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Optimization of Protein Content in Earthworm-based Fish Feed Formulation for Catfish *(Clarius gariepinus)*

Pengoptimuman Kandungan Protein di dalam Formulasi Makanan Ikan-Berasaskan Cacing Tanah untuk Ikan Keli (*Clarius gariepinus*)

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ABSTRACT

The optimization of protein content in earthworm-based fish feed formulation was investigated in the study. Full factorial design (FFD) and central composite design (CCD) were used as a statistical tool to screen the range of the selected parameters and to optimize the process, respectively. Three components were combined in the fish feed formulation namely earthworm powder, chicken guts and soybean waste as the protein source. The optimum nutritional values were obtained at 25%, 25% and 5.95% for earthworm powder, soybean waste and chicken guts, respectively, to give an optimum protein content of 35.97%. Formulation of fish feed from earthworm powder can be an alternative in aquaculture industry in the near future.

Keywords: Catfish; earth worm powder; fish feed formulation; optimization

ABSTRAK

Pengoptimuman kandungan protein dalam formulasi makanan ikan berasaskan cacing tanah dikaji. Reka bentuk Faktorial Penuh dan Reka bentuk Komposit Berpusat telah digunakan untuk analisis dalam kajian ini bagi mengenal pasti julat parameter terpilih serta mengoptimum proses masing-masing. Tiga komponen telah digabungkan dalam formulasi makanan ikan iaitu tepung cacing tanah, usus ayam dan sisa kacang soya sebagai sumber protein. Nilai nutrisi optimum yang diperoleh adalah masing-masing 25%, 25% dan 5.95% bagi tepung cacing tanah, sisa kacang soya dan usus ayam yang memberikan kandungan protein optimum 35.97%. Formulasi makanan ikan dengan tepung cacing tanah boleh menjadi alternatif dalam industri akuakultur di masa hadapan.

Kata kunci: Formulasi makanan ikan; ikan keli; pengoptimuman; tepung cacing tanah

INTRODUCTION

In Malaysia, "keli" (*Clarias* spp.) or catfish is the most popular freshwater fish beside Red tilapia (*Oreochromis* sp. red hybrids) and "patin" (*Pangasius sutchii*) due to the high nutritional value and good taste. It is widely cultivated in earthen ponds, floating net cages and pools. From 2003 to 2007, the demand for freshwater fish has increased by 37% (Info Fish International 2010) which reflects the growing demand for the freshwater fish compared with sea fish because of the inconsistent supply and price (Saalah et al. 2009).

Fish feed contributes to more than one-half of the variable operating cost in the aquaculture sector, thus, knowledge on nutrition and practical feeding of fish are essential to a successful aquaculture. The formulation of fish feed especially for catfish should meet the standard requirement of protein (32% to 35%) (Robinson & Li 2007). This is important in order to supply all essential nutrients and energy for the maintenance of fish's growth, reproduction and health. Commonly fish meal (FM) are used in the fish feed formulation based on the high protein and lipid sources (Tacon 1990). The high value of amino acid, vitamin content and other growth factors make FM a better choice (Tacon 1990). Moreover, it was reported that the fish feed formulated with FM will increase the organic enrichment in the pond (Bransden et al. 2003; Xu et al. 2007) hence, affecting the fish growth.

There are numerous studies undertaken to look for alternative of protein sources in fish feed formulation. The shrimp industry waste (Cavalheiro et al. 2007), soybean meal (Carter & Hauler 2000), aquatic weeds (Mukherjee et al. 2010), soybean waste and chicken guts (Refstie et al. 2006) are among potential dietary protein sources that have been documented. However, earthworm powder-based for fish feed formulation is rarely reported. It was reported that the earthworm contains 60-70% protein and high in essential amino acid (lysine and methionine) compared with meat or fish meal (Paoletti et al. 2003). Earthworm powder-based also contains 6-11% of fat, 5-12% of carbohydrate and 2-3% of minerals and various types of vitamins. The protein content in the earthworm powder was reported to improve sexual performance and stimulate

the appetite of the fingerling marble goby *(Oxyeleotris marmoratus)* and tra catfish *(Pangasius hypophthalmus* (Nhi 2000). Moreover, it has been used in agriculture for soil fertility (Medina et al. 2003), in pharmaceutical as an anticancer, antibiotic, anti-hyperglycemia, anti-diabetes, anti-hypertension and anti-hypotension (Ishii & Mihara 1992; Paoletti et al. 2003) and also in cosmetic industries (Sinha et al. 2010). Therefore, earthworm is viewed as a potential ingredient and a good source of protein in the fish feed formulation.

