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ASSESSMENT OF CRUDE PROTEIN CONTENT IN COMMERCIAL LIVESTOCK FEEDS IN BHUTAN: A RETROSPECTIVE STUDY

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Abstract: *A retrospective analysis of % crude protein (% CP) content was conducted on 410 commercial concentrate feed samples (2018–2022) analyzed at the Animal Nutrition Laboratory, Bhutan. CP was determined using the Kjeldahl method and compared against Minimum Nutrient Requirement Standards (MNRS, BSAF 2025). Overall, 52.92% of samples met MNRS, while 47.08% were below standard. A Python 3 software (spicy, numpy, matplotlib) was run to test for Shapiro–Wilk (normality); Levene's test (homogeneity); Kruskal–Wallis H test (non-parametric ANOVA equivalent); Dunn's post-hoc test with Bonferroni correction; one-sample Wilcoxon signed-rank test with $\alpha = 0.05$ throughout to assess the variation of % CP against MNRS. Kruskal–Wallis test showed significant differences among 13 feed sub-categories ($H = 83.77$, $p < 0.001$). Piggery feeds had significantly lower CP than cattle and poultry feeds ($p < 0.001$), while no difference existed between cattle and poultry. Dunn's test confirmed that all significant pairwise differences involved pig feeds. Wilcoxon tests showed sow ration and pig starter were significantly below MNRS. These findings indicate systemic protein deficiency in pig feeds and highlight the need for improved feed formulation and regulatory control.*

Keywords: Bhutan; Commercial livestock feed; Crude protein content.

1. INTRODUCTION

Livestock rearing has been an integral part of farming in Bhutan. The availability of quality feeding resources in adequate quantity is critical to deriving optimal production from animals (Namgay 2013). Proper management of feeding resources can lower costs by improving feed efficiency. Feed quality is assessed through laboratory analysis and helps to understand the nutritive value of feed provided to animals (Zaroug 1985). Over the years, the demand for commercial concentrate feed has increased markedly due to the expansion of livestock farms across the country. By 2022, the cattle, poultry, and piggery populations had increased relative to baseline levels (DoL Annual Report 2022), resulting in the establishment of four additional feed mills, bringing the total to 10 mills nationwide, with only five operating in

2026. This rapid expansion of the feed industry and its difficulty in withstanding market requirements raises important concerns regarding consistency and adequacy of feed quality, particularly in terms of meeting minimum nutrient standards.

The nutrient utilization theory, first described by Antoine Lavoisier (Saha and Pathak 2021), establishes that livestock productivity declines when diets are deficient in essential nutrients. Feed quality is therefore a key determinant of animal health, growth, productivity, and farm economics. Nutrient-dense feeds have higher nutritive and economic value and support efficient feed conversion and production of high-value animal protein (Baris 2023).

Dietary crude protein (CP) is critical for protein synthesis and overall physiological function. Rocha et al. (2022) report that inadequate CP intake, even in the presence of balanced essential amino acids, limits the synthesis of non-essential amino acids, resulting in reduced growth, production, health, and reproductive performance. In dairy cattle, CP adequacy is necessary to sustain rumen microbial activity, metabolizable protein flow, and milk production (NRC 2001). In poultry, lower level of CP result in a significant increase in feed conversion ratio (FCR), reducing economic return (Badawi et al. 2019). In swine, low dietary CP leads to increased fat deposition as the energy cannot be utilized for lean tissue synthesis due to insufficient nitrogen availability (Rocha et al. 2022; Wang et al. 2018).

Despite these recognized consequences, literature on commercial livestock feed quality in Bhutan remains scarce. An early study by Nidup and Wangchuk (2007), based on limited data from 2007, provided baseline evidence that domestically produced broiler feeds did not meet CP requirements by an average of 1.31% for broiler starter. More broadly, Wangchuk and Dorji (2008), Namgay (2018), and Wangdi et al. (2022) noted persistent concerns about the quality and adequacy of livestock feeding resources in the country. However, no comprehensive systematic assessment of CP content across all commercial feed types in Bhutan has been published to date.

