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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.

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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℃) 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.

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

Components	SM*	TSM†	Soybean meal[1]
	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[1]	1230[1]	2400

[1] Standard Tables of Feed Composition in Japan (NARO, 2009).
* SM=untreated shrimp meal; † TSM=treated shrimp meal.

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

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

Items	Control	SM* (%)			TSM† (%)		
		5	10	15	5	10	15
Ingredients							
Commercial diet[1]	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[2]	15.5	15.5	15.5	15.5	15.5	15.5	15.5
Calculated composition (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 phosphorus	4.8	5.3	6.3	6.7	4.8	5.1	5.5
Analysed composition (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

* SM=untreated shrimp meal; † TSM=treated shrimp meal.
[1] Broiler starter diet (CP≥23.5%, ME≥3050 kcal/kg, Nippon Formula Feed Mfg. Kanagawa, Japan).
[2] 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.

Table 3. **The effects of dietary untreated and treated shrimp meal on growth performance in broilers[1]**

Parameters	Control	SM* (%)			TSM† (%)		
		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/b/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 ($n=6$).
* SM=untreated shrimp meal; † TSM=treated shrimp meal.
[a–d] Means in a row with different superscripts are significantly different ($P<0.05$).

Table 4. **The effects of dietary untreated and treated shrimp meal on nutrient digestibilities and N retention in broilers[1]**

Parameters	Control	SM* (%)			TSM† (%)		
		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, %	0	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 ($n=6$).
* SM=untreated shrimp meal; † TSM=treated shrimp meal.
[a–d] Means in a row with different superscripts are significantly different ($P<0.05$).

of the formic acid leaching the minerals from the shrimp exoskeleton, and accordingly, the CP content increasing in the TSM, similar to previous studies (Fox *et al*., 1994; 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).

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-responsively, 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-Ghousein 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.

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.

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

Parameters	Slope		Intercept	
	SM*	TSM †	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 ($n=6$).
* SM=untreated shrimp meal; † TSM=treated shrimp meal.
[a–b] Means in a row with different superscripts are significantly different ($P<0.05$).

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 from 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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