Formulation of fish feed is usually undertaken by using one variable at a time (OVAT) experiments (Ruohonen & Kettunen 2004), in which a parameter of dietary components is varied while others are kept constant. Other method used in the formulation of fish feed was using Pearson's Chi Square (Llenart et al. 2003). However, statistical optimization tools like Response Surface Methodology (RSM) offers an advantage over OVAT experiments and Pearson's Chi Square where, more information of the interaction between the parameters and the optimization conditions could be studied (Myers & Montgomery 2002).

In this study, the integration of earth worm powder, soybean waste and chicken guts as the protein sources in the fish feed formulation for catfish was investigated. Response surface methodology (RSM) via full factorial design (FFD) and Central Composite Design (CCD) were used to optimize the protein content in the fish feed formulation.

Materials and Method

Earthworm powder was purchased from local company (Parkman Agro Resource, Kuala Lumpur). Tapioca flour, waste of soybeans, chicken gut and rice bran were purchased from local source in Arau, Perlis. The vitamin mixture, calcium bis-(dihydrogen phosphate) and the Kjeldahl tablets were purchased from MerckCorp (USA).

Preparation of Earthworm Powder, Chicken Guts and Soybean Waste

The soybeans and chicken guts were dried at 65°C for 72 h and 40°C for 2 weeks, respectively. The dried soybeans, chicken guts and rice bran were ground and sieved to 500 μm in size. The earthworm powder, soybeans, chicken guts, rice bran, vitamin mixture, calcium bis-(dihydrogen phosphate) and tapioca flour were slowly mixed with hot water at approximately 70°C to accomplish agglutination (Cavalheiro et al. 2007).The percentage of vitamin mixture, calcium bis-(dihydrogen phosphate) and tapioca flour were added based on Kenneth et al. (2005) while, percentage of rice bran was obtained by deduction of 100% of mixture among earthworm powder, soybeans and chicken guts. The dough of the formulation mixture was dried at 40°C for 24 h. The dried fish feed formulation were stored in plastic bags in the refrigerator at 5°C for protein analysis.

Analysis of Protein

The Kjeldhal method was used to analyse the nitrogen and protein content in the fish feed formulation as described by Aksnes et al. (1997).

Pre-Optimization Study (2^K)

Pre-optimization study was conducted through Full Factorial Design (FFD) by using Design Expert Software, Version 7.0 (State-Ease, Inc). This 2^k study could eliminate insignificant parameters involved in the study, thus, providing an efficient regression model. The regression model of the FFD is presented by following equation:

$$y = \beta_o + \sum_{i=1}^{k} \beta_i x_i + \sum_{1 \le i \le j}^{k} \beta_{ij} x_i x_j + \varepsilon, \quad (1)$$

where y is the protein content, β_o is the constant term, β_i is the linear parameters, x_i are the parameters (earthworm powder, soybeans, chicken guts, respectively), β_{ij} is the interaction coefficient of the parameters and ε is the error.

A 2^k with three parameters (k = 3), consisting of earthworm powder (10-20%), soybean waste (10-20%) and chicken guts (5-10%) with three center points were carried out. The regression model obtained in the FFD study according to (1) is presented as follows:

$$\begin{aligned} \%\ \text{Protein} &= 31.94 + 2.11A + 1.48B + 0.037C \\ &\quad - 0.45AB - 0.015AC + 0.060BC + 0.085A^2 \\ &\quad + 0.010B^2 + 0.78C^2, \end{aligned} \quad (2)$$

where A represents earthworm powder (%), B represents soybean waste (%) and C represents chicken guts (%). Factors A, B and C were referred as main effect; while, AB, BC and AC were represented as interactions effect. The result obtained was analyzed by Analysis of Variance (ANOVA) through R^2, F-value, p-value and Lack of Fit (LOF) prior to the optimization studies by using CCD. R^2 value which closed to 1 is used to indicate the model fitness, while parameters that have high F-value is preferred as it gives high influence to the process. The model and parameters which have p-value <0.05 are desired in the process in order to ensure they are statistically significant, meanwhile not significant of LOF represents that data in the experimental domain is included in the regression.

Optimization Study by CCD

Optimization study was conducted through CCD to find the optimum mixture (%) of the involved parameters in the fish feed formulation according to the following regression model:

$$y = \beta_o + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{1 \le i \le j}^{k} \beta_{ij} x_i x_j + \varepsilon, \quad (3)$$

where β_{ii} represents the coefficient of quadratic parameters.