This knowledge gap, i.e the lack of documented evidence on whether commercial concentrate feeds in Bhutan consistently meet the MNRS for CP, forms the basis of the present study. The MNRS for %CP is established in the Bhutan Standards for Animal Feeds 2025 (BSAF 2025) (DoL 2025), a regulatory standard endorsed by the Department of Livestock (DoL) for implementation by feed millers and importers to ensure that all feeds produced and imported meet the minimum nutrient requirements of livestock. The BSAF 2025 specifies MNRS for %CP for all livestock types and their sub-categories (refer Table 1). This study, therefore, aimed to retrospectively assess the CP quality of commercial concentrate feed samples analyzed by the Animal Nutrition Laboratory (ANL) from 2018 to 2022, by comparing measured %CP values against the MNRS in BSAF 2025.

2. MATERIALS AND METHODS

2.1 Study area and sample origin

This study involved commercial concentrate feed samples submitted from dairy, poultry, and piggery farms under DoL. Feed was supplied to the livestock farms by domestic feed millers following the annual animal feed tender process. Laboratory analyses of feed samples were carried out at the ANL, which is under the National Development Centre for Animal Nutrition (NDCAN), Bumthang. The study period corresponds to the 12th Five Year Plan (12th FYP) of Bhutan, covering 2018 to 2022.

2.2 Data collection and sample selection

A retrospective study of laboratory records was conducted. From the ANL database, a total of 410 commercial concentrate feed sample results were identified and included in the analysis. Samples were included if they had (i) a recorded laboratory result for %CP, and (ii) clear identification of feed type, livestock type, and sub-category (i.e., animal stage). Samples with incomplete or unclear information on feed type, livestock type, or sub-category were excluded from the analysis. These feed samples were submitted by different livestock farms under DoL between 2018 and 2022 for routine quality monitoring purposes. The 410 samples represented 13 sub-categories spanning cattle, poultry, and piggery feeds.

2.3 Analytical method

All samples were analyzed for CP content at the ANL using the Kjeldahl method (Association of Official Analytical Chemists [AOAC] 2005). The procedure involved three steps: (i) Digestion: samples were digested in concentrated sulfuric acid (H_2SO_4) at high temperature (~420°C) in the presence of a catalyst (potassium sulfate and copper sulfate) to convert organic nitrogen to ammonium sulfate; (ii) Distillation: the digested sample was made alkaline by addition of sodium hydroxide (NaOH), and ammonia released was steam-distilled into a boric acid receiving solution; and (iii) Titration: the ammonia in the boric acid solution was titrated against standardized hydrochloric acid (HCl) to determine nitrogen content. Quality control measures applied included: analysis of duplicate samples for each sample analysis. The nitrogen content (%) thus determined was converted to %CP by multiplying by the protein conversion factor of 6.25. All duplicate results were averaged before further analysis.

2.4 Comparison with MNRS and statistical analysis

The MNRS for %CP for each feed sub-category was obtained from the BSAF 2025 (DoL 2025). Simple descriptive statistical analysis was carried out using excel. All statistical analyses were performed using Python 3 (SciPy v1.x; Virtanen et al., 2020) package. Differences in %CP across feed sub-categories were assessed using the Kruskal–Wallis test, a non-parametric equivalent of one-way ANOVA (Kruskal & Wallis, 1952) and then followed by Dunn's post-hoc test with Bonferroni correction (Dunn, 1964).

3. RESULTS AND DISCUSSION

3.1 Descriptive statistics of the study

Table 1 presents the descriptive statistics/ data of the study. Of the total 410 samples, 84 samples were from cattle feed sub-categories (cattle concentrate, milk ration and calf feed), 92 samples from poultry feed (chick starter, grower, layer and breeder) and 234 samples were from pig feeds (creep, starter, grower, finisher, sow and boar/ gilt).

Basic comparison of test results to the MNRS showed that only 36.4% of creep feed, followed by 41.1% of pig starter, 41.2 chick starter and 43.3% of sow ration met the MNRS for % CP. More than 50% of the samples from other feed categories met their respective MNRS for % CP. Further, highest standard deviation in %CP was observed in Creep feed followed by pig starter at 14.67± 7.02 and 14.44 ± 4.89, respectively.

