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GLOBAL ACADEMIC EXCELLENCE

DEVELOPMENT OF TILAPIA GROWER FEED (TGF) WITH APPLICATION OF SUSTAINABLE AGRICULTURE MATERIAL

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Abstract:

The price volatility of tilapia fish's feed in the market is strongly positively associated with its price increase. Thus, a possible approach is to generate food for from agricultural waste materials. Hence, this work aimed to generate TGF from black soldier fly larvae (BSFL), defatted kenaf seed (DKS), and broken rice (BR), with the primary objective of obtaining a yield with elevated protein and energy content. As part of our statistical analysis platform, we utilised the Design-Expert software application, which adheres to the response surface approach and mixed design strategy. The study's results showed that the combination of BSFL at 0.01 w/w%, DKS at 0.74 w/w%, and BR at 0.17 w/w% generated TGF that satisfies the Tilapia fish diet conditions, with a crude protein (CP%) content of 37.45% and energy content of 2970.26 kcal. The data obtained in this study is derived from laboratory conditions, therefore it is crucial to provide data obtained from living organisms in order to evaluate the effectiveness of the produced items.

Keywords:

Tilapia Grower Feed; Black Soldier Fly Larvae; Defatted Kenaf Seeds; Broken Rice; Sustainable

Introduction

The problem of rising and unpredictable prices of raw materials, particularly Tilapia, can be quite perplexing for various stakeholders. Users are greatly affected by this issue. According to a comprehensive analysis of scientific literature spanning from 2014 to 2024, it has been determined that the root cause of this problem can be attributed to the volatility in prices of animal food (Vera et al., 2020). It is crucial to address this issue seriously, as it poses a significant threat to the safety of the country's food ecosystem (Li et al., 2020; Soto-Sánchez et al., 2023). The analysis of articles on the reasons behind the increase in animal food reveals that multiple factors are driving this trend. These include the ongoing trade war between China and the United States (David et al., 2022; Hoc et al., 2021), conflicts between major food producers like Russia and Ukraine (Eysteinsson et al., 2020; Mikołajczak et al., 2022), reduced demand for biodiesel products which are key ingredients in animal food (Montero, Moyano, et al., 2023) , unpredictable weather and climate patterns that have impacted the availability of feed ingredients (Rawski et al., 2021; J. Wang et al., 2023)like soybean meal and maize meal (S. Wang et al., 2022), efforts by a few parties.

Regarding the issues mentioned in the excerpt above, it becomes apparent that the high cost of animal food is primarily due to a heavy reliance on external protein sources, particularly soybean meal (Pavlopoulos et al., 2023). Thus, the utilisation of protein sources from the local region is a part of the solution. The findings of studies Karlsen & Skov (2022) and Torrecillas et al. (2021) have provided additional support for this endeavour. In fact, these studies have confirmed that producing animal food locally can effectively lower the expenses associated with managing farmed animals (Adibrata et al., 2022). When it comes to producing animal food from local sources, there are two key aspects to consider. First, it is important to include ingredients that contribute to the protein content (Antonelli et al., 2023; Zatti et al., 2023). Additionally, incorporating ingredients that provide energy is crucial (Mohan et al., 2022; Montero, Carvalho, et al., 2023). It is essential to ensure that the selection of ingredients for protein and energy contribution aligns with the specific dietary requirements of the animal.

After conducting a thorough Scopus search, it was found that a significant number of articles delve into the utilisation of alternative ingredients that contain protein and energy sources for the production of Tilapia fish food. These sources encompass a wide range of materials, including those derived from insects, underutilised agricultural resources, and byproducts of industrial processes. Black soldier fly larvae (BSFL) are commonly utilised in the production of animal food as an insect ingredient (Nankervis et al., 2022; Paul et al., 2023). There are numerous benefits associated with utilising BSFL in animal food production. These include its rich CP%, making it an excellent source of nutrition (Bich et al., 2020). Additionally, BSFL is well-suited for farming in tropical and equatorial climates, allowing for consistent production. The insect's ability to consume a wide range of waste materials, excluding high fibre plants, further contributes to its economic and efficient nature (Virmani et al., 2023). Moreover, the low operational costs of maintaining livestock facilities make BSFL an attractive option (Palomba et al., 2022).

