Feed Formulation of Improved Egg Custard formulation using Response Surface Methodology (RSM)

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Abstract. Macrobrachium rosenbergii or also known as Giant Freshwater Prawn is crustacean species that can naturally be found in Malaysia. Previous studies were carried out on developing alternative feed for M. rosenbergii larvae in order to lower the feed cost and improve growth rate. This project proposed to analyze the nutrition value of M. rosenbergii larvae formulated feed using 40% of egg custard, 3% of eggshells, 1% of Curcuma longa (Turmeric) and 17.5% of Moringa oleifera. There were 13 different feed formulations by using Response Surface Methodology (RSM) in Design Expert Software version 10 which M. oleifera and C. longa were the variables coded while egg custard and eggshells were the based feed of the formulation. Proximate analysis has been conducted to observe the nutrition composition of the formulated feed and compared with the nutrient requirement of M. rosenbergii larvae which were protein, lipid, mineral and carbohydrates requirement. The result by Design Expert Software version 10 revealed that M. oleifera and C. longa gave the significant effect to protein and mineral requirement but not to lipid and carbohydrate requirement. The software also suggested the optimize formulation that near to the nutrient requirement of M. rosenbergii was Formulation 9, suggested percentage of 17.10% M. oleifera and 1% C. longa with 0.738 desirability.

1. Introduction

Giant freshwater prawn that is prominent in size compare to other freshwater crustacean species has make it one of luxurious seafood in the market [5]. Larvae stage of aquaculture species especially Macrobrachium rosenbergii larvae stage is a sensitive stage that need more attention during nursery period. The larvae need optimum water quality with a good management practice to survive including stocking density [6]. Feeding management is the most important aspect in aquaculture farming as feed take over than 50% of the production cost in aquaculture [4]. Common feed used to for M. rosenbergii larvae is live Artemia. However, due to the high cost of Artemia, the common practice in the nursery is to feed the larvae with alternative feed which is egg custard to reduce the feed cost. In normal practices, farmer will give egg custard in the morning and afternoon while Artemia is given in the night. A new formulated feed must need the optimum nutrient requirement for the species. Thus, this study...
investigates the potential application of *Moringa oleifera*, *Curcuma longa* and eggshell as a suitable ingredient to partially replace the protein content in the egg custard formulation.

2. Materials and Methods

2.1 Preparation of raw materials

*Moringa oleifera* and *Curcuma longa* were obtained from local market, dried for 24 h, at 105 °C in air-forced oven and being processed into powder form. The eggshells were collected from local stalls. The eggshells were cleaned through running water, air dried before drying process in the air-forced oven for 24 h, 105 °C and crushed into powder form. The egg custard was prepared by adding together the eggs, semi-skimmed milk powder, eggshell, *M. oleifera* and *C. longa* with percentage suggested by [11]. Then mix thoroughly using electrical mixer and steamed on the water bath for 45 mins. The egg custard was kept at 4°C for 24 h and sieved into powder, using mesh size of 0.01 mm.

2.2 Feed formulation using Response Surface Methodology (RSM)

The improved egg custard formulation for *M. rosenbergii* larvae was designed using Response Surface Methodology (RSM) with the Central Composite Design (CCD) model. RSM suggested 13 formulations for two variables. The amount of suggested *M. oleifera* and *C. longa* from RSM were added with 40% of egg custard and 3% eggshells powder as the based feed.

2.3 Chemical analysis

Chemical composition of improved egg custard was determined using proximate analysis. The analysis was carried out using AOAC (1990) procedures. Each sample was run in triplicates.

2.4 Optimization studies and data analysis

The optimization studies were performed by using Design Expert Software version 10 by comparing the actual experimental results with the predicted data suggested by the software. There were four (4) experimental responses (R) being analysed which were Protein Requirement (%), Lipid Requirement (%), Mineral requirement (%) and Carbohydrate Requirement (%); each was labelled as R1, R2, R3 and R4, respectively. All the responses were evaluated using analysis of variance (ANOVA), development of polynomial regression model equation, diagnostic plot for predicted value versus actual values and diagnostic plot for normal probability plots of residual. The experimental data was observed and analysed using interaction plot, 3D surface plot generated by Design Expert Software version 10.

3. Result and Discussion

3.1 Chemical Composition of Improved Egg Custard Formulation

Table 3.1 showed that the most repeated formulation was formulation 10 in which the nutrient content such as crude protein, crude fat and ash was 17.8731%, 2.5776% and 8.6905% respectively, differ to egg custard poultry by product (PBM) formulation by [11], protein content was 55.63%, fat content was 13.41% and ash content was 8.23%. The huge differ in crude protein and crude fat content value between formulation 10 and egg custard PBM may because of the high protein and fat value contain in PBM meal which about 78.89% and 15.32% respectively [11].
Table 1. Experimental factors with level coded

