Valorization of horticultural agro-residues and marine for sustainable poultry nutrition: a response surface methodology-based approach with argan press cake and anchovy bone residues

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Abstract

Although rich in proteins and minerals, the salty by-products of anchovy processing, especially bones, are usually discarded because of their high sodium chloride (NaCl) content. This study developed a process to desalt anchovy bone residues and combine them with rinsed argan press cake, an agro-industrial co-product used in animal feed, to formulate a functional poultry diet. Simple solid–liquid extraction with tap water (1:1, m/v) reduced NaCl from 15.4% to 4.7%. A Response Surface Methodology (RSM) then evaluated mixtures of desalted bones (10–90%) and rinsed argan press cake (10–30%). Nutritional parameters measured included NaCl, calcium, phosphorus, ash and total nitrogenous matter. The optimal diet, with 10% desalted bones and 10% press cake, achieved a final NaCl content of 0.49% while maintaining essential nutrients. These findings demonstrate the feasibility of integrating marine and agro-industrial co-products into sustainable poultry feed, supporting circular economy principles.

Keywords: animal feed, desalination, fishery products, food quality

Introduction

Fish is a staple dietary component in many maritime countries, with anchovy (*Engraulis encrasicolus*) being among the most abundantly fished species worldwide (Nurulnadia et al., 2021; Fernández Corredor et al., 2021) However, anchovy fillet production generates a significant volume of biological waste, particularly bones, which are often discarded (Paone et al., 2021). Additionally, the salting process involved in anchovy preservation produces saline residues that are typically considered non-recyclable due to their high sodium chloride (NaCl) content (Marchetti et al., 2021). This presents a challenge for valorizing these by-products, especially in the context of sustainable food systems. Despite their rich nutritional profile, salted fish waste remains underexplored for animal nutrition purposes (Hasan et al., 2019). Fish waste, which constitutes up to 70% of fish processing outputs, holds significant potential for nutrient recovery and sustainability through circular bioeconomy approaches, aligning with SDG 12.3. Recent research has demonstrated that anchovy fillet residues can be sustainably valorized through green extraction, and waste-to-energy systems, reinforcing their potential in a circular economy context aligned with the UN Agenda 2030. On the other hand, increasing attention is being given to the use of agro-industrial by-products as affordable and sustainable sources of nutrients and bioactive compounds (Taarji et al., 2018) the solid residue obtained after oil extraction from *Argania spinosa* kernels. Argan press cake constitutes approximately 50% of the original seed mass and is commonly reused in animal nutrition due to its high protein content (Mirpoor et al., 2021; Mirpoor et al., 2022). Recent studies have emphasized the bioactive potential and antioxidant richness of argan press cake (Paone et al., 2021; Hallouch et al.,2025), underlining its suitability as a cold-pressed

oilseed by-product and its potential for exploitation as a feed additive in both monogastric and ruminant diets (Rakita et al., 2023). In Moroccan rural communities, particularly among the Amazigh population, argan press cake has traditionally been used both as a livestock feed and a biofuel (Lakram et al., 2019; Moutik et al., 2021). Its historical and ongoing use in animal diets highlights its potential as a nutritionally valuable feed ingredient (Koufan et al, 2020; Ibourki et al., 2021). Faced with the growing demand for sustainable and organic animal products, argan press cake is also being used to replace some of the imported raw materials such as soybean meal, It becomes necessary to develop new natural feed formulations capable of improving animal growth and health without resorting to synthetic additives (Abd El-Hack et al., 2020).The aim of the present study is to develop a natural and economical process for desalting salted anchovy bone residues by aqueous extraction, and to formulate a nutritionally enriched poultry feed by combining these desalted bones with rinsed argan press cake. The rinsing phase reduces anti-nutritional factors, in particular saponins, present in argan press cake. These substances, known for their surface-active properties, are associated with a bitter, astringent taste, likely to reduce food consumption (Lakram et al. 2019). Application of a solid/liquid ratio of 1:12.5 (g/mL) eliminates up to 91% of the saponin content, making the final product more palatable and nutritionally appropriate (Lakram et al. 2019). The experimental design is based on a Response Surface Methodology (RSM) to optimize the proportions of these two components (anchovy bone residues: 10 to 90%; argan press cake: 10 to 30%). By combining desalinated marine waste and detoxified plant residues, this study proposes a quasi-organic poultry feed solution with an acceptable low salt content and reduced anti-nutritional factors. Previous work on sodium requirements in poultry diets (Sassa et al., 2022; Gao et al., 2021) supports the feasibility of this formulation, reinforcing its relevance as an alternative to conventional feeds in modern poultry farming.

