GoldMehl® FM as fishmeal replacer in diets for white shrimps (*Penaeus vannamei*)

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Abstract

A feeding trial of substituting fish meal by feather meal in white shrimp (Penaeus vannamei) was conducted in 2 experiments.
In experiment I, 8 experimental diets were fed shrimp in the collection test tank under lab (aquarium) conditions. The control diets had 30% fishmeal (FM) and 21.16% soybean meal (SBM) as a principal source of protein, and diets were prepared by replacing fishmeal by GoldMehl® (feather meal hydrolysed) at 0%, 15%, 30%, 30% plus Lys + Met, 50% and 50% plus Lys + Met (diets 1-6). Diet 7 has same level of protein and lipid compared to fish meal source.
A commercial diet is included in diet 8. In experiment II, shrimp were fed under pond conditions. Four experimental diets (control 0%, 15%, 30% and 30% plus Lys + Met feather meal replacing fish meal) were selected from experiment I.
The result of experiment II indicated that these diets did not cause any significant (P>0.05) impact on growth performance, ADG, FCR and digestive enzyme activity. Another indicator of growth and feed conversion, Hepatosomatic index (HSI), also showed no significantly (P>0.05) different effects from the experimental diets.

Introduction

Feed cost is a key obstacle in shrimp production particularly in the past two years when oil price is taking a non-stop rise resulting in unbearable price for feed production and shrimp culture as a whole.
Although a search for alternative protein sources has been carried out globally for many years, only few protein sources can be used to completely substitute fishmeal in fish diets. For shrimp diets particularly those for marine shrimp, full replacement for fishmeal has been unsuccessful and good candidates are often marine animal proteins such as squid meal and shrimp meal which would face the same problem as fishmeal in terms of cost and availability in the future. This study therefore aims to demonstrate a potential of GoldMehl® FM as fishmeal replacer in shrimp diets.

Objective

1. To study growth performance, survival, FCR and protein utilization of white shrimp in juvenile stage in both aquarium and practical system diets were fed with varying levels of GoldMehl® FM.

2. To evaluate protein digestibility and enzyme activity of white shrimp that are fed diet with increasing levels of GoldMehl® FM.
3. To assess the highest suitable levels of GoldMehl® FM that can be incorporated in shrimp diets in comparison with other animal protein sources.

Material and methods

Animal
Species: White shrimp (*P. vannamei*)
Weight: 1-2 g initial weight
No Vaccination / Disease history

Product and specification

The product to be tested is GoldMehl® FM which is a processed protein produced under gentle drying condition (low temperature) feathers composing of 85% crude protein, 8% crude fat and 4% crude ash.

Design

The study is composed of two experiments:
1. Optimum level of GoldMehl® FM as fishmeal replacer in diets for white shrimp.
2. Utilization of GoldMehl FM® as fishmeal replacer in diets for white shrimp in practical culture conditions.

Experiment I: Optimum level of GoldMehl® FM as fishmeal replacer in diets for white shrimp (*Penaeus vannamei*)

The trial is to evaluate the growth performance, survival, FCR, protein utilization and protein digestibility in juvenile white shrimp under lab conditions (aquarium).

Test animal and culture system

Healthy white shrimp (*Penaeus vannamei*) with average body weight of 1-2g. Shrimp have been acclimatized to the lab conditions at least 2 weeks prior to the start of the

