42[b]

[1]Values for each parameter represent mean ± SE values with 6 observations.

[2]SM=untreated shrimp meal; [3]TSM=treated shrimp meal.

[a-d]Means in a row with different superscripts are significantly different (P<0.05).

Table 15. **Results of the regressions of digestibilities and N retention on chitin levels in untreated and treated shrimp meal diets**[1]

Parameters	Slope		Intercept	
	SM[2]	TSM[3]	SM	TSM
DM digestibility, %	-2.27±0.44[a]	-1.22±0.21[b]	78.9±0.85	78.7±0.35
Ash digestibility, %	-7.27±0.42	-5.95±0.63	47.1±0.79	49.2±1.07
Chitin digestibility, %	-5.73±0.47	-5.52±0.59	34.9±0.91	37.7±1.00
N retention, %	-6.25±0.29[a]	-4.81±0.31[b]	69.9±0.56	72.3±0.53

[1]Values for each parameter represent mean ± SE values with 6 observations.

[2]SM=untreated shrimp meal; [3]TSM=treated shrimp meal.

[a-b]Means within the same row with different superscripts are significantly different ($P<0.05$).

Table 16. **The effect of untreated and treated shrimp meal on carcass quality of broilers**[1]

Parameters	Control	SM[2] (%)			TSM[3] (%)		
		5	10	15	5	10	15
Live weight, g	2135.8±14.1[a]	2094.2±14.4[ab]	1923.3±33.8[cd]	1795.0±38.7[d]	2188.7±14.7[a]	2120.3±46.4[ab]	1995.9±21.9[bc]
Carcass weight, g	1442.5±34.7[a]	1417.7±25.3[ab]	1271.7±47.4[bc]	1175.8±21.9[c]	1495.7±41.0[a]	1415.8±41.4[a]	1338.3±31.9[a]
Intestinal length, cm	150.2±0.79	152.3±4.16	154.5±7.04	147.3±2.56	144.8±2.90	154.3±3.24	156.5±1.84
				% of live weight			
Carcass[4]	67.5±1.27	67.7±1.36	66.0±1.40	65.5±0.63	68.4±1.74	66.8±1.00	67.0±1.03
Giblets	3.7±0.13	3.6±0.12	3.7±0.13	3.5±0.05	3.8±0.13	3.6±0.15	3.7±0.08
Abdominal fat	1.3±0.12	1.2±0.12	1.4±0.16	1.5±0.07	1.2±0.08	1.3±0.12	1.4±0.12
				% of eviscerated carcass			
Head	3.7±0.16	3.8±0.12	3.6±0.19	4.0±0.24	3.8±0.16	3.9±0.16	4.1±0.24
Shank	6.5±0.13	6.5±0.12	6.8±0.18	6.9±0.14	6.2±0.05	6.8±0.08	6.9±0.36

[1]value for each parameter represents mean ± SE values with 6 observations.

[2]SM=untreated shrimp meal; [3]TSM=treated shrimp meal.

[4](carcass weight without internal organs, head and shank/live weight) ×100.

[a-d]Means in a row with different superscripts are significantly different (P<0.05).

Discussion

Growth Performance

In the control group, final BW, BWG, FI, and FCR were similar to those noted in the broiler performance objectives (Aviagen, 2007), but these values deteriorated, dose-responsively, with increasing levels of the SM. Rahman and Koh (2016) reported similar findings, noting decreased growth performance for broilers that received diets containing more than 5% SM. These results suggest that decreased growth performance in the SM group may be, in part, due to decreased FI, and that SM contains one or more anorectic factors. Similarly, chapter V confirmed that FI was decreased with increasing levels of SM than TSM in the diets. Regarding the FCR, generally, this value improves when FI decreases (Rosenfeld *et al*., 1997; Gernat, 2001; El-Ghousein and Al-Beitawi, 2009), but in the present study showed the opposite trend, which may be explained by the decreased DM digestibility. On the other hand, in the TSM group, although final BW and BWG decreased with increasing levels of SM, this trend was more pronounced in the SM group. In addition, FI and FCR were better in the TSM group than in the SM group. In this connection, decreased DM digestibility in SM group was restored in TSM group. Based on these results, it appears that the formic acid treatment improves the growth performance of broilers by reducing the effects of the anorectic and anti-digestive factors contained in the SM.