Material and methods
Raw Materials

This study was conducted at the Food Sciences Laboratory, Faculty of Science and Technology, Mohammedia, Morocco. Two primary materials were used in this study: salted anchovy bone residues and argan press cake. Anchovy bones (*Engraulis encrasicolus*) were obtained from a Moroccan industrial processing facility in January 2023. To preserve their quality, bones were transported in insulated coolers at approximately

4°C. Upon arrival, residual muscle tissue was manually removed, and bones were dried at 60 °C for 24 h following established protocols [26]. Dried bones were ground using a laboratory mill and sieved to a uniform particle size of 800 μm to ensure homogeneity (Samuelsen et al., 2013). Argan press cake, a by-product of *Argania spinosa* kernel oil extraction, was obtained from a women's cooperative in Essaouira, Morocco, and stored at −20 °C to prevent oxidative degradation (Taraji et al., 2020). Prior to use, the argan press cake was ground using a high-speed blender to a particle size below 0.5mm (Taraji et al., 2020), then subjected to an aqueous heat treatment designed to reduce saponin content. Specifically, the powder was rinsed in distilled water at 80°C for 25 minutes using a solid-to-liquid ratio of 1:12.5 (g/mL) (Lakram et al., 2019).

Desalination and Feed Formulation Process

Desalination of anchovy bone powder was conducted using a solid–liquid extraction technique in potable tap water. The desalted anchovy residues (DAR) were prepared at concentrations ranging from 10% to 90% (w/v), and mixed with argan press cake (APC) at proportions between 10% and 30% (w/v). A preliminary extraction was also performed using a fixed ratio of 10% DAR and 10% APC (w/v), combined with water in a 1:1 material-to-solvent ratio, to evaluate baseline salt removal efficiency. All extractions were carried out at a constant temperature of 25°C, and suspensions were stirred continuously for 24 hours using a magnetic stirrer to ensure optimal mass transfer (Muñoz-Guerrero et al., 2010; Taarji et al., 2018). After the extraction period, the mixtures were filtered to remove solids, and the resulting filtrates were stored at 4°C prior to physicochemical analysis. This preparation process was repeated for various formulations, which were later subjected to nutrient profiling and optimization via Response Surface Methodology (RSM), to determine the ideal balance between desalination efficiency and nutritional quality for poultry feed applications.

Physicochemical Analysis

All measurements were performed in triplicate, and results were expressed as the mean ± standard deviation (Shi et al., 2009). Salt (NaCl) content was determined using the Mohr titration method with silver nitrate as titrant and potassium chromate as indicator (Ababouch et al., 2009). Ash content was measured by incineration at 550 °C for 12 h in a muffle (Ababouch et al., 2009). This process allowed for the determination of the total mineral residue, indicating the overall inorganic matter in the sample. Total nitrogen was assessed via

the Kjeldahl method and converted to protein content using N × 6.25 (Ababouch et al., 2009). Calcium (Ca) and phosphorus (P) were quantified according to AOAC-approved methods (Ababouch et al., 2009) ; Ca by complexometric titration with EDTA and P using the vanadomolybdate colorimetric method. These analyses provided a comprehensive profile of salt, protein, ash, and essential minerals, critical for evaluating feed suitability.

Experimental Design

To evaluate the influence of independent variables on the physicochemical properties of the formulated poultry feed, a Response Surface Methodology (RSM) was employed. The experimental design and statistical analyses were conducted using Design-Expert® software version 13.0.14.0 (Stat-Ease Inc., Minneapolis, USA). The objective of RSM was to optimize the process parameters and to model the relationship between the dependent variables (responses) and the independent variables, which included the proportion of desalted anchovy residues (DAR, 10–90% w/v) and argan press cake (APC, 10–30% w/v) (El Kaourat et al., 2024). These two factors were selected based on preliminary trials and their nutritional relevance. A second-order polynomial regression model was fitted to the experimental data to describe the response surfaces. Each response variable Yi (e.g., NaCl content, total ash, total nitrogen, calcium, phosphorus) was modeled as a function of the two factors using the following general quadratic equation:

Where:

$$Y = f(y) = \beta_0 + \beta_1 A + \beta_2 B + \beta_{12} AB + \beta_{11} A^2 + \beta_{22} B^2 + \varepsilon$$

Yi is the predicted response, used to relate the independent variables A and B

, β_0 represents the constant term, β_{ij} (i = 1, 2 and j = 1, 2), β_{ii} (i = 1, 2) represents the interactions between the variables and the quadratic terms,

ε is the residual error.