The three parameters namely earthworm powder (15-25%), soybean waste (15-25%) and chicken guts (4-6%) were employed to give 17 runs (Table 1).

TABLE 1. Parameter levels for the Central Composite Design (CCD)

Parameters (%)	Low level (-)	High level (+)
Earthworm powder	15.00	25.00
Soybean waste	15.00	25.00
Chicken gut	4.00	6.00

A regression analysis was performed to fit the empirical model based on the second order of equation (3) and presented by the following equation:

$$\% \text{ protein} = 32.071 + 0.663\,A + 0.582B - 7.947C - 0.018\,AB - 0.003\,AC + 0.012\,BC + 0.003\,A^2 + 0.0004B^2 + 0.780C^2, \quad (4)$$

where, terms A^2, B^2 and C^2 are referred as the quadratic terms.

VALIDATION OF THE MODEL

A validation process was carried out to verify the optimum mixture of earthworm powders, soybean waste and chicken guts to give maximum protein content. The goal for each parameter was set to be in the range level whereas, the responds which is the protein content was set to be at the maximum level.

RESULTS AND DISCUSSION

FULL FACTORIAL DESIGN (FFD) STUDY

The result obtained from FFD study is listed in Table 2. It shows that high percentage of earthworm and soybean combined with low percentage of chicken guts might result in high percentage of protein (Run no 1-3). However, when the percentage for all the parameters were increased, the observed protein content in the prepared fish feed decreased (Run no 4-6), but slightly similar to the protein content at the center point level (Run no 25-27).

It is suggested that high percentage of earthworm powder and soybean waste ratio will maximize the protein content in the formulation. It was found that only small amount of chicken guts (around 6 %) was required for the best protein formulation. From Table 2, we suggest that the content of earthworm powder and soybean waste need to be increased (>20%).

The main factors and interactions between the three parameters involved in the fish feed formulation were plotted on the half normal probability plot as presented in Figure 1.

TABLE 2. Protein concentration (%) obtained from FFD

Formulation	Factor 1 A: Earthworm Powder (%)	Factor 2 B: Soybeans waste (%)	Factor 3 C: Chicken guts (%)	Protein (%)
1	20	20	5	31.61
2	20	20	5	31.32
3	20	20	5	30.83
4	20	20	10	28.71
5	20	20	10	28.92
6	20	20	10	28.84
7	20	10	10	30.53
8	20	10	10	30.62
9	20	10	10	30.81
10	20	10	5	27.63
11	20	10	5	28.34
12	20	10	5	27.52
13	10	20	10	24.31
14	10	20	10	24.14
15	10	20	10	24.83
16	10	20	5	25.73
17	10	20	5	25.54
18	10	20	5	24.52
19	10	10	10	23.13
20	10	10	10	23.51
21	10	10	10	23.33
22	10	10	5	23.32
23	10	10	5	23.73
24	10	10	5	23.63
25	15	15	7.5	28.74
26	15	15	7.5	28.83
27	15	15	7.5	28.62