| Formulation | Dry Matter (%) | Crude Protein (%) | Crude Fat (%) | Crude Fiber (%) | Ash (Minera) (%) | Nitrogen Free Extract (%) | Moisture (%) |
|--------------|----------------|-------------------|---------------|-----------------|-----------------|--------------------------|-------------|
| 1            | 95.66          | 26.64             | 4.04          | 4.72            | 13.36           | 46.89                    | 4.34        |
| 2            | 94.69          | 31.08             | 5.22          | 0.26            | 8.64            | 49.49                    | 5.31        |
| 3            | 93.89          | 26.23             | 5.57          | 6.17            | 11.15           | 44.78                    | 6.11        |
| 4            | 93.91          | 27.05             | 5.58          | 6.32            | 11.27           | 43.70                    | 6.09        |
| 5            | 93.95          | 27.18             | 5.91          | 7.11            | 11.04           | 42.71                    | 6.05        |
| 6            | 94.02          | 26.99             | 5.69          | 6.40            | 11.20           | 43.75                    | 5.98        |
| 7            | 93.98          | 27.34             | 5.59          | 5.78            | 11.51           | 43.76                    | 6.02        |
| 8            | 93.99          | 27.14             | 6.18          | 5.83            | 11.00           | 43.84                    | 6.02        |
| 9            | 93.62          | 26.60             | 5.40          | 5.56            | 11.00           | 45.06                    | 6.38        |
| 10           | 91.30          | 17.87             | 2.58          | 3.57            | 08.69           | 58.58                    | 8.90        |
| 11           | 93.51          | 27.51             | 6.01          | 6.07            | 11.44           | 42.49                    | 6.49        |
| 12           | 93.49          | 27.32             | 5.38          | 5.93            | 10.99           | 43.88                    | 6.51        |
| 13           | 93.49          | 27.71             | 5.6           | 6.07            | 12.16           | 41.90                    | 6.51        |

3.2 Effect of Moringa oleifera and Curcuma longa towards Different Responses in RSM

Three dimensions (3D) plot of interaction effect of *M. oleifera* and *C. longa* in the feed formulation towards Protein Requirement (%) was showed in Figure 1. The graph showed that percentage of protein requirement increase at 73.73% with the percentage of *C. longa* was in between 0.00 to 0.25 whereas percentage of *M. oleifera* was in between 4.28 to 8.55. The increment of protein requirement percentage is may because of the percentage of protein source in *M. oleifera* which about 31.5% [7].

![3D response surface graph of interaction effect of Moringa oleifera and Curcuma longa towards R1, Protein Requirement (%)](image)

Figure 1. 3D response surface graph of interaction effect of *Moringa oleifera* and *Curcuma longa* towards R1, Protein Requirement (%)

The effect of *Moringa oleifera* and *Curcuma longa* towards lipid requirement percentage in the feed was showed in Figure 2. Lipid requirement in the feed formulation increase up to 76.60% with the percentage of factor A (*C. longa*) was in between 0.00 to 0.25 while factor B (*M. oleifera*)
was in between 4.28 and 8.55. Finding in previous research on *C. longa* crude lipid content was 6.85 % while *M. oleifera* was 2.5 % [1][7].

![3D response surface graph of interaction effect of Moringa oleifera and Curcuma longa towards R2, Lipid Requirement (%)](image)

**Figure 2.** 3D response surface graph of interaction effect of *Moringa oleifera* and *Curcuma longa* towards R2, Lipid Requirement (%)

The effect of *M. oleifera* and *C. longa* towards mineral requirement percentage was observed in Figure 3. The graph showed that there was only individual interaction that influence the increasing percentage of mineral requirement for linear model. The mineral requirement increases up to 396.07 % in which it exceeded the 100 % of requirement for *M. rosenbergii* larvae if factor B (*M. oleifera*) percentage was in between 12.83 to 17.10. The increasing in mineral value may because of the mineral content in *M. oleifera* which about 6.96 % [7].

![3D response surface graph of interaction effect of Moringa oleifera and Curcuma longa towards R3, Mineral Requirement (%)](image)

**Figure 3.** 3D response surface graph of interaction effect of *Moringa oleifera* and *Curcuma longa* towards R3, Mineral Requirement (%)

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[1] Reference 1
[7] Reference 7
The effect of *M. oleifera* and *C. longa* towards Carbohydrate was shown in Figure 4. Result showed that the percentage of carbohydrate requirement in the formulated feed increase by 136.66% with the increasing percentage of individual factor A, *C. longa* in which it has exceed the 100% of carbohydrate requirement needed by *M. rosenbergii* larvae. This was due to higher carbohydrate content in *C. longa* which was 67.38 % [1].

![3D response surface graph of interaction effect of Moringa oleifera and Curcuma longa towards R4, Carbohydrate Requirement (%)](image)

**Figure 4.** 3D response surface graph of interaction effect of Moringa oleifera and Curcuma longa towards R4, Carbohydrate Requirement (%)

### 3.3 Numerical Optimization of Desirability Function for Factors and Parameters

In this study, Design Expert Software version 10 had suggested the most optimal parameters condition with the optimal percentage of factors for this study as shown in Figure 5. To guide the estimation of desirability, a linear ramp has been created by the software between the minimum value and the maximum value of factors and parameters. The optimal percentage of *C. longa* and *M. oleifera* were 1.00 and 17.10, respectively with the optimum percentage of protein requirement, lipid requirement, mineral requirement and carbohydrate requirement were 70.55, 72.86, 396.6 and 129.42, respectively. The overall desirability value was 0.738 that near to 1.00 indicates that it was a quite desirable formulation to fulfill the nutrient requirement of *M. rosenbergii* larvae.

![The desirability function ramp for all factors and parameters](image)

**Figure 5.** The desirability function ramp for all factors and parameters
4. Conclusion
In conclusion, the most desirable formulation was formulation 9 which contain 40% egg custard and 3% eggshell for overall feed formulation and 1.00% C. longa and 17.10% M. oleifera from the suggested formulation percentage by Design Expert Software version 10.

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