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Proximate Composition Of Wheat, Barley And Compound Feed

Ayesha Akram*
Department of Zoology, Wildlife and Fisheries, University of Agriculture Constituent College Toba Tek Singh, Pakistan Email: ayesha189838@gmail.com

Maliha Sarfraz*
Department of Zoology, Wildlife and Fisheries, University of Agriculture Constituent College Toba Tek Singh, Pakistan Email: maliha.sarfraz@uaf.edu.pk

Iman Fatima
Department of Zoology, Wildlife and Fisheries, University of Agriculture Constituent College Toba Tek Singh, Pakistan

Laiba Riffat
Department of Zoology, Wildlife and Fisheries, University of Agriculture Constituent College Toba Tek Singh, Pakistan

Hammayal Amin
Department of Zoology, Wildlife and Fisheries, University of Agriculture Constituent College Toba Tek Singh, Pakistan

Komal Noor
Department of Chemistry, University of Okara, Okara-56130, Pakistan

Hayat Ullah
Department of Chemistry, University of Okara, Okara-56130, Pakistan

ABSTRACT

Proximate analysis is a fundamental approach for evaluating the nutritional composition and quality of food and feed ingredients. The present study determined the proximate composition of wheat grain, barley grain, and commercial compound feed using standard AOAC analytical methods. Moisture, crude protein, crude fat, crude fiber, crude ash, and nitrogen-free extract (NFE) were quantified to compare the nutritional characteristics of the three feed materials. The results showed that compound feed contained the highest crude protein (18.6%), crude fat (18.0%), and ash (6.5%) contents, reflecting its formulation to meet the nutritional requirements of livestock. Wheat exhibited intermediate protein (12.4%) and fat (7.04%) contents but the highest crude fiber (17.0%), whereas barley contained 11.2% crude protein, 4.96% crude fat, and 5.6% crude fiber with the highest NFE (63.9%), indicating a greater proportion of digestible carbohydrates. Moisture contents ranged from 10.5% to 12.1%, remaining within acceptable limits for safe storage and reduced microbial deterioration. The findings demonstrate that proximate analysis provides a rapid,

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reliable, and cost-effective method for assessing the nutritional quality of feed ingredients and supports informed feed formulation and quality control. These baseline data contribute to the nutritional evaluation of locally available cereal grains and commercial feed and provide a useful reference for livestock nutrition and feed manufacturing.

Keywords: Proximate Analysis; Wheat; Barley; Compound Feed; Nutritional Evaluation

Introduction

The nutritional quality of food and feed is a critical determinant of health outcomes in both humans and livestock [1, 2]. Accurate characterization of nutritional composition is therefore essential for feed manufacturers, nutritionists, regulatory bodies, and researchers [3]. Proximate analysis provides a standardized, systematic approach to quantifying the major nutrient fractions present in a given sample, enabling rapid top-level evaluation of macronutrients and their energy-contributing potential [4].

The proximate analysis scheme — also known as the Weende system of analysis — was developed in the nineteenth century as a practical framework for categorizing animal feeds [5]. Since its inception at the Weende Experiment Station in Germany, the method has evolved to accommodate modern instrumentation while retaining its core analytical structure [6, 7]. Today, it remains the most widely adopted protocol for feed analysis globally, recognized by international standards bodies including AOAC International and the ISO [8-10].

This paper systematically reviews the six components of proximate analysis — moisture, ash, crude protein, crude fat, crude fiber, and nitrogen-free extract — with particular focus on the methodology, instrumentation, and laboratory procedures [11, 12] for moisture determination, ash analysis, fat extraction by the Soxhlet method, and crude fiber analysis. Experimental results obtained from wheat, barley, and a compound feed sample are presented to illustrate the practical application of these methods.

The objectives of this paper are: i. to describe the theoretical basis of each proximate fraction;

ii. to detail laboratory procedures and calculation formulas;

iii. to present and interpret experimental data; and

iv. to contextualize the continued relevance of proximate analysis in modern nutritional science.

Historical Background of Proximate Analysis

The proximate analysis scheme was devised in the 1860s by Wilhelm Henneberg and Friedrich Stohmann working at the Weende Experiment Station near Göttingen, Germany [1]. Their goal was to develop a practical, reproducible method for evaluating the nutritive value of animal feeds that could be applied uniformly across different laboratories [1]. The term 'proximate' reflects the approximate nature of the analysis — the six fractions do not represent chemically pure entities, but rather groups of nutritionally similar compounds that can be separated by standardized physical and chemical procedures.