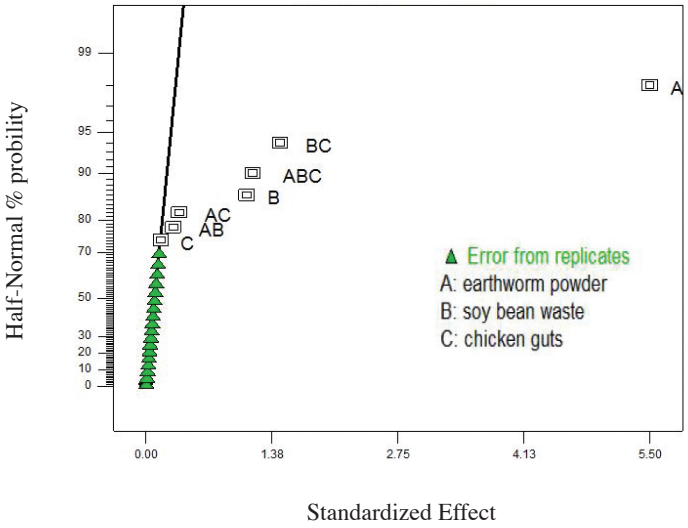

FIGURE 1. Half normal probability plot in fish feed formulation

The factors that fall along the straight line were considered as noise and any factors that were located away from the line were considered to give a significant effect in the process. From the experimental run, A, B, C, BC, AB, AC and ABC were significant and have high potentials to the fish feed formulation results. The analysis of variance (ANOVA) as presented in Table 3 was used to obtain the quantitative information for each effect. The fitness of the model is expressed by R^2 which was 0.9904 and thus; shows a good satisfactory of the regression model. The model F-value of 265.54 and the p-value < 0.001 implies that the model is valid and significant for further optimization study by Central Composite Design (CCD). Terms A, AB, AC, BC and ABC give significant result to the fish formulation based on the p value < 0.05. Term A which represents the earthworm powder shows the highest F value (1596.25) which reflects to the strong effectof the parameter compared with soybean waste and chicken guts. Based on the analysis of variance (ANOVA) result, term A, B and C would be considered in the optimization study.

CENTRAL COMPOSITE DESIGN (CCD) STUDY

The result obtained from CCD studies is presented in Table 4. It shows that the earthworm powder at high level (25%), gives the highest protein content (35.87% protein). However, when the earthworm powder was at the low level (15%), the protein content was also low (28.76% protein). This shows that the earthworm powder could be a good substitute of protein source compared with other imported protein sourcelike fish meal for fish feed formulation.

The coefficient determination, R^2 (Table 5) obtained is 0.9816 which presented that only 1.84% of the total parameters were not explained by the model and, thus; confirming the accuracy of the model which represents the fitness of the model.

The adequacy of the model is statistically analyzed by ANOVA. It shows that the F-values of 41.49 were significant and there were only 0.01% of chances that the model would not be significant due to the noise. Terms A, B and AB showed a significant value based on the p value < 0.05. Earthworm gave the highest F- value (233.93) which

TABLE 3. Analysis of Variance (ANOVA) obtained from FFD study

Source	Sum of Squares	*df*	Mean Square	*F* Value	*p*-value	Prob> *F*
Model	211.35	7	30.19	265.54	< 0.0001	significant
Earthworm powder (%)	181.50	1	181.50	1596.25	< 0.0001	
Soybean waste(%)	7.26	1	7.26	63.85	< 0.0001	
Chicken Guts (%)	0.17	1	0.17	1.47	0.2417	
AB	0.54	1	0.54	4.75	0.0428	
AC	0.81	1	0.81	7.09	0.0158	
BC	12.91	1	12.91	113.51	< 0.0001	
ABC	8.17	1	8.17	71.82	< 0.0001	
Curvature	8.96	1	8.96	78.83	< 0.0001	significant
Pure Error	2.05	18	0.11			
Cor Total	222.36	26				

R^2 = 0.9904

TABLE 4. Protein concentration (%) obtained from CCD

Run	Factor 1 A: Earthworm powder (%)	Factor 2 B: Soybeans waste (%)	Factor 3 C: Chicken guts (%)	% Protein
1	25.00	25.00	4.00	35.76
2	25.00	25.00	6.00	35.87
3	25.00	15.00	6.00	33.98
4	25.00	15.00	4.00	33.68
5	28.00	20.00	5.00	34.58
6	20.00	12.00	5.00	30.56
7	20.00	20.00	5.00	31.43
8	20.00	20.00	5.00	31.56
9	20.00	20.00	5.00	31.84
10	20.00	28.00	5.00	33.85
11	20.00	20.00	3.00	33.26
12	20.00	20.00	7.00	32.69
13	15.00	25.00	4.00	32.29
14	15.00	15.00	4.00	28.83
15	15.00	15.00	6.00	28.76
16	15.00	25.00	6.00	32.89
17	12.00	20.00	5.00	29.98

TABLE 5. Analysis of Variance (ANOVA) obtained from CCD study

Source	Sum of Squares	*df*	Mean Square	*F* Value	*p*-value	Prob> *F*
Model	71.20	9	7.91	41.49	< 0.0001	Significant
Earthworm powder (%)	44.61	1	44.61	233.93	< 0.0001	
Soybean waste(%)	22.05	1	22.05	115.65	< 0.0001	
Chicken Guts (%)	0.014	1	0.014	0.072	0.7965	
AB	1.64	1	1.64	8.59	0.0220	
AC	1.800×10^{-3}	1	1.800×10^{-3}	9.440×10^{-3}	0.9253	
BC	0.029	1	0.029	0.15	0.7091	
A^2	0.020	1	0.020	0.10	0.7584	
B^2	2.871×10^{-4}	1	2.871×10^{-4}	1.506×10^{-4}	0.0222	
C^2	1.63	1	1.63	8.56	0.1564	Not significant
Residual	1.33	7	0.19	0.9701		
Lack of Fit	1.25	5	0.25	5.68		
Pure Error	0.088	2	0.044			
Cor Total	72.53	16				

$R^2 = 0.9816$

represents a strong effect and important parameters in the fish feed formulation. The lack of fit test conducted was not significant. Thus it showed that the model adequately fits the data well.