Table 1: Experimental diets: GoldMehl® FM replacing fishmeal in the formulation

Treatment	Amount (%)							
	T1	T2	T3	T4	T5	T6	T7	T8
Protein Source	Fishmeal and soybean meal						Fishmeal	Commercial
Ingredients								
GoldMehl® FM	0.00	4.50	9.00	9.00	15.00	15.00	0.00	
Fishmeal	30.00	25.50	21.00	21.00	15.00	15.00	40.57	
Soybean meal	21.16	18.24	15.32	16.69	11.42	12.80	0.00	
Wheat flour	31.92	34.83	37.64	33.26	41.52	37.08	43.58	
Gluten	2.00	2.00	2.00	2.00	2.00	2.00	2.00	
Squid meal	5.00	5.00	5.00	5.00	5.00	5.00	5.00	
Squid liver powder	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
Squid ink	0.15	0.15	0.15	0.15	0.15	0.15	0.15	
Fish oil	3.36	3.42	3.48	3.54	3.55	2.61	2.34	
Lecithin	2.00	2.00	2.00	2.00	2.00	2.00	2.00	
PCS	1.60	1.60	1.60	1.60	1.60	1.60	1.60	1.60
Potassium chloride	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
Choline chloride	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27
MonoKP0_4	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24
Sodium Chloride	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
Fylax (Antimold)	0.15	0.10	0.15	0.10	0.10	0.10	0.10	0.1
Binder	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.1
Ethoxyquin	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
PVM	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.1
Lys	0.00	0.00	0.00	2.00	0.00	2.00	0.00	0
Met	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.05

experiment. Twenty shrimps will be stocked into aquaria with water supply system and aeration.

Test diets

Eight diets will be used in the experiment. Diets 1-6 is formulated with ingredients similar to those commercial feed industry (in this case, provided by INTEQC FEED) to contain 38% protein and 8% fat.
Fishmeal and soybean meal are used as principal protein sources but GoldMehl® is incorporated at an expense of fishmeal at 0%, 15%, 30%, 30% plus Lys + Met, 50% and 50% plus Lys + Met for diets 1-6, respectively. Diet 7 has the same level of protein and lipid and contain same ingredients as those of diets 1-6 except fish meal will be the sole source of protein.
A commercial diet is included to the experiment as diet 8 (Table 1). Chromic oxide is used as a marker in experimental diets for studying protein digestibility.

Experimental design

The experimental aquaria are randomly selected for the treatments. Each treatment comprises 3 aquaria.
Shrimp are fed diets 4 times daily (8:00, 12:00, 16:00 and 20:00) and feed is fine adjusted at each meal based on actual consumption.
Daily feed consumption of shrimp in each aquarium is recorded. The feeding trial runs for 8 weeks.
Shrimp are weighted at beginning (week 0) and 8 weeks of the trial. Growth rate, survival, FCR and feed intake are calculated accordingly. Feed conversion ratio (FCR) was calculated by dividing total feed intake per tank by total body weight gain per tank:
Feed intake (%) = (F*100) / ((W1 + W2 / 2) * (N1 + N2/2) * t),
F= total feed consumption,
W1 = average initial weight,
W2 = average final weight,
N1= no. of shrimp at begin of experimental,
N2= no. of shrimp at the end of experimental,
t = days of rearing period

$$\text{Weight gain (\%)} = \frac{\text{Total final weight} - \text{Total initial weight}}{\text{Total initial weight}} \times 100$$

$$\text{ADG} = \frac{\text{Final weight} - \text{Initial weight day of rearing period}}{\text{Total initial weight}}$$

$$\text{FCR} = \frac{\text{Total feed intake}}{\text{Total body weight gain}}$$

Sample collection

Feces collection for protein digestibility determination are performed throughout the trial.
Feces samples are dried, ground and analysed for protein and chromic oxide using wet-acid digestion method (Furukawa and Tsukahara, 1996).
All chemical analyses of each sample are done in triplicate. Apparent digestibility coefficient for protein is calculated according to standard calculation (Lee, 2002).

Chemical analyses

Feed and shrimp samples are analysed for dry matter, crude ash, crude protein, crude fat and crude fiber.
Collected feces are analysed only for dry matter.
Protein content is measured using Kjeldahl method.
Crude fat is determined using Soxhlet extraction with petroleum ether.
Crude Ash is determined after combustion at 600°C (AOAC, 1990).