In the present study and the chapter II confirmed that chitin and Ca levels were reduced by formic acid treatment, but both of these constituents may not be the anorectic factor, because of the following reasons: decreased FI was not found in broilers given a diet containing purified chitin at the same levels of as chitin in the SM diets (Khempaka *et al*., 2006b); and increasing the dietary level of Ca up to 2.12%

(Shafey and McDonald, 1990) and 3.0% (Smith and Kabaiji, 1985) did not cause any detrimental effects on FI or the growth performance of broilers.

Digestibilities and N Retention

In the control group, DM and ash digestibilities, and N retention were 78.5, 42.5, and 68.1%, respectively, which are reasonable values for 35 d old broilers (Apata, 2008; Khempaka *et al*., 2011). Similar to the previous results (Fanimo *et al*., 2004; Khempaka *et al*., 2006b), these values in the SM group decreased with increasing levels of SM. Although a similar trend was observed in the TSM group, the trend was less prominent, except for ash digestibility, which was similar between the SM and TSM groups. These results were supported by the *in vitro* study (chapter II), which revealed higher DM and CP digestibilities in the TSM than in the SM. As previously discussed, the higher digestibilities, and N retention in the TSM group may be the reason for the better growth performance in this group.

Chitin digestibility in the SM group ranged from 19.3% (15% group) to 29.3% (5% group), and decreased with increasing levels of SM (Table 14). Similar findings are reported by Rahman and Koh (2016). This trend was also observed in the TSM group, but was less prominent. As previously mentioned, there was lower amount of chitin, the factor responsible for decreased digestibility (Fox *et al*., 1994; Rahman and Koh, 2016), in the TSM than in the SM, and thus it may be interesting to examine whether the improved digestibilities in the TSM group can be explained by the decreased chitin level. Therefore, the regression analyses were conducted to determine the relationships between dietary chitin levels, and digestibilities and N retention in the SM and the TSM groups (Table 15). The results showed that the slopes for DM digestibility and N retention were gentler in the TSM group than in the SM group ($P<0.05$), which not only chitin, but also some other unknown factor(s) may be involved in the improved

digestibility and N retention in the TSM group. The decreased chitin level in the TSM suggests a partially degraded chitin-protein complex in the shrimp shell, which would lead to an increased level of free protein in the shell (*i.e.* a more digestible from of protein).

Carcass Quality

There was significant ($P<0.05$) differences recorded in live weight largely explain the variation observed in carcass weight in the SM groups. This parameter, however, did not change in the broilers fed TSM diets ($P>0.05$). However, percentages of dressing yield decreased numerically, but not significantly, with increasing levels of the SM then TSM in the diets ($P>0.05$). Similarly, SM and TSM had no significant effect on percentage of giblets yield among the dietary treatment groups ($P>0.05$). This agrees with the findings of Fanimo *et al*. (1996), who reported that dressing percentage did not change significantly with increasing level of the SM in the diets. Abdominal fat percentage, however, increased numerically in the SM and TSM groups, but not significantly, with the increasing levels of the SM and TSM in the diets. Although it has been reported that abdominal fat percent was reduced when fed chitin (derived from crustacean) to the broilers (Hossain and Blair, 2007). Furthermore, percentage of head and shank weight did not show significant difference ($P>0.05$) among the dietary groups.

Conclusion

The results obtained here confirmed that broilers received high levels of dietary SM, such as at and above 5%, exhibited adverse effects on body weight gain, FI, FCR and nutrient digestibility when compared to the corresponding values of the control group. Instead, the beneficiary effects on growth performance along with improved nutrient digestibilities and N retention were observed in the TSM groups, which was similar to the control group. In conclusion, it is suggested that TSM can be used as a potential protein source in broiler diets as far as it is included at and below 10% without showing any negative effects on growth performance, nutrient digestibilities and retention.

