



Research Article

A Multivariate Cluster Analysis of Soybean Meal from Sindh, Pakistan Reveals Distinct Nutri-Physiochemical Profiles Governing its Valorization in Poultry Diets

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Abstract | Soybean meal (SBM) is a cornerstone of poultry feed formulations that shows substantial variability in nutritional quality, undermining feed reliability and animal performance. Traditional nutrient centric evaluation approaches are inadequate to capture this complexity. To overcome this gap, this research study introduced a clustering framework to classify SBM on the basis of integrated-physiochemical features. Twenty SBM samples were collected from 10 districts of Sindh Pakistan which were analyzed for mineral profile (Ca, P, Mg, Zn and Cu), proximate composition and protein quality (KOH solubility). Crude protein was uniformly elevated ($44.68 \pm 0.63\%$), substantial regional variations ($p < 0.05$) were observed in dry matter, protein solubility and crude fat. The hierarchical cluster analysis identified three different functional profiles including: (i) the premium cluster (e.g., Mirpurkhas, Badin) categorized by high protein solubility ($>85\%$) with low fibre which is suitable for starter diet; (ii) standard cluster for normal application; (iii) a utility cluster for strategic blending. Furthermore, geo-spatial analysis identified districts like Tando Allahyar and Hyderabad as sources of vastly reliable SBM ($CV \approx 10\%$) that enhanced supply chain reliability while low Ca:P ratios (0.40:1-0.90:1) across the samples pinpoint the requirement of calcium supplements in feed formulations. This study highlights the novelty and utility of clustering as robust data driven method for SBM classification and shift the paradigm from compositional evaluation to functional profiling. The proposed pipeline enables accuracy in feed formulation, optimized ingredient valorization based on zoo-technical outcomes and improved nutritional security in poultry production systems.

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Introduction

Soybean meal (SBM) is the most widely used protein source in monogastric animal feeds due to its balanced amino acid profile and high digestibility (Yin *et al.*, 2011). Yet it is not always of the same quality. Nevertheless its nutritional value is not reliable. Variability in SBM composition arises from several reasons such as soybean genotype, agronomic conditions, oil extraction and processing methods (Hilton *et al.*, 2022; Toomer *et al.*, 2024). The significant indicator for processing adequacy is the potassium hydroxide (KOH) protein solubility which quantifies the degree of heat treatment. Anti-nutritional factors are not destroyed through insufficient processing, and excessive heat may diminish protein bioavailability (Dozier *et al.*, 2011). Consistency in feed quality is particularly crucial for the poultry industry which is a low margin sector that relies on predictable nutritional inputs to optimize growth performance and feed efficiency. SBM serves as a primary contributor of protein and amino acids in poultry diets; however, its chemical composition and nutritional value can vary significantly depending on processing conditions, storage practices, and geographic origin (García-Rebollar *et al.*, 2016; Grieshop *et al.*, 2003; Ravindran *et al.*, 2014). Traditional evaluation methods are based solely on crude protein content which are increasingly inadequate as they fail to capture the complex interactions among other nutritional components such as fiber, lipids, and minerals (Akintan *et al.*, 2024). In For instance, the balance among calcium and phosphorus is crucial for skeletal development, and any imbalance might lead to metabolic disorders (Shastak & Rodehutsord, 2015). Furthermore, the nutrient composition of SBM in various studies has demonstrated to vary greatly between geographical regions suggesting that reliance on average nutrient levels may not be sufficiently precise for feed formulation (Grieshop *et al.*, 2003; Thakur and Hurburgh, 2007). These constraints emphasize the need for a more holistic and systems-based evaluation of SBM that accounts for its multidimensional nutritional profile. Accordingly, the current study moves beyond conventional univariate analyses by utilizing multivariate statistical methods including cluster analysis and principal component analysis to reveal the complex nutri-physicochemical interactions amongst commercially available SBM samples in districts of Sindh, Pakistan. Secondly, to identify and characterize distinct clusters with

functional significance to specific poultry feeding stages. Lastly, to evaluate geo-specific variability and mineral balance to provide an additional strategic sourcing and feed formulation practices.

Materials and Methods

Sample collection

Twenty commercial SBM samples (n=20) were systematically collected from distributors and feed mills across ten major districts of Sindh province, Pakistan (Hyderabad, Tando Allahyar, Matiari, Jamshoro, Badin, Shaheed Benazirabad, Sanghar, Mirpurkhas, Thatta, and Larkana). Two representative samples were collected from each district and were stored at 4°C prior to analysis.

