Effects of Formic Acid-Treated Shrimp Meal on Growth Performance and Nutrient Digestibility in Broilers

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This study was conducted to know the effect of formic acid-treated shrimp meal as a protein source on growth performance, digestibilities, and nitrogen (N) retention for broilers. Shrimp meal (SM) was treated with 3\% formic acid (w/v) at room temperature for 20 minutes, sun-dried, ground through a 1.0 mm mesh screen, and then ready to use as the treated SM (TSM). 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. Feed conversion ratio 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). Dry matter 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 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.

Key words: broiler, digestibilities, formic acid, growth performance, shrimp meal

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Introduction

In general, the nutritional quality of shrimp meal (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, 2016a). 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, our previous in vitro study (Rahman and Koh, 2016b) revealed that formic acid could successfully reduce the chitin level in SM, and in vitro digestibility was greater for formic acid-treated SM (TSM) than for untreated SM. Therefore, TSM is a promising protein source for broiler diets.

The purpose of our present study was to measure growth performance, digestibilities, and nitrogen (N) retention in broilers that received diets containing TSM, and to discuss the suitability of this shrimp meal as a 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 sun-dried SM, composed of heads and hulls of black tiger shrimp (Penaeus monodon), was treated with formic acid. In brief, approximately 100 g of SM was suspended with 300 mL of 3\% formic acid at room temperature for 20 minutes. The SM was then sun-dried and ground through a 1.0 mm mesh screen, and was then ready to use as the TSM.
Proximate components, calcium (Ca), phosphorus, and chitin content of the SM and TSM were analysed according to AOAC (1990) and Ghanem et al. (2003) methods, respectively (Table 1).

**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 2). 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 36 d of age and stored in a freezer (−20°C) until analysis.

**Chemical Analysis**

Dry matter (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 and Discussion**

**Nutrient Composition of SM and TSM**

The results of the chemical analysis of the SM and TSM, (Table 1), revealed that there was higher CP and lower crude ash levels in the TSM than in the SM. This may be the result of the treatments applied to SM.

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**Table 1. Chemical composition of untreated and treated shrimp meal and soybean meal (air dry matter basis)**

| Components          | SM* g/kg | TSM† g/kg | Soybean meal† 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†    | 1230†     | 2400               |

*SM* = untreated shrimp meal; †TSM = treated shrimp meal.

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**Table 2. Ingredients and chemical composition of experimental diets (g/kg)**

| Ingredients         | Control | SM* (%) 5 10 15 | TSM† (%) 5 10 15 |
|---------------------|---------|-----------------|------------------|
| Commercial diet     | 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†             | 15.5    | 15.5 15.5 15.5  | 15.5 15.5 15.5  |
| 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 phosphorus| 4.8     | 5.3 6.3 6.7     | 4.8 5.1 5.5      |
| 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    |

*SM* = untreated shrimp meal; †TSM = treated shrimp meal.

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

†Premix (units/kg): vitamin A, 5,00,000 IU; vitamin D₃, 1,00,000 IU; vitamin E, 50 IU; vitamin K₃, 100 mg; vitamin B₁₂, 5.4 mg; pantothenic acid, 800 mg; niacin, 800 mg; choline chloride, 20,000 mg; folate, 104 mg; phosphorus, 106 mg; iron, 2 mg; copper, 362 mg; zinc, 3368 mg; manganese, 2,560 mg; iodine, 45 mg.
the TSM, similar to previous studies (Fox et al., 1999; Oduguwa et al., 1998; Rahman and Koh, 2016b), we confirmed that chitin and Ca levels were reduced in TSM group compared to SM group. 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. The results of the present study are in agreement with previous studies (Rahman and Koh, 2016b), which revealed 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 and our previous studies (Rahman and Koh, 2016b), we 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.

**Growth Performance**

In the control group, final BW, body weight gain (BWG), FI, and FCR were similar to those noted in the broiler performance objectives (Aviagen, 2007), but these values deteriorated, dose-dependently, with increasing levels of the SM (Table 3). Rahman and Koh (2016a) 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. Regarding the FCR, generally, this value improves when FI decreases (Rosenfeld et al., 1997; Gernat, 2001; El-Ghousain and Al-Beitawi, 2009), but our data showed the opposite trend, which may be explained by the decreased DM digestibility (Table 4). 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 (Table 4). 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.