Only main effect of A and B are significant ($p<0.05$). Similar result was obtained with the interaction effect, where only interaction AB is only significant compared with interaction between AC and BC. Parameter C (chicken guts) does not contribute to the main effect in the protein content of fish feed formulation. Therefore, the percentage of C in the fish feed formulation should be low due to the insignificant result obtained in the CCD study as compared to the percentage of earthworm powder and soybean waste. The result was supported by Khan (1994) who reported that chicken gut is poorly digested by the fish.

It was found that high % of protein in the fish feed formulation could be obtained at high % of earthworm powder and soybean, respectively (Figure 2). The optimization process by CCD showed the main effect and the interaction of the parameters which contributed to the highest protein content in the fish feed formulation.

VALIDATION OF THE MODEL

The optimized mixture were found to be 25% of earthworm powders, 25% of soybean waste and 5.94% of chicken gut to produce 35.95% of protein (predicted value). Confirmation runs were carried out to determine the observed value of protein content (Table 6). The protein content obtained in the experiment was 35.93%. The small standard deviation which was 0.06 suggested that the predicted value and

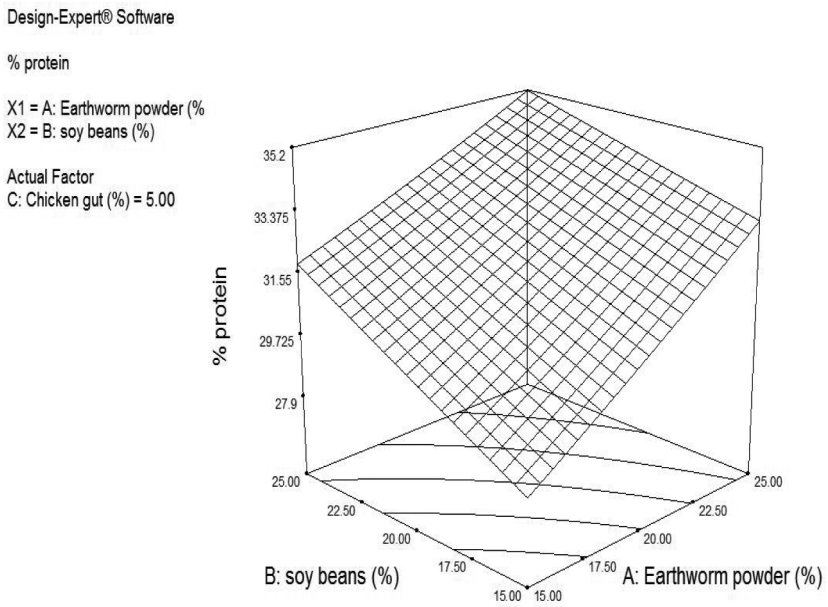

FIGURE 2. Interaction effect between A, earthworm powder and B, soybean waste when C, chicken guts is set at its middle level (5%)

TABLE 6. The confirmation run for protein content in the fish feed formulation-earthworm based

Run	Earthworm powder (%)	Soybeans waste (%)	Chicken guts (%)	Protein (%)		% Error	σ
				Predicted	Experimental		
1	25.00	25.00	5.94	35.95	35.89	0.15	0.06
2	25.00	25.00	5.94	35.95	35.97	0.05	
3	25.00	25.00	5.94	35.95	35.93	0.05	

the observed value were in good agreement. The result obtained in the optimization studies showed an increment of 12% from the FFD studies which achieved only 31.61% of protein in the fish feed formulation.

CONCLUSION

The fish feed formulation based on the mixture of earthworm powder, soybean waste and chicken gut has statistically improved the protein content. The earthworm powders play a significant role in the formulation based on its high protein content compared with chicken gut and soybean waste. This study enabled the determination of the range for selected ingredients based on their availability and cost. This result showed that the fish feed formulation-earthworm powder based has significant protein content and could be an alternative in the catfish aquaculture industry.

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