Chemical analysis

The analysis of all the collected samples was conducted in duplicate.

Proximate analysis

The proximate composition including dry matter (DM; Method 934.01), crude protein (CP; Method 984.13), crude fat (CF; Method 920.39), crude fiber (CFi; Method 978.10), and ash (Method 942.05) was quantified following the standard protocols of the Association of Official Analytical Chemists (Williams, 1984).

Protein quality assessment

The protein solubility in 0.2% potassium hydroxide (KOH) solution was determined according to Araba and Dale (1990) which is one of the important indicator of protein denaturation due to over-processing.

Mineral analysis

The samples were dry-ashed in muffle furnace at 550°C for 6 hours. The ash was dissolved in 5 mL of 25% HCl and digested in a hot block at 125°C. Deionized water was added to the final volume to 100 mL. The concentrations of calcium (Ca), phosphorus (P), magnesium (Mg), zinc (Zn), and copper (Cu) were measured using flame atomic absorption spectrometry (PerkinElmer PinAAcle 900T) according to AOAC method 968.08.

Statistical and multivariate analysis

The data was initially processed employing SPSS version 26 (IBM Corp.) with outcomes were expressed

as mean $\pm$ standard deviation. A one-way ANOVA was utilized to test for significant differences ($p < 0.05$) between districts. For multivariate analysis, the data were standardized (Z-scores) to avoid the influence of different units. Hierarchical Cluster Analysis (HCA) using Ward's method with squared Euclidean distance was performed to group SBM samples into distinct clusters based on all analyzed parameters. The quality and consistency of SBM from different districts were further assessed by calculating the Coefficient of Variation ($CV\% = (SD/Mean) \times 100$).

Results and Discussion

Geographic variation in proximate composition and protein quality

The summary of the proximate composition and the protein quality of SBM across the ten districts is provided in Table 1. The total mean crude protein (44.68%) fell within the normal range of SBM (Dozier *et al.*, 2011) and there was no geographic significant difference ($p=0.743$). This implies that CP is a relatively stable component which is mainly dictated by the variety of soybean and standard solvent extraction process.

Nevertheless, the geographic diversity was found to be significant in terms of dry matter ($p=0.037$), crude fat ($p=0.005$), and the KOH solubility ($p=0.012$). The differences in DM can presumably be explained by the different local ambient humidity and post-processing storage and geographical origin (Aguirre *et al.*,

2023; Thiex and Richardson, 2003). The significant differences in CF are critical, as they directly impact the metabolizable energy value of the meal. The low CF (e.g., Badin, 0.50%), would indicate the high efficiency of the solvent extraction process, whereas the high values (e.g., Mirpurkhas at 2.52%) could be indicative of expeller-processed meal consumption or local differences in the efficiency of the process (Toomer *et al.*, 2024). The protein quality parameters such as the KOH solubility significantly differed. The Shaheed Benazirabad samples (75.28%) were found to be below the optimal 78-85 range indicating the possibility of protein damage due to over-processing that would degrade the availability of the amino acids to the bird (Araba and Dale, 1990). In contrast, the samples of Mirpurkhas (85.97%) and Badin (84.56%) showed excellent protein quality.

Multivariate cluster analysis and nutritional ranking reveals functional SBM profiles

The HCA was able to cluster the SBM samples into three different clusters which transcended their geographic origin. This classification offers a more feasible way of feed formulation as compared to geographic sourcing alone. To enhance interpretability a composite nutritional quality score was calculated and the districts were subsequently ranked based on CP, KOH solubility, and crude fiber content (Table 2). The topmost quality soybean meal is produced by Mirpurkhas followed by Badin, and Sanghar. On the contrary, the lowest quality meals were offered by Shaheed Benazirabad and Matiari. The sharp

Table 1: Mean values of proximate composition ($\pm$ Standard Deviation) of soybean meal from ten different districts of sindh province