Chapter VII

General Discussion

There have been two major problems for the poultry producers in the South and South-East Asian countries, like Bangladesh, India, Thailand and Indonesia which are unstable supply and high price of feed ingredients. Accordingly, the formulation of chicken diets has become a difficult task in this region. Consideration is given to the use of unconventional protein source ingredients that are locally available, cheap and safe. In this context, earlier studies have been investigated the utilisation of the SM as a protein source in chicken diets, but the performance differed among reports. This may be due to differences in nutritional quality which depend on shrimp species, waste composition, processing method, and their nutrient availability. Taking this point in consideration, the primary objective of this study was to boost up nutritional quality of SM as a potential protein source for chicken feed, and hence the following trials were conducted to make a concrete conclusion.

At first, chemical composition and *in vitro* digestibility of the SM after receiving autoclaving or chemical treatments have been investigated in chapter II. The results revealed that autoclaving failed to improve the nutritional quality of SM, which may suggest that this level of autoclaving condition is not effective to alter the chemical composition of crustacean meal. NaOH treatment exhibited decreased CP level, increased CA level and unchanged *in vitro* CP digestibility, which was contrast to the results of Septinova *et al*. (2010). However, prolonged incubation time with NaOH improved digestibility of feather meal (Kim *et al*., 2002), which indicates that further study is needed to confirm the effect of NaOH treatment on nutritional quality of SM.

Beneficial effects were obtained in acids treatment: significantly higher CP and lower CA levels were found in both acids-treated SM. This may be resulted from leaching the minerals, such as Ca, in exoskeleton (No *et al*., 1989; Fox *et al*., 1994; Oduguwa *et al*., 1998), and accordingly, relative content of CP increased. Interestingly, formic acid treatment decreased CF and chitin levels, suggested that chitin, main source of CF and possible factor to decrease digestibility (Austin *et al*., 1981; Fanimo *et al*., 2006; Khempaka *et al*., 2006b), was leached from SM by formic acid treatment. In fact, it is suggested that formic acid treatment is promising to improve the nutritional quality of SM and seems to be used as a potential source of protein in chicken diets.

The chemical composition of untreated or treated some unconventional protein sources in previous studies have been reported as follows: squilla meal contains 34% CP and 2% EE (Reddy *et al*., 1997), tannery waste contains 77% CP, 1% CF, 3% EE and 7% ash (Alam *et al*., 2002), silkworm pupae contains 23% CP, 2% CA and 10% EE (Rangacharyulu *et al*., 2003), SM contains 39% CP, 12% CF, 14% CA and 2.7% EE (Fanimo *et al*., 2000), HCl treated SM contains 32% CP, 17% CF, 22% CA (Septinova *et al*., 2010), formic acid-treated SM contains 51% CP, 20% CA and 11% EE (Fox *et al*., 1994), tofu-by product contains 24% CP, 15% fat, 17% CF and 4% ash (Tarachai *et al*., 1999; Tarachai and Yamauchi, 2001). Comparing these by-products, formic acid-treated SM would appear to be a better source of protein, and could be used as a potential protein source in chicken diets.

Based on the results obtained in chapter II, the effects of dietary SM and TSM on palatability (choice feeding) for laying hens was investigated in chapter III. It was observed that the FI and preference of feed decreased with increasing levels of the dietary SM than TSM ($P<0.05$), which means some factor(s) might influence the selection of SM-based diets. In comparison to the control group, all TSM groups did not

show any significant effects on FI and preference of feed (P>0.05). These results may believe that formic acid can remove or degrade the anti-digestive factor(s) of SM and consequently improved its acceptability. In this regards, Alenier and Combs (1981) reported that feeding preferences for diets containing practical feedstuffs popularly associated with unidentified growth factor activities. Thus, these results suggest that formic acid treated SM is more palatable than SM, and it can be used as a good protein source ingredient for chicken feed.

Following the results obtained in chapter III, the effects of dietary TSM as a protein source on laying performance and egg quality in laying hens were investigated in chapter IV. The results indicated that the laying performance was significantly affected in SM groups when it was included at and above 10% in the diets, while all the TSM groups did not show such detrimental effects observed in the SM group, even though it was included up to 15% in the diets (P<0.05). The difference in performances between the SM and TSM groups may be two reasons: one is decreased digestibility, and another decreased FI. The former may result, in part, from chitin in the SM, which has been reported to decrease digestibility in broilers by means of its own low digestibility (Rahman and Koh, 2016). The latter may not be due to chitin, because chitin affected little to FI in broilers (Khempaka *et al*., 2006b), but the factor to decrease FI is still unclear.