Kenaf seeds have shown promise as a viable ingredient for animal food production, particularly for underutilised agricultural sources (Rachmawati et al., 2023). There has been a significant amount of research and literature published on the potential use of kenaf seeds as an ingredient in animal food. In addition to predictive studies, there has been extensive research on the amino acid and fatty acid profiles found in kenaf seeds (Piazzon et al., 2022). Interestingly, certain varieties of kenaf seeds have a higher fat content compared to their protein content (Gasco et al., 2020). Sarkar et al. (2022) discusses how the protein value of the kenaf seed can be enhanced through the use of defatting, specifically by extracting fat using supercritical fluid technology (Miebach et al., 2023). Kenaf seeds, rich in protein, play a crucial role in fulfilling the nutritional requirements of animals. Additionally, the removal of fat from these seeds has pronoun it as defatted kenaf seeds (DKS) that prevent the development of rancidity in the end product.

In addition to protein sources, the composition of Tilapia food also relies on the inclusion of ingredients and energy sources (Liu et al., 2023). Encouraging the use of energy sources instead of agricultural waste materials is a viable alternative. Not only does this promote the production of sustainable products, but it also contributes to the achievement of the 12^{th} DSG goal of responsible consumption and production. Broken rice (BR) is a waste material that can serve as an energy source for Tilapia (Vandeputte et al., 2022). The utilisation of BR is a well-established practice that has found extensive application in the feeding of livestock, particularly chickens and ducks. This material is a result of rice processing, and its physical breakdown is not suitable for general sale. The projected revenue for packaging crushed rice is 30%. This role holds significant value for small-scale rice entrepreneurs, as it involves the utilisation of crushed original rice to develop new products (Liang et al., 2022). This approach is a positive step towards addressing the consequences of crushed rice.

The use of response surface methodology (RSM) in food product processing is a well-established practice in the field of research (El-Naggar et al., 2022). There are several established products that have been produced using this technique, such as beverage products

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(Wan et al., 2023), emulsions(Khieokhajonkhet et al., 2022), and protein extracts(Vicente et al., 2022). There are several benefits that can be derived from utilising this analysis instrument. These include the potential to reduce material costs by minimising the amount of materials used and the ability to expedite the study period (Naik et al., 2021). Considering the fact that Tilapia fish food should have a high protein and energy content, the utilisation of RSM in this research might hinder the accurate determination of the optimal ratio of ingredients (BSFL, DKS, and BR) needed to meet the study's goals of maximising the CP% and Energy of TGF.

Methodology

Materials
The production process of TGF involves a combination of three distinct ingredients: BSFL, DKS, and BR. The researcher obtained 5 kg of BSFL from a local supplier in the Sri Aman district of Sarawak. The DKS used in this study was obtained from a local kenaf seed supplier in Kelantan, Malaysia. Prior to the experiment, the DKS underwent a defatting process using supercritical fluid processing at University Putra Malaysia (UPM), Selangor. The BR used in this study was sourced from premises located around Sibu, Sarawak.

Methods
The production process of TGF is initiated by combining the three ingredients based on the specified measurements provided in Table 1. The mixture is subsequently blended using a mixer in order to attain uniformity. Subsequently, the TGF mixture was transferred to a ziplock bag and carefully placed in a temperature-controlled chiller room at a temperature of 4 ± 2 ^{o}C. The mixture was kept in this environment until it was ready for further analysis.

Determination of CP% and Energy
The determination of the crude protein value is typically conducted using the Kjedahl technique, which involves analysing the nitrogen content. In this method, a conversion rate of 6.25 is commonly employed to calculate the protein content based on the percentage of nitrogen present (Pouil et al., 2020). Meanwhile, the Energy value has been determined according to the technique that has been practiced by Adebisi & Stephen (2017).

Statistical Analysis
This study utilises two data analysis techniques available in the Design - Expert 11 software: RSM and Mixture Design. The objective is to examine the impact of varying rates of three materials on the CP% and Energy values of the final product. In addition, this study also examines the interaction relationship between each variable included: independent variables (BSFL, DKS, and BR) % w/w, and dependent variables (CP% and Energy). This study utilises a three-factor design with three levels for each factor and incorporates modelling parameters based on Design-Expert 11. According to the data in table 1, the statistical software suggests a total of 14 run numbers. The study has one central point located on the 6th run. The purpose of implementing the middle point of the run is to estimate the variance result of each dependent variable [46]. The study focusses on selecting CP% and Energy as dependent variables to align with the objective of producing high-protein and high-energy TGF that is suitable for the Tilapia grower's diet. Efforts have been made to optimise the value of CP% and Energy in TGF by taking into account all three factors within their respective ranges.