3.2 Normality testing (Shapiro–Wilk)

This analysis revealed that three of 13 sub-categories deviated significantly from a normal distribution, and Levene's test confirmed heterogeneous variance across groups (Table 2). The non-parametric Kruskal–Wallis test was therefore the appropriate choice over one-way ANOVA.

3.3 The Kruskal–Wallis test

The Kruskal-Wallis (Figure 1) test showed highly significant differences in %CP across all 13 sub-categories ($H=83.77$, $df=12$, $p<0.001$), and across the three feed types ($H=62.26$, $df=2$, $p<0.001$).

3.4 Dunn's post-hoc test (Bonferroni)

The Dunn's post-hoc test (Bonferroni) identified 13 of 78 pairwise comparisons as significant. All significant contrasts involved piggery feeds (especially Sow Ration, Pig Starter, Pig Grower, and Pig Finisher) versus cattle or poultry feeds with higher %CP. No significant differences were found within cattle or within poultry sub-categories (Table 3 and 4).

The test showed that by livestock type, piggery %CP was significantly lower than both cattle ($p<0.001$) and poultry ($p<0.001$). Cattle and poultry did not differ significantly from each other ($p=0.584$).

3.5 Wilcoxon signed-rank tests

The Wilcoxon signed-rank tests against MNRS revealed that Sow Ration ($p=0.012$) and Pig Starter ($p=0.036$) were significantly below their respective MNRS, while Boar/Gilt ($p=0.001$) and Calf Feed ($p=0.031$) were significantly above their MNRS. The remaining nine sub-categories did not show

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Table 1: Descriptive statistics for %CP in each feed sub-category.

Feed Sub-category	Type	n	Mean ± SD (%CP)	Median	Min	Max	≥MNRS (n)	<MNRS (n)	% Meeting MNRS	MNRS (%CP)
Cattle Concentrate	Cattle	56	15.69 ± 3.83	15.99	7.24	23.40	35	21	62.5%	15
Milk Ration	Cattle	22	18.42 ± 1.88	18.69	15.00	21.89	13	8	61.9%	18
Calf Feed	Cattle	7	17.77 ± 1.50	17.30	15.49	19.86	6	1	85.7%	16
Chick Starter	Poultry	17	18.41 ± 1.91	18.85	13.58	22.48	7	10	41.2%	19
Chick Grower	Poultry	14	16.47 ± 2.68	16.59	9.52	20.71	10	4	71.4%	16
Layer	Poultry	43	17.39 ± 3.55	17.51	8.21	29.13	27	16	62.8%	17
Breeder	Poultry	17	17.52 ± 3.60	16.75	12.62	24.75	10	7	58.8%	16
Sow Ration	Piggery	60	12.40 ± 4.48	12.20	2.51	22.39	26	34	43.3%	14
Creep Feed	Piggery	11	14.67 ± 7.02	18.69	5.76	24.41	4	7	36.4%	20
Pig Starter	Piggery	56	14.44 ± 4.89	14.55	2.97	23.06	23	33	41.1%	16
Pig Grower	Piggery	39	13.73 ± 4.70	14.31	2.90	24.75	21	18	53.8%	14
Pig Finisher	Piggery	54	13.32 ± 4.49	14.31	3.12	22.05	29	25	53.7%	13
Boar/Gilt	Piggery	14	15.70 ± 1.81	15.15	13.63	20.71	14	0	100.0%	13

MNRS = Minimum Nutrient Requirement Standard; **SD** = standard deviation calculated with n−1 (unbiased).

Table 2: Shapiro–Wilk test for normality on each feed sub-category.

Feed Sub-category	p-value	Result
Cattle Concentrate	0.2457	Normal
Milk Ration	0.6144	Normal
Calf Feed	0.6797	Normal
Chick Starter	0.1151	Normal
Chick Grower	0.1748	Normal
Layer	0.0205*	Non-normal
Breeder	0.3666	Normal
Sow Ration	0.5431	Normal
Creep Feed	0.0393*	Non-normal
Pig Starter	0.3855	Normal
Pig Grower	0.7347	Normal
Pig Finisher	0.4909	Normal
Boar/Gilt	0.0028**	Non-normal

* $p<0.05$; ** $p<0.01$. Three sub-categories (Layer, Creep Feed, Boar/Gilt) showed significant departure from normality.