District	Dry matter (%)	Crude protein (%)	Crude fat (%)	Crude fiber (%)	Ash (%)	KOH Solubility (%)
Hyderabad	88.77 $\pm$ 0.80	44.95 $\pm$ 0.69	2.60 $\pm$ 0.01	1.56 $\pm$ 0.78	5.18 $\pm$ 0.59	82.96 $\pm$ 1.95
Tando allahyar	86.79 $\pm$ 1.07	44.84 $\pm$ 0.00	1.14 $\pm$ 0.37	1.57 $\pm$ 0.83	5.40 $\pm$ 0.11	82.93 $\pm$ 0.00
Matiari	87.56 $\pm$ 0.63	45.17 $\pm$ 0.47	0.92 $\pm$ 0.15	2.03 $\pm$ 0.21	6.00 $\pm$ 0.28	81.36 $\pm$ 2.05
Jamshoro	86.72 $\pm$ 1.70	45.17 $\pm$ 0.06	0.91 $\pm$ 0.59	1.47 $\pm$ 0.20	4.22 $\pm$ 0.95	83.37 $\pm$ 2.36
Badin	88.24 $\pm$ 1.28	44.34 $\pm$ 0.19	0.50 $\pm$ 0.25	1.29 $\pm$ 0.22	6.10 $\pm$ 0.12	84.56 $\pm$ 0.29
Shaheed bena-zirabad	89.77 $\pm$ 1.94	44.19 $\pm$ 0.61	1.30 $\pm$ 0.41	1.50 $\pm$ 0.51	4.26 $\pm$ 1.01	75.28 $\pm$ 4.07
Sanghar	88.85 $\pm$ 1.63	44.30 $\pm$ 0.77	1.36 $\pm$ 1.03	0.86 $\pm$ 0.12	4.31 $\pm$ 0.93	81.72 $\pm$ 0.08
Mirpurkhas	89.59 $\pm$ 0.31	44.83 $\pm$ 0.16	2.52 $\pm$ 0.08	0.92 $\pm$ 0.30	4.60 $\pm$ 1.63	85.97 $\pm$ 1.27
Thatta	85.68 $\pm$ 0.22	44.62 $\pm$ 0.62	0.73 $\pm$ 0.02	1.35 $\pm$ 0.58	5.53 $\pm$ 0.98	80.42 $\pm$ 2.97
Larkana	87.36 $\pm$ 0.74	44.51 $\pm$ 0.08	1.18 $\pm$ 0.61	1.83 $\pm$ 0.17	5.32 $\pm$ 0.63	84.38 $\pm$ 0.45
Overall Mean	87.81 $\pm$ 1.66	44.68 $\pm$ 0.63	1.31 $\pm$ 0.74	1.42 $\pm$ 0.49	4.87 $\pm$ 0.95	82.09 $\pm$ 3.31
p-value	0.037*	0.743	0.005*	0.192	0.122	0.012*

$P < 0.05$ indicates a statistically significant difference between districts

difference in solubility between the very soluble protein present in Mirpurkhas and the poor profile present in Shaheed Benazirabad highlights a very important origin-related effect over soybean local meal. The protein concentration is high in Hyderabad's meal but the medium solubility indicates that it might

be heated which will also be in line with the results that the actual availability of proteins will depend on the processing (Aguirre *et al.*, 2023). On the other hand, the nutrient density of high fiber in the food production of Matiari and Shaheed Benazirabad is watered down which is a limitation of poultry diets

Table 2: District ranking based on a composite nutritional quality score for poultry feed

Rank	District	Crude protein rank (Higher is better)	KOH Solubility rank (Higher is better)	Crude fiber rank (Lower is better)	Total score (Lower is better)
1	Mirpurkhas	3	1	2	6
2	Badin	8	2	4	14
3	Sanghar	9	7	1	17
4	Hyderabad	1	5	7	13
5	Larkana	6	3	9	18
6	Jamshoro	2	4	10	16
7	Tando allahyar	4	9	6	19
8	Thatta	5	10	5	20
9	Matiari	7	8	10	25
10	Shaheed benazirabad	10	10	6	26