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Results

In order to assign each data in Table 1 to the most suitable mathematical model, the Design - Expert software has conducted tests on seven different mathematical models. The process of projecting acquired data to this activity is commonly referred to as model fitting. The summary of the fitting model is presented in Table 2. Initial research indicates that the data set is appropriate for coding using the L-pseudo model. Through data matching and model fitting, it has been demonstrated that the variables CP% and Energy are conform to the linear models. The data and mathematical models are matched based on the significance of each variable, indicated by p-values below 0.05. The data's accuracy is reinforced by the insignificance of the Lack of Fit values for each selected model (CP%: 0.8096 and Energy: 0.1259).

The adjusted R^2 values (CP%: 0.9998, Energy: 0.9760) and predicted R^2 values (CP%: 0.9997, Energy: 0.9613) obtained for the two dependent variables are deemed satisfactory. The satisfactory level is determined by the difference of less than 0.2 between the two components and each dependent variable [47]. In addition, there are two model sources that are commonly regarded as being similar: quartic vs cubic and quartic vs sp quartic. The labelling is implemented due to the limited availability of experimental studies to provide a more precise estimation of the material. Thus, the L-pseudo models within the linear models were chosen for the subsequent ANOVA analysis.

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Table 1: The Summary Data of Three Independent Variables (A, B And C) and Two Dependent Variables (CP% And Energy)

Run	A	B	C	CP%	Energy
1	0.5	0.5	0	29.63	2212.96
2	0	0	1	3.29	3217.02
3	1	0	0	12.76	4079.26
4	0	0	1	3.46	3217.02
5	0.17	0.66	0.17	33.95	1472.79
6	0.33	0.33	0.34	20.69	2522.19
7	0.17	0.17	0.66	12.39	2907.81
8	0.5	0.5	0	30.25	2218.35
9	0	1	0	46.58	346.97
10	0.66	0.17	0.17	17.00	3338.80
11	0	0.5	0.5	25.02	1782.21
12	0	1	0	46.87	348.26
13	0.5	0	0.5	8.07	3648.01
14	1	0	0	12.68	4082.50

Note* A: BSFL, B: DKS, C: BR

Table 2: Model Fitting for CP% and CF%

Source	Sequential p-value		Lack of Fit p-value		Adjusted R^2		Predicted R^2		Status	
	CP%	Energy	CP%	Energy	CP%	Energy	CP%	Energy	CP%	Energy
Linear	<0.000	0.0008	0.8096	0.1259	0.9998	0.9760	0.9997	0.9613	Suggested	
Quadratic	0.8035	0.1019	0.6585	0.2142	0.9997	0.7864	0.9996	0.5165		
Special Cubic	0.5192	0.2133	0.5872	0.2319	0.9997	0.8074	0.9990	-0.1338		
Cubic	0.9069	0.1235	0.2358	0.0932	0.9996	1.000	0.9942	0.0943	Aliased	
Sp Quartic vs Quadratic	0.9053	0.0885	0.2358	0.9214	0.9996	0.8977	0.9942	0.8312		
Quartic vs Cubic	0.2358	0.9214			0.9997	0.8724			Aliased	
Quartic vs Sp Quartic	0.2358	0.9214			0.9997	0.8724			Aliased	

The ANOVA results in Table 3 indicate that only one significant finding for all the responses examined, such as Energy (AC). Every interaction is deemed significant if its p-value is below 0.05. In addition, these important interactions play a crucial role in achieving the study's objective of producing TGF. Regarding other interactions, there are no level of data shows as it purely reflect to significance of each data, where as for Energy only two interactions (AB and AC) are deemed to be insiginificant, as their p-value exceeding 0.05. This failure to meet the study's objectives is worth noting. The F value obtained for each response (CP%: 0.49, Energy: 2.35) indicates that the LOF term is not statistically significant when compared to the pure error. In addition, the LOF values for each dependent variable (CP%: 81%, Energy: 21%) suggest that the data source may be influenced by random variation. Having a non-significant LOF position is crucial in demonstrating that each model meets the criteria for a good fit [48].

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In addition, to gain a clearer understanding of each interaction, it is necessary to have knowledge of three dimensional plots for each response.