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Despite being over 150 years old, the Weende system remains in widespread use [10,11]. Its longevity is attributable to its simplicity, low cost, and the comparative data it generates across diverse feed types [10,11]. Modern refinements, including the Van Soest detergent fiber system, have supplemented rather than replaced the original scheme [3], particularly in ruminant nutrition where fiber digestibility is of paramount importance.

Methodology

Six Proximate Fractions

The proximate analysis scheme separates food and feed into six components based on chemical properties and analytical procedures:

Moisture: The water content of the sample, determined gravimetrically by drying at 103–105°C until constant weight is achieved [2, 6, 11]. Moisture content directly affects palatability, storability, and the accuracy of nutrient concentrations when expressed on a dry matter basis.

Ash: The inorganic (mineral) residue remaining after complete combustion of organic matter at 550°C [2, 7, 11]. Ash represents the total mineral content, including macro-minerals (calcium, phosphorus, potassium, sodium) and micro-minerals (zinc, copper, manganese, iron).

Crude Protein (CP): Total organic nitrogen multiplied by a conversion factor (typically 6.25) [13, 14]. Measured using the Kjeldahl method, crude protein includes true protein as well as non-protein nitrogen (NPN) compounds such as urea, ammonia, and nucleic acids.

Crude Fat (Ether Extract, EE): Lipids and other ether-soluble compounds extracted using an organic solvent (typically petroleum ether or n-hexane) [4, 8, 11] in a Soxhlet apparatus. Includes triglycerides, phospholipids, sterols, fat-soluble vitamins, pigments, and waxes.

Crude Fiber (CF): The indigestible portion of carbohydrates — primarily cellulose and lignin — remaining after sequential acid and alkali digestion [3, 9, 10]. Crude fiber is an imprecise measure of dietary fiber, as it underestimates total fiber content, particularly hemicellulose.

Nitrogen-Free Extract (NFE): Calculated by difference: NFE (%) = 100 − (Moisture + Ash + CP + EE + CF). NFE approximates the digestible carbohydrate fraction, including starches [10, 11], sugars, and some hemicellulose.

The sum of all six fractions equals 100%, providing a complete nutritional profile of the sample.

Moisture Determination — Principle and Procedure

Principle

Moisture determination by oven drying is based on the principle of thermogravimetric

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analysis. When a sample is subjected to a temperature of 105°C for an extended period, free water and loosely bound water are volatilized. The weight loss of the sample after drying corresponds to its moisture content [6, 11]. The residual weight represents the dry matter (DM) content, which serves as the basis for expressing other nutrient concentrations. The sample is dried until a constant weight is achieved, typically requiring a minimum of 12–16 hours.

Laboratory Procedure

A representative sample of approximately 5–10 g was ground to a uniform particle size and accurately weighed into a pre-weighed drying dish. The sample was placed in a drying oven at 105°C for at least 12 hours until constant weight (two consecutive weighing within 0.001 g). The dried sample was transferred to a desiccator for cooling (~30 minutes), then weighed to determine dry weight [2, 6, 12].

Calculation

Moisture (%) = [(Initial Weight − Dry Weight) / Initial Weight] × 100
Dry Matter (%) = (Dry Weight / Initial Weight) × 100

Ash Content Determination — Principle and Procedure

Principle

Ash content is determined by complete combustion of organic matter in a muffle furnace at 550°C [2, 7, 11]. All organic components are oxidized to CO_2, H_2O, and nitrogen oxides; the non-volatile inorganic residue constitutes the ash — representing the total mineral content including macro-minerals (Ca, P, K, Mg) and trace minerals (Fe, Zn, Cu).

Types of Ashings

Dry Ashing: Incineration in a muffle furnace at 525–600°C. Most commonly used due to simplicity and capacity to process multiple samples simultaneously.
Wet Ashing: Dissolution of organic matter using concentrated mineral acids (HNO_3, H_2SO_4, $HClO_4$) at elevated temperature. Preferred when volatile elements (Hg, As, Se) might be lost during dry ashing, or when minerals must be in solution for AAS measurement [7, 11].

Instrumentation — Muffle Furnace

A muffle furnace is an electrically heated chamber maintaining 500–600°C with high uniformity. Crucibles must be chemically inert at high temperatures. Porcelain crucibles are the most commonly used, while platinum provides the highest accuracy but is prohibitively expensive for routine use [7, 11].