Table 2: Diet composition

	Amount (%)				
Treatment	Moisture	Crude Protein	Crude Fat	Crude Fiber	Ash
T1	10.49 ± 0.11	40.56 ± 0.21	7.41 ± 0.54	1.72 ± 0.16	7.51 ± 0.03
T2	9.63 ± 0.11	40.31 ± 0.30	6.53 ± 0.70	1.33 ± 0.11	8.09 ± 0.14
T3	10.00 ± 0.16	39.49 ± 0.42	6.58 ± 0.08	1.30 ± 0.02	7.18 ± 0.01
T4	8.36 ± 0.04	43.30 ± 0.46	6.90 ± 1.11	1.07 ± 0.18	7.42 ± 0.07
T5	8.50 ± 0.17	39.73 ± 0.72	7.28 ± 0.01	0.86 ± 0.11	6.26 ± 0.00
T6	8.29 ± 0.15	43.18 ± 0.08	6.69 ± 0.04	1.02 ± 0.11	6.29 ± 0.00
T7	8.30 ± 0.20	40.86 ± 0.13	7.82 ± 0.57	0.26 ± 0.05	9.10 ± 0.03
T8	9.22 ± 0.32	38.77 ± 0.01	5.98 ± 0.19	2.47 ± 0.19	11.37 ± 0.05

Mean ± SD of 3 replicates

Table 3: Response of white shrimp after 8 weeks							
Treatment	Initial weight (g)	Final weight (g)	Weight gain (%)	ADG (g/shrimp/day)	FCR	Feed intake (%)	Survival rate (%)
T1	7.13 ± 0.02	17.33 ± 0.65	143.23 ± 9.69	0.18 ± 0.01	2.10 ± 0.18	2.76 ± 0.09	91.67 ± 2.89
T2	7.12 ± 0.02	17.14 ± 0.45	140.63 ± 7.03	0.19 ± 0.01	2.02 ± 0.24	2.75 ± 0.12	93.33 ± 5.77
T3	7.13 ± 0.02	17.50 ± 0.96	145.53 ± 13.92	0.18 ± 0.01	2.26 ± 0.70	2.82 ± 0.25	91.67 ± 10.41
T4	7.14 ± 0.02	17.75 ± 0.67	149.23 ± 9.55	0.18 ± 0.02	2.06 ± 0.09	2.76 ± 0.05	93.33 ± 2.89
T5	7.13 ± 0.01	17.80 ± 0.63	149.70 ± 9.16	0.18 ± 0.00	2.55 ± 0.57	2.95 ± 0.18	96.67 ± 5.77
T6	7.13 ± 0.01	17.55 ± 0.19	145.91 ± 2.67	0.17 ± 0.01	2.46 ± 0.69	2.93 ± 0.21	85.00 ± 8.66
T7	7.12 ± 0.01	17.22 ± 0.71	141.55 ± 9.65	0.18 ± 0.01	2.19 ± 0.38	2.86 ± 0.11	93.33 ± 2.89
T8	7.12 ± 0.01	17.37 ± 0.82	143.68 ± 11.39	0.18 ± 0.01	2.06 ± 0.12	2.80 ± 0.05	83.33 ± 10.41

Mean ± SD of 3 replicates

Digestibility measurement

Apparent digestibility coefficients (ADC) of protein is determined for all diets using internal marker (0.5% chromic oxide) in the diets after feces collection.
ADC = 100 x (1- (% marker in feed / % marker in feces) (% protein in feces / % protein in feed).

Results

Experiment I: Optimum level of GoldMehl® FM as fishmeal replacer in diets for white shrimp.

After 8 weeks feeding trial, the initial weight, final weight, weight gain, ADG, FCR and feed intake of shrimp is showed in **Table 3** and apparent digestibility coefficient of protein (%) in **Table 4**.

Growth performance of shrimps was not significantly affected by experimental diets. Survival rate of shrimps in the experiment was not affected from experimental diets, but from cannibalism during shrimp molting.

Table 4: Apparent digestibility coefficients of protein	
Treatment	ADC (%) of protein
T1	23.66 ± 3.94
T2	24.80 ± 0.91
T3	25.85 ± 2.53
T4	23.64 ± 4.80
T5	26.92 ± 2.44
T6	26.57 ± 5.11
T7	25.86 ± 2.95
T8	23.57 ± 0.87

Mean ± SD of 3 replicates

Experiment II:
Utilization of GoldMehl® FM as fishmeal replacer in diets for white shrimp *(Penaeus vannamei)* in earthen pond

The trial is to evaluate the growth performance, feed utilization and enzyme activities of juvenile white shrimp under practical culture conditions. Culture conditions are similar to the grow-out pond.