This statistical approach allowed for the identification of optimal mixing ratios that minimize salt content while preserving key nutritional qualities of the blend. The significance of the model and its terms was assessed using analysis of variance (ANOVA) at a 95% confidence level. The adequacy of the model was evaluated based on R² values, lack-of-fit tests, and residual analyses. Insignificant terms (p > 0.05) were

systematically eliminated via backward elimination using Design-Expert® software to enhance model precision and predictive power. Three-dimensional response surface plots were then generated to visualize the interactions between variables and their effects on the measured responses. The RSM approach was applied specifically to optimize and model the nutritional parameters of the experimental poultry feed formulations. The independent variables included the proportion of desalted anchovy bones (DAR) and argan press cake (APC), while the dependent variables (responses) were: NaCl content, total phosphorus (g/kg), total nitrogen matter (TNM, %), ash content (%), and calcium content (g/kg). As summarized in **Table 1**, the coded levels of the independent variables were represented numerically: "1" for the factorial points (high and low values), and "0" for the central point, which served as the reference for evaluating model curvature and reproducibility.

(2) to formulate an optimized, nutrient-dense poultry feed from desalted anchovy bone residues, contributing to both waste valorization and the development of cost-effective, organic animal feed solutions.

Results and Discussion

Nutritional Composition of Raw Materials

The fish canning industry generates substantial volumes of high-salinity by-products, particularly from the processing of anchovy (*Engraulis encrasicolus*) (Marchetti et al., 2021). The elevated salt content of these by-products presents specific technical challenges for downstream utilization. In this context, the present study explored desalting as a targeted valorization strategy to improve resource recovery potential and support sustainable management practices. The proximate chemical composition of three raw materials Salted Anchovy Residue (SAR), Desalted Anchovy Residue (DAR), and Argan Press Cake (APC) is presented in **Table 2**. The desalting process, based on solid-liquid extraction using potable water, proved effective in significantly reducing the salt content of SAR from 15.4% to 4.7%, without causing substantial alterations in its other nutritional attributes such as protein, ash, or mineral content. This reduction in NaCl content demonstrates the feasibility of a simple, cost-effective, and environmentally friendly desalination

Table 1. Factor levels of independent variables for poultry feed synthesis

Independent variables	Variables coded	Unit	Range and level of actual and coded values		
			-1	0	1
Desalted Anchovies Residues (DAR) (%)	A	%	10	50	90
Argan Press Cake (APC) (%)	B	%	10	20	30

Table 2. Chemical composition of the products used

Settings	Samples		
	SAR	DAR	APC
Salt%	15.4	4.70	0.3
ASH%	16.09	13.98	4.23
TNM %	34.12	29.73	28.42
Ca (g/Kg)	34.80	27.15	6.7
P (g/Kg)	17.4	14.2	6.3

SAR: salted anchovies residues; **DAR:** desalted anchovies residues, and **APC:** argan press cake

Table 3. Real value of independent factors with responses

Factors		Reponses				
DAR (%)	APC (%)	Salt (%)	Ca(g/Kg)	P(g/Kg)	ASH (%)	TNM (%)
90	10	4.24	25.115	13.31	13.005	29.699
50	20	2.51	14.915	8.306	7.836	20.449
10	10	0.49	3.305	2	1.771	5.7815
10	30	0.59	4.725	3.35	2.567	11.899
90	30	4.27	26.445	14.57	13.9051	35.3283
50	20	2.41	14.8815	8.4	7.816	20.4549
50	20	2.31	14.925	8.36	7.839	20.549
50	20	2.49	14.905	8.26	7.7836	20.649
10	20	0.52	4.155	2.73	2.294	8.557
50	10	2.38	14.295	7.653	7.3413	17.807
90	20	4.36	25.675	14.14	13.328	32.341
50	30	2.47	15.605	9.07	8.269	23.4391

method that preserves the nutritional integrity of the anchovy residue. The incorporation of argan press cake, known for its high protein content and traditional use as animal feed, further enhances the nutritional profile of the final feed formulation. These findings support the potential dual valorization of both anchovy bone residues and argan press cake, contributing to circular economy principles in agro-industrial systems while enabling the production of an organic and nutritionally balanced poultry feed. Nonetheless, studies have emphasized the need to quantify antinutritional factors and evaluate their residual presence after processing, particularly saponins .These elements were not addressed in this study and should be considered in future optimizations for feed palatability and nutrient availability.