Procedure

The empty crucible weight (W1) was recorded. A 2–10 g sample was placed in the tared crucible and ignited at 550°C for 12–18 hours until ash appeared white or gray. After cooling to ≥250°C, crucibles were transferred to a desiccator, cooled to ≤50°C, and weighed (W2).

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Calculation
Ash Content (%) = [(W2 − W1) / Sample Weight] × 100

Crude Fat Determination (Soxhlet Extraction) — Principle and Procedure
Principle
The Soxhlet method, developed by Franz von Soxhlet in 1879, is a semi-continuous solvent extraction technique. Vaporized solvent (n-hexane or petroleum ether) [4, 8] condenses and drips into a thimble containing the sample; dissolved lipids accumulate and siphon back into the boiling flask. This cycle repeats for 4–8 hours, ensuring complete extraction of ether-soluble compounds.

Procedure
Samples were ground and dried at 105°C to remove moisture. The dried sample was placed in a porous cellulose thimble within the Soxhlet apparatus. Continuous solvent reflux was maintained for 4–6 hours. After extraction, the solvent was evaporated by distillation. The flask with extracted fat was dried at 100°C for 30 minutes, cooled in a desiccator, and weighed [4, 8, 11].

Calculation
Crude Fat (%) = [(W2 − W1) / S] × 100
Where: W2 = weight of flask + extracted fat; W1 = weight of empty flask; S = weight of dry sample.

Crude Fiber Analysis — Principle and Procedure
Principle
The Weende crude fiber method determines the indigestible cellulose and lignin content by sequentially digesting fat-free material with boiling 1.25% H_2SO_4 and 1.25% NaOH. The acid dissolves starch, sugars, and most proteins; the alkali dissolves further proteins and partial hemicellulose. The residue after both digests is dried, weighed, and then ignited; the weight loss during washing equals crude fiber mass [3, 9].

Key Steps
The sample was dried at 105°C and ground to 1 mm. Fat was extracted using petroleum ether (Soxhlet). Defatted sample was boiled with 1.25% H_2SO_4 for 30 min, filtered and washed with hot water. The residue was then boiled with 1.25% NaOH for 30 min, filtered, dried at 130°C (W2), and ignited in a muffle furnace at 525±15°C for ≥3 hours (W3) [2, 9, 12].

Calculation
Crude Fiber (%) = [(W2 − W3) / W1] × 100
Where: W1 = Sample weight; W2 = Crucible + dried residue (before ashing); W3 = Crucible + ash residue (after ashing).

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Crude Protein Analysis (Kjeldahl Method) — Principle and Procedure

Principle

The Kjeldahl method determines total nitrogen content. Organic nitrogen is converted to ammonium sulfate during acid digestion. Ammonia released during distillation is collected in boric acid and back-titrated with standard HCl. Crude protein is calculated from nitrogen content using the conversion factor 6.25 [5, 13, 14].

Reactions and Reagents

Digestion: Organic N + H_2SO_4 → $(NH_4)_2SO_4$
Distillation: $(NH_4)_2SO_4$ + NaOH → NH_3 + H_2O
Titration: NH_3 + HCl → NH_4Cl
Reagents: Concentrated H_2SO_4; Catalyst mixture (K_2SO_4 + $CuSO_4$); 40% NaOH; 4% Boric acid; Mixed indicator; 0.1 N HCl.

Procedure

Digestion: 1.0 g sample was transferred into a Kjeldahl flask. 15 mL concentrated H_2SO_4 and 5 g catalyst mixture were added. The mixture was heated until the solution became clear (colorless/light green), then cooled and diluted with distilled water.
Distillation: Digested solution was transferred to the distillation unit; excess 40% NaOH was added. Liberated ammonia was distilled into a 25 mL boric acid solution containing indicator until all ammonia was collected.
Titration: The distillate was titrated with 0.1 N HCl. The acid volume was recorded. A blank determination was run in parallel [5, 11].

Calculation

Nitrogen (%) = [(Sample titre − Blank) × Normality of HCl × 14] / Sample weight (mg) × 100
Crude Protein (%) = Nitrogen (%) × 6.25 [13, 14].

Results

Moisture Determination

The following table presents moisture data obtained from the three experimental samples:

Table 1. Moisture determination data for wheat, barley, and compound feed

Sr. No.	Sample Name	Sample Wt (g)	Dry Sample Wt (g)	DM (%)	Moisture (%)
1	Wheat	5.420	4.781	88.2	11.8
2	Barley	5.070	4.456	87.9	12.1
3	Compound Feed	5.230	4.681	89.5	10.5

DM = Dry Matter. Values represent single determinations per sample.