The egg quality parameters did not change among the treatment groups, except eggshell strength and yolk colour, which were significantly increased in both SM and TSM groups when compared with the control group. The former can be explained, in part, by the difference in Ca availability between Ca in SM and regent $CaCO_3$, since Ca was contained at the same level in all dietary groups and the latter one responsible for

astaxanthin in SM. Similar results have been reported in laying hen (Gernat, 2001) and in broilers (Chawan and Gerry, 1974; Khempaka *et al*., 2006b).

It has been reported that astaxanthin is a pigment that belongs to the family of the xanthophyll's, and oxygenated derivatives of carotenoids. In addition, one of the most important properties of astaxanthin is its antioxidant properties which have been reported to surpass those of β-carotene or even α-tocopherol (Miki, 1991). It has many high potent pharmacological activities, such as antioxidative activity (Fukuhara *et al*., 1998; Kobayashi, 2000), anti-tumor and anti-cancer effects (Chew *et al*., 1999), and anti- diabetic (Naito *et al*., 2004) and anti-inflammatory actions (Bennedsen *et al*., 1999; Lee *et al*., 2003). Therefore, there is room for future study with astaxanthin as functional foods for human health benefits. In fact, it may conclude that the dietary TSM had the beneficial effects on laying performance, along with improved egg quality, and this SM can be included up to the level of 15% in laying hen diets.

Therefore, the influences of dietary SM and TSM on palatability (choice feeding) in broilers were investigated in chapter V. The results revealed that FI and feed preference were decreased with increasing levels of SM in the diets, and the lowest value found in 15% group ($P<0.05$). It seems likely that SM contain high proportion of CF (mainly as chitin) and CA which reduced the FI in broilers. However, these parameter did not change in TSM groups when TSM level was at and below 15% in the diets. This may be explained, in part, that formic acid treatment can remove the anti-digestive factor(s) contained in the SM, and consequently improved its acceptability. Therefore, it is suggested that TSM can be used as a potential protein source in broiler diets.

According to the results obtained in chapter V, the effects of dietary SM and TSM on growth performance, nutrient digestibility and N retention in broilers were

investigated in chapter VI. The results indicated that growth performances were decreased with increasing levels of SM in the diets, and it was pronounced when diets contained more than 5% SM. In contrast, these parameters were not affected in the TSM groups when TSM contained up to the level of 10% in the diets. These results suggest that decreased growth performance in the SM group may be, in part, due to decrease in FI. This is similar to the results of Oduguwa *et al*. (2004), which showed that diets containing SM decreased BWG and FI in broilers. In contrast, high dietary levels of the SM had no adverse effect on FI and growth performance in broilers (Islam *et al*., 1994; Rosenfeld *et al*., 1997).

Chitin digestibility of SM and TSM based diets were approximately 25% and 29%, respectively in broilers, which was comparable with the results obtained in the previous studies (Khempaka *et al*., 2006a, b; Khempaka *et al*., 2011; Rahman and Koh, 2016). The mechanism of chitin utilisation in birds is not fully understood, although chitinolytic activity occurred in mucosa of the proventriculus in broilers (Koh and Iwamae, 2013). Acidic digestive fluid in the proventriculus and gizzard may degrade the shrimp shell to release chitin, and some chitin may simply dissolve. Recently, it has been reported that chitinase is secreted in the proventriculus and gizzard of birds (Han *et al*., 1997). Formic acid treatment can reduce the chitin content in the SM which was also confirmed by chapter II. Taking into account, the dietary TSM had the beneficial effects on growth performance, along with improved digestibility and retention, even though it can be included up to the level of 10% in the diets.

Considering the practical use of TSM, the inclusion levels of this SM were lower in broilers than laying hen diets (10% vs. 15%, respectively). Therefore, it is necessary to clarify the reason why this difference? From the viewpoint of nutritional requirement, high levels of Ca in SM i possible factors involved in the lower inclusion level in

broilers, because still Ca level was higher in broiler diets than laying hen diets, which was confirmed by chapter III and chapter IV. According to Japanese feeding standard for poultry (2011), Ca requirement for broilers is 0.9% which was consistent when diets contained 10% TSM, and beyond this level resulted in negative effects on performance (chapter VI). Similarly, it has been reported that high levels (1.3-1.5%) of Ca decreased FI and growth rate in chickens (Smith and Taylor, 1961; Watkins *et al*., 1989).