An in-depth overview of the experimental parameters and levels of independent variables is provided in Table 1 of this study. The subsequent table presents the observed responses for the different formulations tested during the experimental runs. The primary objective was to develop a robust regression model capable of accurately capturing the relationships between the independent variables and the response variables. To achieve this, the Response Surface Methodology (RSM) was employed, following a series of preliminary trials to refine and optimize the experimental protocol. Prior to constructing the mathematical model and identifying the optimal formulation conditions, the dependent variables were carefully measured and analyzed, with the results summarized in **Table 3**. The experimental design investigated the interaction effects between two key factors: the proportion of desalted anchovy bone residues (Factor A) and the proportion of argan press cake (Factor B). These interactions were evaluated with respect to their influence on several critical response variables: sodium chloride (NaCl) content (%), calcium (g/kg), phosphorus (g/kg), total ash content (%), and total nitrogen matter (TNM, %). Each factor was tested at two distinct coded levels, allowing for the construction of a predictive model and the identification of conditions that optimize the nutritional composition of the resulting poultry feed formulation (Demirel et al., 2012).

Based on the experimental results, the relationships between the independent variables and the five response variables: salt content (%), calcium (g/kg), phosphorus (g/kg), ash content (%), and total nitrogen matter (TNM, %) were modeled using second-order polynomial equations. The amounts of desalted anchovy residue and argan press cake were coded as factors A and B, respectively. The quadratic effects of each factor were represented by the second-order terms (A^2 and B^2), while the interaction between the two factors was denoted by the cross-product term (AB). The linear coefficients (A and B) reflect the individual contributions of each component. In this context, positive regression coefficients indicate a synergistic effect on the response variable, whereas negative coefficients suggest an antagonistic effect (Filli et al., 2010).

Analysis of Variance (ANOVA)

To evaluate the statistical significance and adequacy of the model, Analysis of Variance (ANOVA) was applied to the central composite design (CCD). ANOVA provided insights into model performance by generating sequential P-values, non-adjusted and adjusted P-values, as well as the adjusted and predicted R^2 values. The summary of the results (Table 4) confirmed the relevance and statistical significance of the proposed quadratic model, supporting its suitability for predicting and optimizing the responses within the experimental domain.

Recent studies show that in evaluating the adequacy of RSM models, both R^2 and adjusted R^2 were used to assess descriptive reliability (Mohammed et al., 2024), while the comparison between predicted R^2 and adjusted R^2 was highlighted as a validation tool when the difference between them is less than 0.2(Mohammed et al., 2024). Analysis of variance (ANOVA) was employed to

Table 4. Evaluation of the second-order polynomial equation derived for the five responses

Response variables	Second order polynomial models	Regression coefficient	
		R^2	R_{adj}^2
SALT %	$+2.44+0.0367A+1.88B -0.0175AB-0.0238A^2 -0.0087B^2$	0.9985	0.9973
TNM%	$+20.50+2.90A+11.86B-0.1220AB+0.1628A^2-0.0113B^2$	0.9999	0.9999
ASH%	$+7.82+0.4373A+5.60B+0.0260AB-0.0062A^2-0.0004B^2$	0.9999	0.9998
Ca (g/Kg)	$+14.92+0.6767A+10.84B-0.0225AB+0.0129A^2-0.0221B^2$	1.0000	0.9999
P(g/Kg)	$+8.36+0.6712 A +5.66B-0.0225AB-0.0487A^2 +0.0248B^2$	0.9998	0.9996

evaluate the statistical significance and adequacy of the fitted models. Specifically, F-value tests were used to assess the relevance of each model type. The F-test examines the null hypothesis that a given model does not explain a significant proportion of the variability in the response data. A high F-value, in conjunction with a low P-value, indicates that the model is statistically significant and provides a good fit for the observed data. This approach is widely used in response surface methodology (RSM) to compare the predictive power of different model forms and identify the most appropriate model structure for process optimization (Bhatti et al., 2011) (**Table 5**).

According to the results, both linear and quadratic terms exerted statistically significant effects on the responses under investigation. The model's capacity to explain the variability in the data was evaluated using the adjusted coefficient of determination (R^2), which accounts for the number of predictors included in the model. This metric emphasizes that merely increasing the number of terms does not necessarily enhance model performance or predictive accuracy (Taran et al., 2015). The P-values associated with each coefficient were used to determine their statistical significance, highlighting the critical interactions and individual contributions of the studied components (Shrivastava et al., 2008). A high F-value coupled with a low P-value indicates a higher significance of the corresponding model term, as confirmed by the findings of (Taran et al., 2015).The coefficient of variation (CV), defined as the ratio of the standard deviation to the mean response, was employed to assess the model's repeatability and reliability. The CV values obtained—2.98% for salt, 0.4322% for calcium, 0.9660% for phosphorus, 0.8325% for ash, and 0.4918% for total nitrogen matter (TNM) were all within acceptable limits, indicating low variability and good model reproducibility. A comparison of the predicted values with experimental data (**Table**) demonstrated a strong correlation, thereby validating the model's predictive capacity and supporting the feasibility of the proposed feed formulation strategy for poultry nutrition.