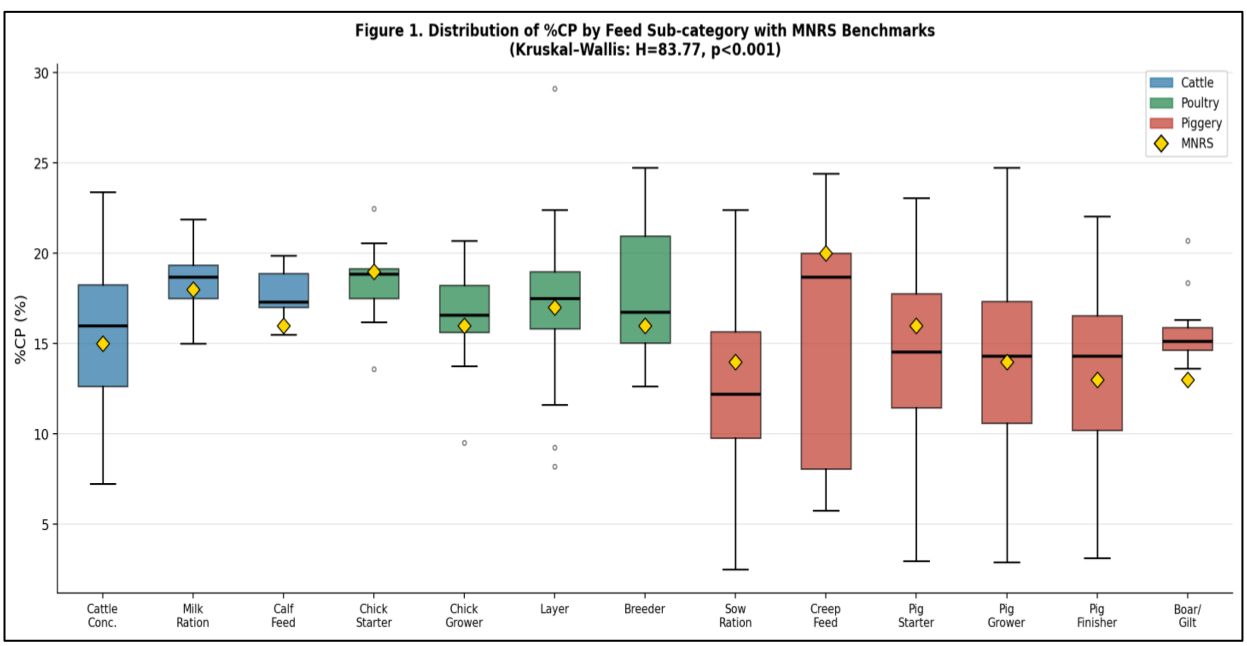

Figure 1: Kruskal-Wallis Test; distribution of % CP by feed sub-category with MNRS Benchmarks.

Table 3: Comparison of %CP across three feed types.

Livestock Type	n	Mean ± SD (%CP)	Dunn vs Piggery
Cattle	84	16.54 ± 3.50	p < 0.001 ***
Poultry	92	17.46 ± 3.19	p < 0.001 ***
Piggery	234	13.63 ± 4.70	Reference

Cattle vs. Poultry: Z=1.30, p_bonf=0.584 (ns). *** = p<0.001.

Table 4: Dunn's post-hoc test and statistical significance at p<0.05 in pig feed sub-category.

Group A	Group B	p (Bonferroni)
Cattle Concentrate	Sow Ration	0.0109
Milk Ration	Sow Ration	<0.0001
Milk Ration	Pig Starter	0.0064
Milk Ration	Pig Grower	0.0012
Milk Ration	Pig Finisher	<0.0001
Chick Starter	Sow Ration	<0.0001
Chick Starter	Pig Starter	0.0173
Chick Starter	Pig Grower	0.0035
Chick Starter	Pig Finisher	0.0004
Layer	Sow Ration	<0.0001
Layer	Pig Grower	0.0147
Layer	Pig Finisher	0.0006
Breeder	Sow Ration	0.0084

statistically significant deviation from their MNRS at α=0.05, although Creep Feed showed a large negative mean deviation (−5.33%) with borderline significance (p=0.063, n=11). This is presented in Table 5.