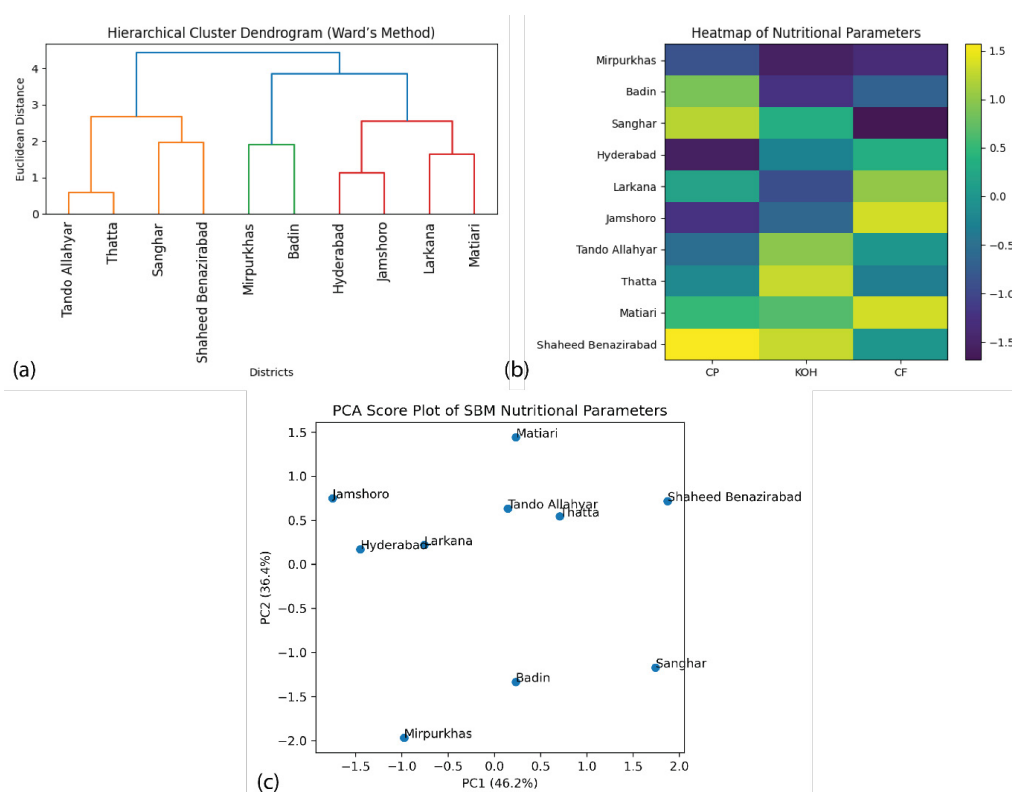


Figure 1: Multivariate analysis of soybean meal (SBM) nutritional features across districts. (a) Hierarchical cluster analysis (HCA) dendrogram generated using Ward's method and Euclidean distance illustrate the grouping of districts based on crude protein (CP), potassium hydroxide (KOH) solubility, and crude fibre (CF) content. Three distinct clusters are observed that indicate variability in nutritional quality among regions. (b) Heatmap of standardized nutritional parameters (CP, KOH, and CF) show relative variation across districts and supporting the clustering patterns identified in the dendrogram. (c) Principal component analysis (PCA) score plot shows two principal components (PC1: 46.2%, PC2: 36.4%) that explains the majority of the variance. The spatial distribution of districts exhibits clear separation consistent with HCA results validating the robustness of the clustering approach.

(Mateos et al., 2012). Thus, feed mills such as Thatta and Tando Allahyar have to focus on protein quality rather than on the content of crude protein. Finally, this geographical grid determines that sourcing out of Mirpurkhas has a unique nutritional benefit over the low-ranking districts.

An extensive multivariate analysis of soil biomass (SBM) nutritional parameters among the districts was studied by hierarchical clustering, heatmap

visualization, and principal component analysis (PCA) illustrated in Figure 1. The dendrogram (a) reveals clear grouping patterns among districts, indicating similarities in their nutritional profiles, with some districts forming closely related clusters while others remain distinct. The heatmap (b) further highlights the variation in key parameters (CP, KOH, and CF), showing contrasting concentration levels across districts through a gradient of color intensities. The PCA score plot (c) explains a substantial

Table 3: *Average mineral content ($\pm$ Standard Deviation) of soybean meal from ten different districts of sindh province*