Test animal and culture system

Healthy P8 white shrimp (*peneaus vannamei*) are acclimatized to the earth pond conditions at least 2 weeks prior to the start of the experiment.
60,000 shrimps/ rai
(37 shrimps / square meter) at initial average body weight of
1 g are stocked into the pond with paddle wheel aeration.
To maintain good water quality, lime, zeolite, organic fertilizer and probiotics are applied to the pond.

Test diets

The best, second and third best diet with GoldMehl® FM substituting fishmeal from Experiment I are tested against meat and bone meal and poultry by-product meal supplied by INTEQC FEED Co. Ltd.
Four diets are used in the experiment.
Diets 1-4 are formulated with ingredients similar to those commercial feed industry (in this case, provide by INTEQC FEED) to contain 38% protein and 8% fat. Fishmeal and soybean meal are used as principal protein sources but GoldMehl® FM is incorporated at an expense of fish meal protein at 0%, 15%, 30%, 30% plus Met + Lys, respectively.
The composition of experimental diets is showed in **Table 5**.

Experimental design

The experimental pond is randomly selected for the treatments. Each treatment comprises of 2 ponds, each pond is equipped with 1 set of paddle wheel to increase DO.
Each pond has 1 feeding tray (80 x 80 x 10cm).
Shrimp will be fed with the test diets 5 times daily and feed will be fine adjusted at each meal based on actual consumption and weight estimation every 2 weeks. Growth rate, survival, feed intake and FCR is calculated accordingly.
The experimental aquaria are randomly selected for the treatments. Each treatment comprises of 2 ponds. Shrimp are

Table 5: Experimental diets GoldMehl® FM replacement fishmeal formulation

	Amount (%)			
Treatment	**T1**	**T2**	**T3**	**T4**
Protein source	Fishmeal and soybean meal replacement			
Ingredients	GM 0%	GM 15%	GM 30%	GM 30% Met + Lys
GoldMehl®	0	4.5	9	9
Fishmeal	30	25.5	21	21
Soybean meal	23.21	20.24	17.37	18.69
Wheat flour	31.92	34.83	37.64	33.26
Squid meal	5	5	5	5
Squid liver powder	1	1	1	1
Squid ink	0.15	0.15	0.15	0.15
Fish oil	3.36	3.42	3.48	3.54
Lys	0	0	0	2
Met	0	0	0	1
Others*	5.36	5.36	5.36	5.36
Total (%)	100	100	100	100
Cost (Bath /kg) (March 2009)	28.86	28.06	27.27	29.69

* Others (%): Lecithin 2, PCS 0.7, KC1 0.7, Choline chloride 0.27, mono $KP0_4$ 0.24, NaC1 0.23, Antimold 0.1, Binder 0.1, Antioxidant 0.2, Premix vitamin-mineral 1

Table 6: Diet composition of 2nd experiment					
	Amount (%)				
Treatment	Moisture	Crude protein	Crude fat	Crude fiber	Crude ash
T1	11.43 ± 0.94	36.68 ± 0.76	7.25 ± 0.27	1.14 ± 0.38	10.10 ± 0.69
T2	11.99 ± 0.66	37.01 ± 0.54	7.78 ± 0.54	0.93 ± 0.32	10.06 ± 0.66
T3	11.93 ± 0.75	36.55 ± 0.79	8.22 ± 0.15	0.92 ± 0.42	8.77 ± 0.15
T4	11.80 ± 0.82	37.46 ± 0.82	7.15± 0.48	0.93 ± 0.32	9.00 ± 0.84

Mean ± SD of 2 replicates

fed diets 4 times daily (8:00, 12:00, 16:00 and 20:00) and feed is fine adjusted at each meal based on actual consumption. Daily feed consumption of shrimp in each aquarium is recorded. The feeding trials run for 8 weeks. Shrimp are weighted at beginning (week 0) and every 2 weeks of the trial.
Growth rate, survival, ADG, FCR and feed intake is calculated according to Experimental I.