These statistical results confirm and strengthen the descriptive findings of the study that piggery feeds, particularly Sow Ration and Pig Starter, consistently fall short of MNRS for %CP at a statistically significant level, and the variability of %CP in piggery feeds is substantially greater than in cattle or poultry feeds.

The impact of lower level of CP content in feed can be substantial over time, primarily through compromised animal productivity. Limiting amino acid requirements, particularly for lysine, threonine, sulfur-containing amino acids, tryptophan, and valine, increase on low CP diets in both pig and poultry, as the demand for nitrogen for endogenous non-essential amino acid synthesis rises when dietary CP is inadequate (Wang et al. 2018). In dairy cattle, CP adequacy is essential for sustaining rumen fermentation and microbial protein synthesis; low CP diets result in

Table 5. One-sample Wilcoxon signed-rank test for each sub-category against its MNRS.

Feed Sub-category	MNRS	Mean Dev. (%CP)	W stat.	p-value	Sig.
Cattle Concentrate	15	+0.69	627.0	0.163	ns
Milk Ration	18	+0.42	86.0	0.305	ns
Calf Feed	16	+1.77	1.0	0.031	*
Chick Starter	19	−0.59	51.5	0.236	ns
Chick Grower	16	+0.47	33.0	0.221	ns
Layer	17	+0.39	364.5	0.277	ns
Breeder	16	+1.52	49.0	0.193	ns
Sow Ration	14	−1.59	553.0	0.012	*
Creep Feed	20	−5.33	9.0	0.063	ns†
Pig Starter	16	−1.56	540.5	0.036	*
Pig Grower	14	−0.27	374.0	0.823	ns
Pig Finisher	13	+0.32	658.0	0.467	ns
Boar/Gilt	13	+2.70	0.0	0.001	**

* $p<0.05$; ** $p<0.01$; ns = not significant; † n=11 (small sample, interpret with caution

deficiency of both rumen-degradable protein and rumen-undegradable protein, decreasing metabolizable energy and protein availability and reducing milk yield (NRC 2001). Imaizumi et al. (2002) reported that increasing dietary CP from 15 to 16% and from 19 to 20% resulted in milk yield increases of 0.94 and 0.42 kg/d, respectively.

The findings of this study indicate that CP content is a significant area of non-compliance in the Bhutanese commercial livestock feed sector. The higher variability observed in pig feeds suggests inconsistent formulation practices which could be driven by variability in raw material quality. Given that protein is one of the most expensive feed components, economic constraints may lead to under-formulation.

However, it should be acknowledged that CP is one of several nutritional parameters, and that compliance or non-compliance with CP alone does not fully characterize overall feed quality; energy content and other nutrients may independently meet or fail to meet standards. Nonetheless, given that CP is among the most critical and costly nutrients in feed formulation, and is part of the MNRS in BSAF 2025, these findings justify the prioritization of CP monitoring in the regulatory framework.

4. CONCLUSION AND RECOMMENDATIONS

A significant proportion (47.08%) of commercial concentrate feed samples analyzed at the ANL during the 12th FYP period (2018–2022) did not meet the MNRS for %CP as stipulated in BSAF 2025. Piggery feeds showed the highest rate of non-compliance. The range of CP deficit in non-compliant samples was wider than the range of CP excess in compliant samples, suggesting greater inconsistency in under-formulated feeds. These findings indicate that inconsistencies in feed quality exist within the Bhutanese commercial feed industry and that strengthened monitoring and quality assurance measures are urgently required.

Based on these findings, the following recommendations are made:

- Regulatory agencies should implement monthly random inspection of feed mills and mandatory pre-market testing of commercial feeds to improve CP compliance.
- Feed mills should be required to employ qualified animal nutritionists and establish in-house feed testing facilities to strengthen formulation accuracy and quality control.
- Since most feed raw materials are imported, millers should be supported to establish procurement protocols that ensure raw material quality, including testing of incoming ingredients for CP content prior to use in formulation.
- Monitoring of CP content in commercial feeds should be systematically expanded and prioritized, with results made available to feed millers as feedback to guide formulation improvements.
- Future feed quality assessments should include additional nutritional parameters such as energy content, fiber, and micronutrients, to provide a more comprehensive evaluation of commercial feed quality in Bhutan.