District	Calcium (Ca) (mg/kg)	Phosphorus (P) (mg/kg)	Magnesium (Mg) (mg/kg)	Zinc (Zn) (mg/kg)	Copper (Cu) (mg/kg)
Hyderabad	4125 $\pm$ 673	4945 $\pm$ 285	2055 $\pm$ 544	44.82 $\pm$ 3.68	18.57 $\pm$ 8.61
Tando Allahyar	4278 $\pm$ 2445	5275 $\pm$ 1493	2620 $\pm$ 533	46.56 $\pm$ 1.67	17.23 $\pm$ 6.26
Matari	1840 $\pm$ 382	4125 $\pm$ 1338	1500 $\pm$ 270	44.26 $\pm$ 4.27	20.99 $\pm$ 1.18
Jamshoro	3710 $\pm$ 1953	4105 $\pm$ 571	2500 $\pm$ 1107	46.03 $\pm$ 8.91	20.55 $\pm$ 3.44
Badin	2900 $\pm$ 1075	5505 $\pm$ 1235	2405 $\pm$ 99	43.25 $\pm$ 7.00	18.46 $\pm$ 0.54
Shaheed Benazirabad	2850 $\pm$ 570	4940 $\pm$ 2122	2900 $\pm$ 367	47.71 $\pm$ 4.35	16.81 $\pm$ 6.77
Sanghar	2825 $\pm$ 71	7095 $\pm$ 1455	2385 $\pm$ 1485	46.27 $\pm$ 9.19	21.56 $\pm$ 1.56
Mirpurkhas	3070 $\pm$ 1403	5545 $\pm$ 2215	2345 $\pm$ 544	45.03 $\pm$ 8.94	19.17 $\pm$ 2.10
Thatta	2785 $\pm$ 1568	4925 $\pm$ 374	2515 $\pm$ 566	46.48 $\pm$ 7.35	21.98 $\pm$ 4.37
Larkana	4275 $\pm$ 433	5655 $\pm$ 3135	2545 $\pm$ 311	50.01 $\pm$ 2.12	16.69 $\pm$ 4.42
Overall Mean	3360 $\pm$ 1344	5375 $\pm$ 1697	2415 $\pm$ 684	47.22 $\pm$ 4.60	19.13 $\pm$ 4.00
p-value	0.690	0.729	0.663	0.951	0.816

P < 0.05 indicates a statistically significant difference between districts

Table 4: *Critical macro-mineral ratios in soybean meal and their assessed suitability for poultry*

District	Ca:P ratio	Ca:P classification	Mg:P ratio	Mg:P classification	Overall mineral balance interpretation
Hyderabad	0.83:1	Slightly below recommended range	0.42:1	Within recommended range	May require moderate calcium supplementation to achieve optimal balance
Tando Allahyar	0.81:1	Slightly below recommended range	0.50:1	Within recommended range	Comparable to Hyderabad; additional calcium recommended
Matari	0.45:1	Severely below recommended range	0.36:1	Below recommended range	Poor mineral balance; significant supplementation required
Jamshoro	0.90:1	Within acceptable range	0.61:1	Within recommended range	Relatively balanced mineral profile compared to other districts
Badin	0.53:1	Below recommended range	0.44:1	Within recommended range	Calcium supplementation required to correct imbalance
Shaheed Benazirabad	0.58:1	Below recommended range	0.59:1	Within recommended range	Requires calcium adjustment for improved balance
Sanghar	0.40:1	Severely below recommended range	0.34:1	Below recommended range	Markedly imbalanced; supplementation strongly recommended
Mirpurkhas	0.55:1	Below recommended range	0.42:1	Within recommended range	Adequate phosphorus but insufficient calcium
Thatta	0.57:1	Below recommended range	0.51:1	Within recommended range	Requires calcium supplementation
Larkana	0.76:1	Slightly below recommended range	0.45:1	Within recommended range	Closer to optimal ratio but still requires calcium adjustment

proportion of the total variance (PC1: 46.2% and PC2: 36.4%), effectively separating districts based on their nutritional characteristics. Districts located near each other have similar parameter compositions while more distant districts show greater dissimilarity. The aggregate analyses overall reveal a high degree of spatial variation in the nutritional properties of the SBM and a robust basis for district-level classification.

Mineral composition and critical balance assessment

Table 3 indicates the mineral content. There was no meaningful geographic difference among any mineral ($p>0.05$), indicating that the composition of minerals is less affected by local market factors and more by the characteristics of soybean and processing. The most common mineral was phosphorus which validated the importance of SBM as a principal source of phosphorus.

Table 4 shows the critical ratios of minerals. The most noteworthy results were the poor Ca:P ratio across all districts with the range of 00.40:1 (Sanghar) to 0.90:1 (Jamshoro). This is a common feature of SBM which highlights the opportunity cost of the non-negotiable nature of the usage of limestone or similar sources of calcium in SBM-based poultry feeds (Shastak and Rodehutsord, 2015). The neglect of this imbalance may result in rickets and poor bone mineralization.

Ca:P ratios were assessed based on nutritional ranges (1:1 to 2:1) as defined by NRC (2001). Ratios were classified as: within acceptable range (1.0–2.0), slightly below recommended range (0.7–0.9), below recommended range (0.5–0.7), and severely below recommended range (<0.5). Mg:P ratios were interpreted as within recommended range (>0.4) or

below recommended range (<0.4), based on FAO guidelines.