Table 7: Average weight of shrimp every 2 weeks				
Average weight	Treatment			
(g)	T1 control	T2 replace 15%	T3 replace 30%	T4 replace 30% Lys + Met
Initial weight	0.78 ± 0.01	0.80 ± 0.14	0.93 ± 0.23	0.82 ± 0.12
2 weeks	3.87 ± 0.21	4.08 ± 1.18	3.75 ± 2.03	4.67 ± 2.11
5 weeks	8.87 ± 0.34	8.51 ± 3.40	7.78 ± 0.18	7.76 ± 1.76
6 weeks	10.71 ± 0.37	8.89 ± 2.23	11.61 ± 1.71	10.29 ± 3.74
8 weeks	17.67 ± 1.33	13.88 ± 2.49	19.67 ± 0.81	15.58 ± 4.53
10 weeks	16.63 ± 0.49	13.36 ± 5.59	17.73 ± 3.29	15.62 ± 5.01
12 weeks	20.43 ± 0.25	14.67 ± 3.97	19.99 ± 5.19	17.16 ± 7.13

Mean ± SD of 2 replicates (shrimp = 100 = samples

Enzyme protease activity

There are two ponds (replicates) for each treatment.
Samples of 30 shrimps are used from each treatment (15 shrimp per pond). Hepatopancreas from three shrimps are pooled for the analysis of protease activity. In total there are 10 data of protease activity for each treatment.
Means of each treatment are reported and statistically compared.

Hepatosomatic index (HSI)

Body and hepatopancreas weight are measured and hepatosomatic index (HSI) may be the indicator of growth rate and the assimilation of feed nutrients, however histology should be studied along for the final conclusion of feed conversion.

HIS (%) = Hepatosomatic weight / Body weight) * 100

Results and discussion

Commercial shrimp feeds are commonly reported to include fishmeal at levels between reported to include fishmeal at levels between 25-50% of the total diet
(Amaya, et al. 2007) as same as this experiment uses 30% of fish meal in control diet.
One of the options proposed to reduce the production cost of the shrimp feed is to replace fishmeal with other protein sources.
In this study, feed costs were slightly reduced as more GoldMehl® FM was

Table 8: Average total length of shrimp every 2 weeks				
Average length	Treatment			
(cm)	T1 control	T2 replace 15%	T3 replace 30%	T4 replace 30% Lys + Met
Initial weight	4.82 ± 0.17	5.14 ± 0.47	4.88 ± 0.12	4.96 ± 0.73
2 weeks	8.26 ± 0.19	8.26 ± 0.66	7.82 ± 1.42	8.71 ± 1.28
5 weeks	10.90 ± 0.06	9.90 ± 0.46	10.94 ± 0.95	10.59 ± 1.02
6 weeks	11.30 ± 0.12	10.51 ± 0.86	11.54 ± 0.58	10.95 ± 1.32
8 weeks	12.93 ± 0.24^{ab}	11.54 ± 0.89^{a}	13.30 ± 0.05^{b}	12.09 ± 1.53^{ab}
10 weeks	13.42 ± 0.10	12.34 ± 1.45	14.91 ± 1.05	13.74 ± 3.25
12 weeks	14.34 ± 0.03	13.37 ± 1.95	14.97 ± 0.75	13.81 ± 2.00

Mean within each column not sharing a common superscript are significantly different (P<0.05). Mean ± SD of 2 replicates (shrimp = 100 samples)

included in the formulation
(**Table 1 and 5**).

Growth, survival and feed performance

The results indicated that the experimental diets replacing fishmeal by GoldMehl® FM 0%, 15%, 30% and 30% plus Lys + Met did not cause any significant (P>0.05) impact on growth performance. Lawrence and Castille (1991) partially replaced fish meal by feather meal at 5 – 10%.
They concluded that feather meal is suitable for shrimp diets as long as the shrimps' requirement for essential amino acids and minerals is met.