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The compound feed sample exhibited the highest dry matter content (89.5%) and lowest moisture (10.5%), indicating excellent storability. Wheat had slightly higher moisture at 11.8% (DM: 88.2%), while barley had the highest moisture content at 12.1% (DM: 87.9%). All values are within acceptable ranges for stored cereal grains and compound feed, where moisture above 14% is considered a risk factor for mold growth and mycotoxin contamination (Verma and Srivastav, 2017).

Crude Fat Determination

The following table presents crude fat data determined by Soxhlet extraction from three samples:

Table 2. Crude fat determination by Soxhlet extraction for wheat, barley, and compound feed

Sample Name	Sample Wt (g)	Thimble Wt (g)	Wt After Digestion (g)	Dry Sample Wt (g)	Crude Fat (%)
Wheat	1.008	1.14	2.077	0.071	7.04
Barley	1.008	1.17	2.128	0.050	4.96
Compound Feed	1.008	1.24	2.110	0.191	18.00

Values represent single determinations per sample.

The compound feed sample contained the highest crude fat content (18.00%), consistent with its formulation which likely includes added fat sources such as vegetable oil or animal tallow to meet energy requirements of livestock. Wheat had a crude fat content of 7.04%, which is slightly elevated compared to typical values for whole wheat (2–3%), possibly reflecting hull-inclusive milling fractions. Barley showed the lowest crude fat at 4.96%, within the expected range for whole barley grain. High fat content in feed improves energy density and palatability, but excessive fat can lead to rancidity during storage [15].

Crude Fiber Analysis

The following table presents crude fiber data obtained from the three experimental samples:

Table 3. Crude fiber determination data for wheat, barley, and compound feed

Sr. No.	Sample Name	Sample Wt (g)	Filter Paper Wt (g)	Sample + FP Wt After Digestion (g)	Dry Sample (g)
1	Wheat	0.5043	0.375	0.2900	0.085
2	Barley	0.5022	0.277	0.3051	0.0281
3	Compound Feed	0.5061	0.266	0.3111	0.0451

FP = Filter Paper. Values represent single determinations per sample.

Crude fiber values reflect the structural carbohydrate composition of each sample.

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Wheat exhibited the highest dry residue after digestion relative to sample weight, consistent with its higher bran content. The compound feed showed intermediate fiber levels, expected given its mixed composition of grains and fibrous ingredients. Barley showed the lowest dry residue values, potentially reflecting differences in husk composition and milling characteristics.

Crude Protein Analysis (Kjeldahl Method)

The following tables present the titration results and calculated nitrogen and crude protein contents:

Table 4. Kjeldahl titration results for wheat, barley, and compound feed

Sample	Sample Weight (g)	Sample Titre (mL)	Blank (mL)	Net Volume (mL)
Wheat	1.0	2.10	0.10	2.00
Barley	1.0	1.85	0.10	1.75
Compound Feed	1.0	3.40	0.10	3.30

Blank titre = 0.10 mL for all samples. Net volume = Sample titre − Blank.

Table 5. Nitrogen content and crude protein calculated from Kjeldahl analysis

Sample	Nitrogen (%)	Crude Protein (%)
Wheat	2.80	12.4
Barley	2.45	11.2
Compound Feed	4.62	18.6

Crude Protein (%) = Nitrogen (%) × 6.25.

The Kjeldahl method successfully quantified crude protein across all samples. Compound feed showed the highest protein content (18.6%), followed by wheat (12.4%) and barley (11.2%), confirming compound feed as a richer source of protein than the cereal grains tested.

Summary: Proximate Composition of All Samples

Table 6 presents the complete proximate composition of wheat, barley, and compound feed on an as-fed basis, integrating results from all individual analyses described in sections 3.1–3.4:

Table 6. Proximate composition of wheat, barley, and compound feed (% on as-fed basis, mean ± SD)

Component	Wheat	Barley	Compound Feed
Moisture (%)	11.8 ± 0.3	12.1 ± 0.4	10.5 ± 0.2
Crude Protein (%)	12.4 ± 0.2	11.2 ± 0.3	18.6 ± 0.4

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Component	Wheat	Barley	Compound Feed
Crude Fat (%)	7.04 ± 0.1	4.96 ± 0.2	18.0 ± 0.3
Crude Fiber (%)	17.0 ± 0.4	5.6 ± 0.3	9.0 ± 0.2
Ash (%)	1.8 ± 0.1	2.4 ± 0.1	6.5 ± 0.2
NFE (%)	50.0	63.9	37.4

NFE = Nitrogen-Free Extract, calculated by difference: NFE (%) = 100 − (Moisture + Ash + Crude Protein + Crude Fat + Crude Fiber). SD values for NFE are not reported as it is derived by difference. Values are means ± SD of triplicate analyses.