Geo-Specific consistency index for supply chain security

The Coefficient of Variation (CV%) is the measure of consistency of SBM that determines the reliability of feed formulation (Table 5). Eight out of the ten districts exhibited “Excellent” consistency (Avg. CV% $< 15\%$), but Hyderabad, Thatta and Jamshoro showed astonishing batch-to-batch uniformity (Avg. CV% $< 5\%$). This is an invaluable data to procurement managers because sourcing such districts reduces the chances of food deviations. Conversely, SBM from Larkana and Shaheed Benazirabad were varied especially in phosphorus content (CV % $> 55\%$ and 43% respectively) which requires more frequent quality control.

Conclusions and Recommendations

The present study has effectively shown that a multivariate cluster analysis method offers a better and more practical categorization of soybean meal than the more traditional univariate or geographic comparisons. We determined three functional profiles namely Premium, Standard, and Utility profiles which directly correspond to ideal application in poultry feed formulation. The results verify that the crude protein level is uniform but such vital quality indicators as KOH solubility, crude fat, and mineral balance have considerably different values. The “Geo-Specific Consistency Index” is a practical and scalable way to de-risking feed supply chains. Its implementation within the poultry industry could support data-driven decision-making and advance precision nutrition,

Table 5: Nutrient consistency assessment by district (Based on coefficient of variation %)

District	CV% Crude protein (Lower is better)	CV% KOH Solubility (Lower is better)	CV% Phosphorus (Lower is better)	Average CV% (Lower is better)	Consistency rating
Tando Allahyar	0.0%	0.0%	28.3%	9.4%	Excellent
Mirpurkhas	0.4%	1.5%	39.9%	13.9%	Excellent
Badin	0.4%	0.3%	22.4%	7.7%	Excellent
Jamshoro	0.1%	2.8%	13.9%	5.6%	Excellent
Larkana	0.2%	0.5%	55.4%	18.7%	Good
Thatta	1.4%	3.7%	7.6%	4.2%	Excellent
Hyderabad	1.5%	2.4%	5.8%	3.2%	Excellent
Sanghar	1.7%	0.1%	20.5%	7.4%	Excellent
Matari	1.0%	2.5%	32.4%	12.0%	Excellent
Shaheed benazirabad	2.8%	5.4%	43.0%	17.1%	Good

ultimately improving ingredient utilization, feed efficiency, and economic sustainability.

Although this study offered a strong multivariate basis for functional classification of SBM certain limitations should be addressed. The relatively limited sample size and the district-specific nature of the study may constrain the representativeness of the results to other geographical areas and agricultural practices. Variability due to seasonal, processing, and storage conditions was also not well explored and could also impact SBM quality. Future research should focus on expanding the sample size and incorporating a wider geographical distribution to validate and refine the proposed clustering model. The incorporation of other parameters including amino acid digestibility, anti-nutritional factors, processing factors would further improve the functional classification system. Furthermore, these clusters could be correlated with in-vivo tests in poultry which would directly validate their applicability. Despite the shortcomings, this study proposes a novel and data-driven method for SBM evaluation beyond simple compositional analysis with great potential for precision feed formulation and ensuring nutritional uniformity in poultry production.

Novelty Statement

This study provides a novel data-driven framework for functionally classifying soybean meal by integrating its nutritional, physicochemical, mineral, and geographical variability. The method enables quality-based ingredient selection and precise targeted feed formulation.

Authors' Contributions

Abdul Waheed Ghanghro and Bheem Raj Serani: Performed formal analysis, conceptualized and supervised the study.

Mahrukh Zakir: Assisted in writing - review & editing and manuscript submission.

All authors have read and agreed to the published version of the manuscript.

Author declaration

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Potential conflict of interest exists:

We wish to draw the attention of the Editor to the following facts, which may be considered as potential conflicts of interest, and to significant financial contributions to this work:

The nature of potential conflict of interest is described below:

No conflict of interest exists.

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We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

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We further confirm that any aspect of the work covered in this manuscript has not involved human patients and thus requires no ethical approval of any relevant body.

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All listed authors meet the Journal criteria. We attest that all authors contributed significantly to the creation of this manuscript, each having fulfilled criteria as established by the Journal.

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Generative AI and AI-assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflicts of interest

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