Model Validation and Adequacy Check
As shown in Table 4, validating the reliability of the response surface methodology (RSM)-based models is essential to ensure accurate predictions under real-world conditions. Two principal methods were employed: a numerical evaluation of goodness-of-fit metrics and a graphical analysis of model residuals. The numerical evaluation focused on the coefficient of determination (R^2) and adjusted R^2 (R^2adj) to assess the explanatory power of the model, while the graphical analysis considered the residual distribution, i.e., the differences between observed and predicted values. This dual verification process is crucial for ensuring that the developed models faithfully represent the experimental system and can be reliably applied in practical applications (Filli et al., 2010).

Model Adequacy and Predictive Performance
Figure 1 presents the scatter plots comparing predicted versus observed values for salt (%), calcium (g/kg), phosphorus (g/kg), total nitrogen matter (TNM, %), and ash (%). These plots are essential to assess the model's adequacy, particularly through residual analysis based on least squares fitting. A strong alignment along the diagonal line in each graph indicates that the developed regression models reliably predict the experimental outcomes. The corresponding regression coefficients (R^2) for salt, calcium, phosphorus, TNM, and ash were 99.85%, 100%, 99.98%, 99.99%, and 99.99%, respectively, confirming the exceptional accuracy and robustness of the fitted models. This high degree of linearity and minimal residual dispersion further supports the model's validity and predictive strength. The statistical relationship between the predicted and actual values reinforces the hypothesis that the experimental system can be effectively modeled using the selected response surface methodology (RSM). These R^2 values substantially exceed the conventional threshold of acceptability. While R^2 values above 78% are generally considered adequate (Chauhan et al., 2004), recommend a minimum of 80% for optimal model reliability. Moreover, the adjusted R^2 values reported in this study remained consistently high, confirming the models' stability and precision even after accounting for the number of predictors (Zaibunnisa et al., 2009).This is in accordance with best practice standards recommending that the difference between R^2 and

Table 5. Analysis and treatment of variance (ANOVA) data for the full quadratic model of response variables