Table 3: ANOVA Analysis for L-Pseudo Model of CP% and CF%

Source	Sum of Squares		Df		Mean Square		F – Value		p – Value		Status	
	CP%	Energy	CP%	Energy	CP%	Energy	CP%	Energy	CP%	Energy	CP%	Energy
Model	2677.96	66.25	2	5	1338.98	13.25	31549.57	10.57	0.00	0.00	Significant	
Linear Mixture	2677.96	55.36	2		1338.98	27.68	31549.57	22.08	0.00	0.00		
AB	NA	4.11	NA	1	NA	4.11	NA	3.28	NA	0.11		
AC	NA	6.78	NA	1	NA	6.78	NA	5.41	NA	0.04		
BC	NA	0.15	NA	1	NA	0.15	NA	0.12	NA	0.74		
A^2BC	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		
AB^2C	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		
ABC^2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		
Residual	0.47	10.03	11	8	0.04	1.25						
LOF	0.21	7.03	7	4	0.03	1.76	0.49	2.35	0.81	0.21	Not Significant	
P.E	0.25	2.99	4		0.06	0.75						
C.T	2678.43	76.27	13									

Note* NA: Not Applicable, LOF: Lack of Fit, P.E: Pure Error, C.T: Cor Total

Figure 1 shows two three-dimensional plots illustrating the relationship between the independent variables (BSFL, KSD, and BR) and the dependent variables (CP% and Energy). Both graph dimensional plots show the same shape since both are modeled on a linear position. But, it is distinguished by the degree of inclination of each graph. Based on observations, it appears that the graph in Fig. 1(a) is more sloped than Fig. 1(b), this is closely related to the relationship between the rate of independent variables and the value of dependent variables.Using colour and shape as indicators can effectively convey the highest and lowest values of each response. Three colours are used to represent data values: red for the maximum, yellow for the medium, and purple for the minimum. Based on the data presented in Fig. 1(a) and (b), the minimum CP% value is observed at 3.73% for A: 0.02 (w/w)%, B: 0.02 (w/w)%, and C: 0.96 (w/w)%, while the maximum state is found at 46.44% for A: 0.01 (w/w)%, B: 0.98 (w/w)%, and C: 0.01 (w/w)%. At the state, the energy value records the minimum and maximum values for 379.18 kcal, A: 0.01 (w/w)%, B: 0.97 (w/w)% and C: 0.01 (w/w)%; 4029.92 kcal, A: 0.97 (w/w)%, B: 0.00 (w/w)%, and C: 0.03 (w/w)%.

The importance of CP% in the diets of animals, especially Tilapia fish, cannot be denied. This component is crucial for the development of the animal's muscular structure [49]. The influence of the muscle-building factor on both growth and final weight measurement in animals is significant. An issue arises when operators establish the sales rate based on the ultimate weight of the animal, and the fish does not grow at its ideal rate because of inadequate consumption of CP% [50]. De Silva et al. [51] indicates that tilapia should attain a crude protein percentage CP% of 30% during the growth phase. Under such circumstances, the tilapia must also ingest a specific proportion of CP%. Surpassing this criterion will result in the animal excreting enormous quantities of pee, therefore polluting the fishpond and rendering it unhygienic [52]. In light of the above described concerns, a possible resolution is to obtain CP% from alternate sources.

The data presented in Fig. 1(a) and the previous results clearly demonstrate a substantial interaction between BSFL and DKS in the making of high-protein TGF. Furthermore, this favourable outcome validates the conclusions of prior investigations, particularly those carried out by Mariod et al. [53] on black soldier fly larvae and Giwa et al. [30] on kenaf seed. Previous