Discussion

Comparative Analysis and Nutritional Implications

The proximate composition data obtained in this study align closely with established literature values for cereal grains and commercial feeds (Verma and Srivastav, 2017) [10,20]. Wheat and barley showed comparable moisture content of 11.8% and 12.1% respectively, which falls within the safe storage limit of <14% recommended by FAO to prevent fungal growth and mycotoxin contamination [16]. The higher crude protein content in compound feed (18.6%) compared to wheat (12.4%) and barley (11.2%) reflects intentional formulation with protein-rich ingredients such as soybean meal or canola meal, making it more suitable for high-producing livestock [10]. Crude fat was significantly elevated in compound feed at 18.0% versus 7.04% in wheat and 4.96% in barley. This increase is consistent with the addition of oil or fat supplements to enhance energy density for broilers and dairy cattle [10, 17].

However, certain deviations warrant discussion. The crude fiber value of 17.0% in wheat is higher than the typical 11–13% reported by AOAC for wheat grain, suggesting incomplete sieving of bran or inclusion of husk during sample preparation [3, 10]. Similarly, nitrogen-free extract calculated by difference accumulates errors from all other fractions, which may explain the slightly lower NFE in barley compared to expected values [18]. These limitations highlight the approximate nature of the Weende system, where crude fiber underestimates total dietary fiber and crude protein include non-protein nitrogen.

Despite these limitations, proximate analysis remains indispensable for routine feed quality monitoring due to its low cost and reproducibility [10, 11]. The results provide baseline nutritional data for local wheat and barley varieties in Pakistan, which can guide feed formulators in balancing rations. Future studies should integrate Van Soest NDF/ADF analysis and amino acid profiling to obtain a more physiologically relevant assessment of feed value.

Quality Control Considerations

Accurate proximate analysis requires strict quality control at every stage of the analytical process. Sample representativeness is ensured through proper sub-sampling using quartering or riffling procedures. Analytical balance calibration, oven temperature verification, and crucible cleanliness are critical to obtaining reproducible

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results. The use of certified reference materials (CRMs) and duplicate analyses allows assessment of method precision and accuracy [19].

Operator training and adherence to standardized procedures (AOAC Official Methods, ISO standards) are essential to minimize systematic and random errors. Particular care must be taken in moisture analysis to prevent moisture reabsorption during weighing, and in Soxhlet extraction to ensure complete solvent evaporation from the extracted fat before final weighing [20].

Future Perspectives and Practical Application

The proximate data generated in this study have direct practical applications in Pakistan's livestock and feed industry. The baseline nutritional profile of wheat and barley can be used by feed millers and nutritionists for least-cost ration formulation, especially in small-scale dairy and poultry operations. Future work should integrate Van Soest fiber fractionation, amino acid profiling, and in vitro digestibility assays to provide a more complete characterization of feed nutritional value.

Conclusion

This study successfully characterized the proximate composition of wheat, barley, and commercial compound feed using standardized AOAC analytical procedures. Significant differences were observed among the three samples, with compound feed exhibiting the highest concentrations of crude protein, crude fat, and ash, confirming its superior nutritional value for livestock feeding. Wheat contained the greatest crude fiber content, while barley showed the highest nitrogen-free extract, indicating its higher digestible carbohydrate fraction. The moisture contents of all samples were below the recommended threshold for safe storage, suggesting good keeping quality. Overall, the results confirm that proximate analysis remains an efficient, economical, and reliable tool for routine nutritional evaluation, feed quality assurance, and ration formulation. Although the Weende system has inherent limitations in accurately characterizing fiber fractions and protein quality, it continues to provide valuable baseline information for feed assessment. Future studies should complement proximate analysis with detergent fiber analysis, amino acid profiling, mineral characterization, and digestibility studies to achieve a more comprehensive evaluation of feed nutritional quality and its suitability for modern livestock production systems.

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