Table 9: Weight gain of shrimp every 2 weeks

Average length	Treatment			
(%)	T1 control	T2 replace 15%	T3 replace 30%	T4 replace 30% Lys + Met
2 weeks	395.99 ± 18.24	430.71 ± 241.43	427.88 ± 446.83	412.47 ± 226.63
5 weeks	1,037.99 ± 63.82	1,018.40 ± 622.85	879.94 ± 359.57	758.92 ± 190.42
6 weeks	1,273,43 ± 72.42	1,053.42 ± 483.14	1,262.77 ± 577.38	1,156.83 ± 576.47
8 weeks	2,166.76 ± 211.58	1,690.15 ± 627,37	2,062.30 ± 579.83	1,901.87 ± 886.73
10 weeks	2,032.33 ± 101.21	1,659.38 ± 1,009.51	1,918.61 ± 856.68	1,872.66 ± 892.79
12 weeks	2,519.05 ± 14.40	1,806.77 ± 833.18	2,185.03 ± 1,113.98	2,093.21 ± 1,199.10

Mean ± SD 2 of replicates (1 replicate = shrimp = 100 samples)

Cheng et al. (2002) reported that the maximum fishmeal protein replacement by feather meal is 33%
(steam pressure hydrolysed) in white shrimp without losing performance. 43% (enzyme treated feather meal) and 66% (steam pressure hydrolysed plus Lys and Met).

Protease activity

The result are shown in **Table 11**. The enzyme activities of all treatments are not significantly different (P>0.05) at both temperatures of incubation. The higher temperature of incubation (37° C) appeared to enhance the activity when compared with lower one (25° C). HIS is also not significantly different (P>0.05) **(Table 12)**.

Table 10: ADG, FCR and survival rate (%) of shrimp at 12 weeks

Treatment	ADG	FCR	Survival rate (%)
T1 control	0.23 ± 0.00	1.80 ± 0.22	75.00 ± 4.06
T2 replace 15%	0.17 ± 0.05	1.85 ± 0.33	72.48 ± 22.55
T3 replace 30%	0.23 ± 0.06	1.45 ± 0.41	59.22 ± 6.09
T4 replace 30% Lys + Met	0.19 ± 0.09	1.69 ± 0.74	74.85 ± 11.66

Mean ± SD of 10 replicates

Conclusions

Growth performance and analysis of protease specific activity from hepatopancreas of Pacific white shrimp *(Penaeus vannamei)* after feeding with experimental diets for certain period of time was conducted in the Laboratory of Aquatic Animal Health Management, Dept. of Aquaculture, Faculty of Fisheries, Kasetsart University. The results indicated that these experimental diets replacing fishmeal with GoldMehl® FM did not cause any significant impact on growth performance, ADG, FCR and digestive enzyme activity. Another indicator of growth and feed conversion, Hepatosomatic index (HIS), also showed no significant effects due to the experimental diets.

Table 11: Protease specific activities from hepatopancreas of *Penaeus vannamei* (unit/ min/ mg protein) fed with different diets at for two incubation temperatures

Treatment	Protease specific activities of hepatopancreas	(Unit/ min/ mg protein)
	37° C	25° C
T1 control	3.5082 ± 0.2989	1.9101 ± 0.1653
T2 replace 15%	3.6823 ± 0.8255	1.9981 ± 0.4258
T3 replace 30%	3.2503 ± 0.2822	1.8019 ± 0.2155
T4 replace 30% Lys + Met	2.9929 ± 1.1978	1.7019 ± 0.5835

Mean ± SD of 10 replicates

Table 12: Body weight, hepatopancreas weight and hepatosomatic index of *Penaeus vannamei*

Treatment	Body weight	hepatopancreas weight (g)	Hepatosomatic index (%)
T1 control	21.98 ± 1.58	0.81 ± 0.07	3.70 ± 0.20
T2 replace 15%	12.15 ± 1.05	0.43 ± 0.04	3.54 ± 0.41
T3 replace 30%	27.06 ± 2.48	0.97 ± 0.14	3.56 ± 0.32
T4 replace 30% Lys + Met	17.36 ± 6.27	0.51 ± 0.09	3.22 ± 0.98

Mean ± SD of 2 replicates

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