Source	Sum of Squares	Df	Mean Square	F-value	p-value	Statistical
Salt Model	21.18	5	4.24	815.83	< 0.0001	Significant
A-A.P.C	0.0081	1	0.0081	1.55	0.2591	
B-D.A.R	21.17	1	21.17	4076.92	< 0.0001	
AB	0.0012	1	0.0012	0.2359	0.6444	
A^2	0.0015	1	0.0015	0.2897	0.6098	
B^2	0.0002	1	0.0002	0.0393	0.8494	
Residual	0.0312	6	0.0052			
Lack of Fit	0.0064	3	0.0021	0.2562	0.8536	Not significant
Pure Error	0.0248	3	0.0083			
Cor Total	21.21	11				
Std. Dev.	0.0721	R^2		0.9985		
Mean	2.42	Adjusted R^2		0.9973		
C.V. %	2.98	Predicted R^2		0.9952		
		Adeq Precision		75.2577		
Ca Model	708.00	5	141.60	34081.03	< 0.0001	Significant
A-A.P.C	2.75	1	2.75	661.23	< 0.0001	
B-D.A.R	705.25	1	705.25	1.697E+05	< 0.0001	
AB	0.0020	1	0.0020	0.4874	0.5112	
A^2	0.0004	1	0.0004	0.1074	0.7542	
B^2	0.0013	1	0.0013	0.3124	0.5964	
Residual	0.0249	6	0.0042			
Lack of Fit	0.0239	3	0.0080	22.93	0.0143	Significant
Pure Error	0.0010	3	0.0003			
Cor Total	708.03	11				
Std. Dev.	0.064	R^2		1.0000		
Mean	14.91	Adjusted R^2		0.9999		
C.V. %	0.4322	Predicted R^2		0.9997		
		Adeq Precision		505.4277		
P Model	194.70	5	38.94	5991.62	< 0.0001	Significant
A-A.P.C	2.70	1	2.70	415.88	< 0.0001	
B-D.A.R	191.99	1	191.99	29540.92	< 0.0001	
AB	0.0020	1	0.0020	0.3116	0.5969	
A^2	0.0063	1	0.0063	0.9751	0.3615	
B^2	0.0016	1	0.0016	0.2513	0.6340	
Residual	0.0390	6	0.0065			
Lack of Fit	0.0277	3	0.0092	2.46	0.2395	not significant
Pure Error	0.0113	3	0.0038			
Cor Total	194.74	11				
Std. Dev.	0.0806	R^2		0.9998		
Mean	8.35	Adjusted R^2		0.9996		
C.V. %	0.9660	Predicted R^2		0.9987		
		Adeq Precision		222.0117		
ASH Model	189.38	5	37.88	8953.21	< 0.0001	Significant
A-A.P.C	1.15	1	1.15	271.22	< 0.0001	
B-D.A.R	188.23	1	188.23	44494.16	< 0.0001	
AB	0.0027	1	0.0027	0.6404	0.4541	
A^2	0.0001	1	0.0001	0.0245	0.8807	
B^2	4.004E-07	1	4.004E-07	0.0001	0.9926	
Residual	0.0254	6	0.0042			
Lack of Fit	0.0234	3	0.0078	12.01	0.0353	Significant
Pure Error	0.0020	3	0.0007			
Cor Total	189.40	11				
Std. Dev.	0.0650	R^2		0.9999		
Mean	7.81	Adjusted R^2		0.9998		
C.V. %	0.8325	Predicted R^2		0.9987		
		Adeq Precision		262.5850		
TNM Model	893.74	5	178.75	17450.47	< 0.0001	Significant
A-A.P.C	50.34	1	50.34	4914.28	< 0.0001	
B-D.A.R	843.27	1	843.27	82324.81	< 0.0001	
AB	0.0596	1	0.0596	5.82	0.0524	
A^2	0.0706	1	0.0706	6.90	0.0393	
B^2	0.0003	1	0.0003	0.0332	0.8615	
Residual	0.0615	6	0.0102			
Lack of Fit	0.0348	3	0.0116	1.31	0.4156	not significant
Pure Error	0.0266	3	0.0089			
Cor Total	893.80	11				
Std. Dev.	0.1012	R^2		0.9999		
Mean	20.58	Adjusted R^2		0.9999		
C.V. %	0.4918	Predicted R^2		0.9996		
		Adeq Precision		412.2569		

adjusted R^2 be minimal (<0.2), and that R^2 predicted is also close to adjusted R^2 to ensure predictive validity(Lakram et al., 2009), It is important to note that a high R^2 alone does not guarantee a valid model, particularly if superfluous terms inflate the fit artificially. However, the adjusted R^2 values exceeding 90% in all cases indicate that the models were not overfitted and retained only statistically meaningful parameters. The parameters *p* and *n* refer to the number of predictors (excluding the intercept) and the number of experimental trials, respectively. In

Table 6. Responses of synthetic poultry feed to predicted values

Run Order	SALT Actual Value	SALT Predicted Value	Ca Actual Value	Ca Predicted Value	P Actual Value	P Predicted Value	TNM Actual Value	TNM Predicted Value	ASH Actual Value	ASH Predicted Value
1	4.24	4.26	25.11	25.10	13.31	13.34	8.56	8.64	13.01	12.95
2	2.51	2.44	14.91	14.92	8.31	8.36	20.65	20.50	7.84	7.82
3	0.490	0.4712	3.31	3.37	2.00	1.98	32.34	32.35	1.77	1.80
4	0.590	0.5796	4.72	4.77	3.35	3.37	23.44	23.56	2.57	2.62
5	4.27	4.30	26.45	26.40	14.57	14.64	20.55	20.50	13.91	13.87
6	2.41	2.44	14.88	14.92	8.40	8.36	29.70	29.74	7.82	7.82
7	2.31	2.44	14.93	14.92	8.36	8.36	5.78	5.78	7.84	7.82
8	2.49	2.44	14.90	14.92	8.26	8.36	20.45	20.50	7.78	7.82
9	0.520	0.5492	4.16	4.05	2.73	2.73	20.45	20.50	2.29	2.21
10	2.38	2.38	14.29	14.25	7.65	7.64	11.90	11.82	7.34	7.37
11	4.36	4.31	25.68	25.74	14.14	14.04	35.33	35.28	13.33	13.42
12	2.47	2.45	15.61	15.61	9.07	8.98	17.81	17.77	8.27	8.25

conclusion, the graphical residual analysis (**Figure 1**) and the high R² values confirm that the developed models accurately capture the experimental dynamics of poultry feed formulation using desalted anchovy bone residues and argan press cake. The uniform distribution and close alignment of data points in all subplots support the model's suitability for optimizing such sustainable feed products.
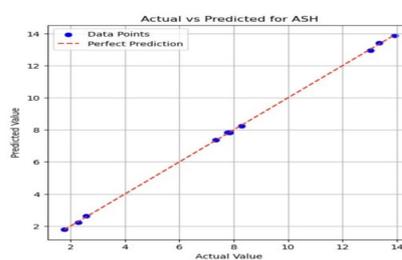
Interactive Factors and Effects of Argan Cake Addition on Desalination