On the other hand, the recommended Ca level is 3.33% for laying hens (Japanese feeding standard for poultry, 2011), which was similar or slightly higher in this study (chapter V) where diets contained up to 15% TSM had no negative effects on performance. Accordingly, Bar *et al*. (2002) reported that Ca requirement for best shell quality in aged (450 to 650 d) hens is slightly higher than NRC (1994) recommendation, and these results agreed with the results obtained in this study. In this context, it is noted that high Ca level is needed, especially for the maintenance of eggshell strength in older layers. So, it may conclude that TSM is better for laying hen than broiler diets, and hence there is a room for further study with TSM-based with low Ca level for broilers.

In practical viewpoint, the potential benefits of improving SM production have to be balanced against capital and running cost. The major part of these costs is associated with the installation and use of chemicals. Because of, the imported conventional protein sources such as soybean meal (approximately 400 USD/ton) are much more expensive which enhance the production cost. Whereas the cost of shrimp wastes (collection and processing) are not so expensive (approximately 125 USD/ton), and locally available of these waste products in the shrimp producing countries. The use of these waste products in chicken diets considerably reduced feed costs, resulting in improved net benefits, as found by Aktar *et al*. (2011). Therefore, it may assume that, by using these waste products as a practical ingredient for chicken feed, approximately

70.0 million USD can be preserved annually in Bangladesh. Additionally, the advantages in the production of TSM, compared to the common protein sources (*i.e.* soybean meal, fish meal): the process is virtually independent form the scale, the technology is simple, the investment is little, even in large-scale production, reduced wastes and odour problems, and finally this process might be more effective for the shrimp producing countries, such as Bangladesh, India, Indonesia and Thailand etc.

In contrast, in order to generate the TSM for the industry, some potential disadvantages of formic acid handling need to be considered. The hazards of formic acid treatment depend on its concentration, with higher concentrations (>10%) considered to be corrosive to skin and eyes, and a risk to unprotected workers (EFSA, 2014). Formic acid is currently listed in the European Union registered feed additives as a technological additive (functional group: preservative) and as a sensory additive (functional group: flavouring compounds) for use in feed for all animal species (EFSA, 2014). It is allowed for the processing of by-products of fish origin (Regulation (EC) No 93/2005), and its use in animal nutrition is safe for the environment (EFSA, 2014). Moreover, formic acid treatment of chicken feed could have important benefits for public health (Humphrey and Lanning, 1988).

Therefore, no adverse effects are to be anticipated when formic acid is used at the maximum proposed dose in feed for pigs (12,000 mg formic acid/kg complete feed), poultry or ruminants (10,000 mg/ formic acid/kg complete feed) (EFSA, 2014). Moreover, EFSA (2014) reported that the turnover of formic acid is rapid, with no evidence of accumulation in body tissues, its use in animal nutrition is not expected to contribute to consumer exposure.

Concluding Remarks

Shrimp aquaculture is the second largest export industries after readymade garments from which plays an important role in the economy of Bangladesh, the author's home country. As shrimp production increase, so does the volume of waste products generated, and no way to reuse, creates an environmental threat. In this regards, the present study was designed to reuse this waste products as a potential protein source for chicken feed. The results obtained in this study suggested that the formic acid treatment is promising to improve the nutritional quality of the SM, and can be used as a potential protein source for chicken feed. In view of that, this SM is useful not only as a protein source for production performance, but it may improve the eggshell strength and yolk colour in laying hens, and nutrient digestibility and retention in broilers. In consequence, by using this SM in chicken diets, many tons of soybean meal would be spared considering the total amount of soybean meal used annually as chicken feed in the author home country. This minor adjustment in feed practical for chickens would help to reduce environmental loading and to preserve the country's foreign-exchange reserves. Finally, this study will provide the imperative information to produce the high quality SM for chicken feed.

For Future Research

From the practical viewpoint, further studies are needed to increase the safety margin along with cost-benefit analyses of this SM in chicken diets. In addition, there is an opportunity to investigate the effects of astaxanthin (contains in SM) as functional foods for human health benefits, because it has strong antioxidant activity.

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