**Digestibilities and N Retention**

In the control group, DM and ash digestibilities, and N retention were 78.5, 42.5, and 68.1% (Table 4), 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 our previous in vitro study (Rahman and Koh, 2016b), which revealed higher DM and CP digestibilities in the TSM than in the SM. As previously discussed, the higher digestibilities, and N

### Table 3. The effects of dietary untreated and treated shrimp meal on growth performance in broilers

| Parameters       | Control       | SM* (%) | TSM† (%) |
|------------------|---------------|---------|----------|
|                  | 5 | 10 | 15 | 5 | 10 | 15 |
| Final BW, g      | 2135.8±14.1 | 2094.2±14.4 | 1923.3±33.8 | 1795.0±38.7 | 2188.7±14.7 | 2120.3±46.4 | 1995.9±21.9 |
| BWG, g           | 1944.6±12.4 | 1912.0±14.9 | 1738.6±32.8 | 1601.3±37.9 | 2001.3±15.0 | 1915.5±51.1 | 1803.6±20.8 |
| Feed intake, g/b/d | 111.5±0.8 | 111.2±0.7 | 105.9±1.5 | 104.4±1.4 | 112.9±0.6 | 110.7±1.6 | 108.5±0.5 |
| FCR, g feed/g BW | 1.61±0.01 | 1.63±0.01 | 1.71±0.01 | 1.83±0.03 | 1.58±0.02 | 1.62±0.02 | 1.67±0.05 |

1Values for each parameter represent mean±SE (n=6).

*SM=untreated shrimp meal; †TSM=treated shrimp meal.

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### Table 4. The effects of dietary untreated and treated shrimp meal on nutrient digestibilities and N retention in broilers

| Parameters       | Control       | SM* (%) | TSM† (%) |
|------------------|---------------|---------|----------|
|                  | 5 | 10 | 15 | 5 | 10 | 15 |
| DM digestibility, % | 78.5±0.53 | 77.2±0.48 | 74.3±0.75 | 73.2±0.98 | 77.7±0.76 | 76.8±0.82 | 75.8±0.84 |
| Ash digestibility, % | 42.5±0.53 | 41.4±0.39 | 35.7±0.55 | 30.3±0.37 | 42.1±0.43 | 40.4±0.40 | 37.4±0.26 |
| Chitin digestibility, % | 0 | 29.3±0.38 | 25.5±0.66 | 19.3±0.55 | 33.6±0.77 | 28.5±0.51 | 25.1±0.61 |
| N retention, %    | 68.1±0.23 | 65.4±0.31 | 58.1±0.17 | 53.7±0.39 | 68.2±0.29 | 66.8±0.49 | 66.0±0.42 |

1Values for each parameter represent mean±SE (n=6).

*SM=untreated shrimp meal; †TSM=treated shrimp meal.

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of the formic acid leaching the minerals from the shrimp exoskeleton, and accordingly, the CP content increased in the TSM, similar to previous studies (Fox et al., 1999; Oduguwa et al., 1998; Rahman and Koh, 2016b). Consequently, levels of crude fibre and chitin were lower in the TSM than in the SM, suggesting that chitin, the main source of crude fibre and the cause of the decrease in digestibility (Khempaka et al., 2006b), was leached from the SM by the formic acid (Rahman and Koh, 2016b). Overall, the TSM used in this study, meets the nutrient requirements for broilers, defined by the Japanese feeding standard for poultry (2011).
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 4). Similar findings are reported by Rahman and Koh (2016a). 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, 2016b), 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, we conducted regression analyses to determine the relationships between dietary chitin levels, and digestibilities and N retention in the SM and the TSM groups (Table 5). 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 form of protein).

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, from all perspectives, the use of formic acid at a 3% level is considered safe (EFSA, 2014).

In conclusion, the beneficiary effects of the TSM (up to the level of 10%) on growth performance, along with improved nutrient digestibilities and N retention, suggest that formic acid-treated SM can be used as a potential protein source in broiler diets.

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