The influence of anchovy bone residues and argan press cake on key nutritional parameters was investigated using response surface methodology (RSM),
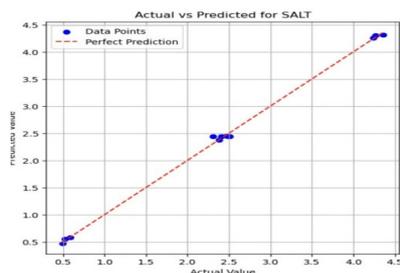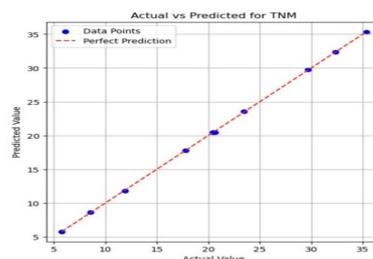
Actual vs Predicted for P
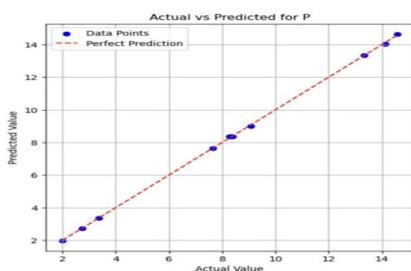
Actual vs Predicted for ASH

Actual vs Predicted for SALT

Actual vs Predicted for TNM
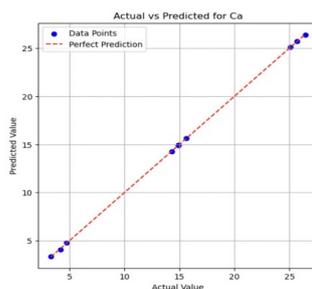

Actual vs Predicted for Ca

Figure 1. Different plots of predicted versus experimental values for SALT, Ca, P, TNM and ASH

3D Surface Plot for Salt (%)

3D Surface plot for Ca (g/Kg)