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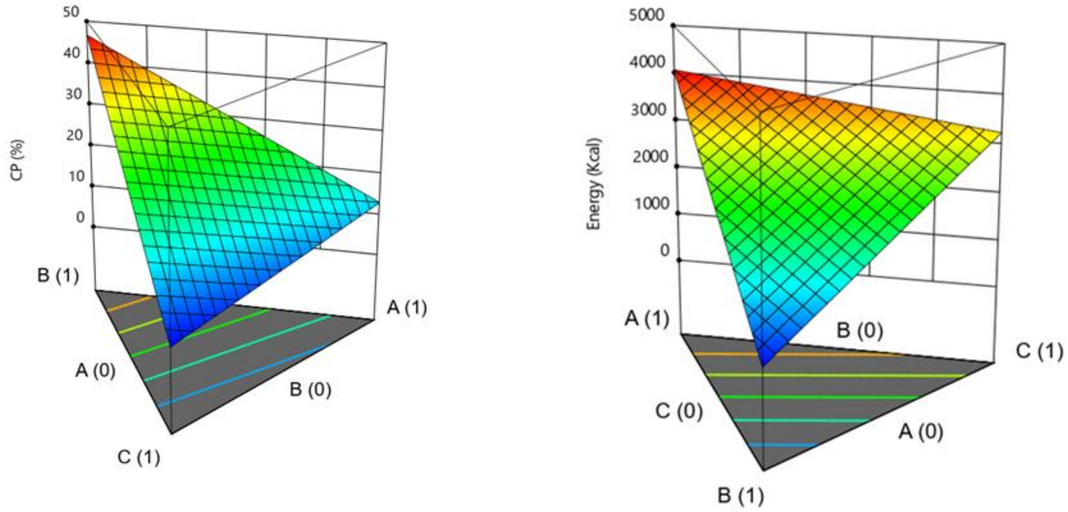
research, such as those utilising BSFL as feed for chicken [54], duck [55], and Pangasius , provide additional confirmation of the suitability of this material as animal feed. Nevertheless, it is worth investigating the possibility of modifying the material by including DKS. It is well-established that kenaf seeds possess a substantial crude fat content, and any processing method such as milling will effectively extract the oil. In order to prevent oxidation, which may lead to rancidity and an unpleasant flavour for the fish, it is advisable to exclude any kenaf seed oil from the material's membrane. Hence, the use of defatting in this work not only reduced the potential harm to the end product, but also displayed a notable enhancement in the CP% value of the initial material to 19.26 [32].

The three-dimensional graph effectively illustrates the impact of BSFL and BR on energy levels. The nutritional composition of BSFL, with a carbohydrate content of around 8 to 24 kcal [57], is a key determinant in its utilisation as a primary source of energy. In addition, the nutritional items he consumes, including agricultural processing waste, crude palm oil cake, and sago dregs, significantly influence the overall energy content. Furthermore, there is now no longer any uncertainty on the impact of BR. The results of this study indicate the considerable promising for the development and commercialisation of this agricultural waste material, especially in the field of animal feed manufacturing companies.



(a) ***(b)***

Figure 1: Three-dimensional surface plots showing the relationship between CP% (a) and Energy (b) as affected by A, B and C

Following the implementation of the formulation to accomplish the study's purpose, the Design Expert program has proposed multiple formulations for the combination values that yield the maximum CP% and Energy values. The suggested formulation is enumerated in Table 4. Solution number 1 has been selected based on its desirability value, which is nearly equal to 1. A desirability value is a forecast of the attainability of the proposed formulation. Moreover, the desirability value that approaches or equals 1 can be attributed to the alignment of the proposed formulation with the specific purpose of the study [58]. In order to prove the consistency of Formulation 1, the experiment involving it and its reaction has been replicated three times. The analysis of the data in Table 5 has determined that there is no statistically significant difference

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among any data point. Moreover, the data points in Table 6 exhibit well-defined values that fall within the recommended range of response values provided by the software.

Table 4: The Suggested Optimised Formulations Generated by Statistical Analysis Software For Research Goals

Solution number	1	2	3	CP%	Energy	Desirability
1	0.01	0.74	0.17	37.45	2969.62	0.99
2	0.33	0.33	0.33	20.98	2554.29	0.98
3	0.67	0.17	0.16	16.83	3319.21	0.97

Table 5: Experimental Values according to the Optimised TGF Mixture

Independent trials	CP%	Energy
Trial 1	37.47	2970.26
Trial 2	37.58	2969.25
Trial 3	37.42	2970.23

Table 6: Model Verification of Mixture Design in Optimizing TGF

Dependent Variables	Predicted Mean	Predicted Median	Standard Deviation	SE Mean	95% CI low for Mean	95% CI high for Mean
CP%	37.56	37.56	0.21	0.09	37.37	37.76
Energy	2955.87	2955.87	17.06	8.66	2935.91	2975.84

Conclusions

In conclusion, the RSM method under mixture design technique, , has successfully produced high-protein and high-energy TGF at (A: 0.01 w/w%, B: 0.74 w/w%, C: 0.17 w/w%; CP%: 37.45% and Energy: 2969.62 Kcal). The next study should concentrate on examining the impact of TGF consumption on the animal's physiology.

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