3D Surface Plot for ASH (%)
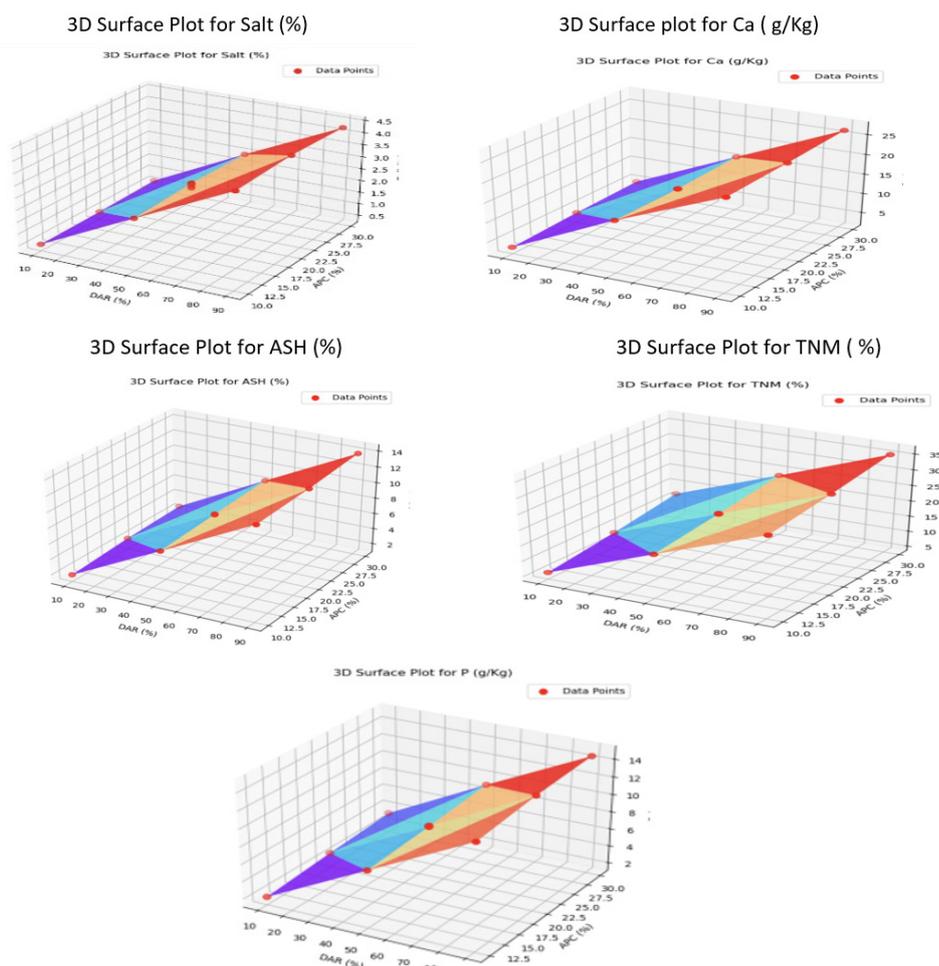

3D Surface Plot for TNM (%)

Figure 2. Interaction 3D entre %APC et % DAR

which generated three-dimensional response surface plots to visualize the interactions between variables. These models, developed using the linear, quadratic, and interaction terms described in Table 4, provided insight into the multifactorial effects of APC and DAR proportions on salt content, calcium, phosphorus, total nitrogen matter (TNM), and ash (Liu et al., 2009). Each 3D surface plot illustrates the effect of varying the proportions of the two independent variables on a given response, while the corresponding contour lines represent levels of constant response, thus enabling a visual interpretation of synergistic or antagonistic interactions. These visualizations were constructed based on extensive experimental data, allowing precise observation of how changes in the independent variables influenced the responses throughout the experimental range. Statistical analysis (Table 5) revealed highly significant interaction effects ($P < 0.0001$) between APC and DAR for all measured responses: salt, calcium, phosphorus, TNM, and ash. Notably, increased DAR levels were positively associated with higher TNM and ash content. Similarly,

higher levels of both APC and DAR significantly enhanced phosphorus and calcium concentrations. In contrast, salt content exhibited a decreasing trend with increasing APC, ranging from 4.24% to 0.49%, suggesting that argan press cake contributed effectively to desalination. These findings are visually corroborated by the surface plots presented in **Figure 2** (f–j), which clearly depict the interaction trends. Preliminary insights from Table 3 support these results, which closely aligned with model predictions. The high degree of agreement between the experimental and predicted values is further validated in Table 6. The models' predictive strength is underscored by exceptionally high coefficients of determination (R^2): 100% for calcium, 99.98% for phosphorus, 99.99% for TNM, 99.99% for ash, and 99.85% for salt. These values indicate that the experimental outcomes were almost perfectly aligned with the projected responses, thereby reinforcing the precision and robustness of the developed models. The close match between observed and predicted values confirms the models' utility in optimizing the formulation of desalinated poultry feed from anchovy bone residues

and argan press cake.

Process Optimisation and Validation of Waste Associations

The process parameter model was streamlined using the response optimisation tool incorporated in Design-Expert software version 13.0.14.0. This tool facilitates the identification of optimal conditions by exploring a broad spectrum of input variable combinations and evaluating associated trade-offs in a highly interactive manner (Agu et al., 2015). The application of response surface methodology (RSM) successfully elucidated the relationships between the key response variables salt content, total nitrogen matter (TNM), ash (ASH), phosphorus (P), and calcium (Ca)and the process parameters, specifically the proportions of desalted anchovy residue (DAR) and argan Press cake (APC). Leveraging the predictive capability of the developed models, the response optimizer efficiently identified the optimal balance of DAR and APC to maximize feed quality parameters while ensuring effective desalination. This approach validates the feasibility of combining these waste products into a valuable, nutrient-rich poultry feed additive, offering an innovative solution to reduce industrial waste and improve feed formulation.

Conclusion

In conclusion, the use of response surface methodology (RSM) successfully optimized the formulation of a co-product-based enriched poultry feed, while ensuring the efficient treatment of organic residues. Fitting a second-degree polynomial model offered reliable prediction of responses within experimental limits, and graphical optimization helped determine ideal conditions for desalting anchovy bones. Among the parameters studied, argan press cake stood out for its ability to play a dual role: natural desalination agent and complementary nutrient source. This result highlights its potential in a circular economy approach applied to animal nutrition. These results pave the way for future investigations, including *in vivo* biological validation and industrial scale-up.

Acknowledgments

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