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References

Association of Official Analytical Chemists [AOAC]. (2005). Official Method 2001.11-2005: Protein (Crude) in Animal Feed, Forage (Combustion Method). Association of Official Analytical Chemists, Washington, DC.

Badawi ME, Ali AH, El-Razik WMA, and Soliman MH. (2019). Influence of low crude protein diets on broiler chickens' performance. Advances in Animal and Veterinary Sciences, 7(2), 26–33.

Baris A. (2023). Impact of feed quality on livestock productivity. Journal of Livestock Policy, 2(1).

Bhutan Standards for Animal Feeds. (2025). Department of Livestock, Ministry of Agriculture and Livestock, Thimphu, Bhutan.

Department of Livestock. (2022). Annual Report 2022. Ministry of Agriculture and Livestock, Thimphu, Bhutan.

Dunn OJ. (1964). Multiple comparisons using rank sums. Technometrics, 6(3), 241–252.

Imaizumi H, Santos FAP, Pires AV, Nussio CMB, Barnabé EC, and Juchen SO. (2002). Avaliação de diferentes fontes e teores de proteína na dieta sobre o desempenho, fermentação ruminal e parâmetros sangüíneos de vacas da raça Holandesa em final de lactação. Acta Scientiarum, 24, 1031–1037.

Kırkpınar F, and Açıkgöz Z. (2018). Feeding. IntechOpen. https://doi.org/10.5772/intechopen.78618

Kruskal WH, and Wallis WA. (1952). Use of ranks in one-criterion variance analysis. Journal of the American Statistical Association, 47(260), 583–621.

Levene H. (1960). Robust tests for equality of variances. In I. Olkin (Ed.), Contributions to Probability and Statistics (pp. 278–292). Stanford University Press.

Namgay K. (2013). Livestock feeds and feeding practices in Bhutan. In

SAARC Agriculture Centre (Ed.), Livestock Feeds and Feeding Practices in South Asia. SAARC Agriculture Centre, Dhaka, Bangladesh.

Namgay K. (2018). Livestock feeds and feeding practices in Bhutan. In SAARC Agriculture Centre (Ed.), Livestock Feeds and Feeding Practices in South Asia (updated ed.). SAARC Agriculture Centre, Dhaka, Bangladesh.

National Research Council. (2001). Nutrient Requirements of Dairy Cattle (7th Rev. Ed.). National Academy Press, Washington, D.C. 408 pp.

Nidup K, and Wangchuk P. (2007). Comparative study of broiler feeds in Bhutan. Bhutan Journal of Rural and Natural Resources (Bhu.J.RNR), 3(1), 11–25.

Rocha GC, Duarte ME, and Kim SW. (2022). Advances, implications, and limitations of low-crude-protein diets in pig production. Animals, 12(24), 3478. https://doi.org/10.3390/ani12243478

Saha SK, and Pathak NN. (2021). The Fundamentals of Animal Nutrition. Springer Singapore. https://doi.org/10.1007/978-981-15-9125-9

Shapiro SS, and Wilk MB. (1965). An analysis of variance test for normality (complete samples). Biometrika, 52(3–4), 591–611.

Virtanen P, Gommers R, Oliphant TE, Haberland M, Reddy T, Cournapeau D, Burovski E, Peterson P, Weckesser W, Bright J, van der Walt SJ, Brett M, Wilson J, Millman KJ, Mayorov N, Nelson ARJ, Jones E, Kern R, Larson E, …van Mulbregt P. (2020). SciPy 1.0: Fundamental algorithms for scientific computing in Python. Nature Methods, 17, 261–272. https://doi.org/10.1038/s41592-019-0686-2

Wang Y, Zhou J, Wang G, Cai S, Zeng X, and Qiao S. (2018). Advances in low-protein diets for swine. Journal of Animal Science and Biotechnology, 9, 60. https://doi.org/10.1186/s40104-018-0276-7

Wangchuk K, and Dorji T. (2008). Animal feed production and management in Bhutan. In Best Practices in Animal Feed Production and Management in SAARC Countries. SAARC Agricultural Centre, Dhaka, Bangladesh. ISBN 984-300-002742-1.

Wangdi K, Zangmo T, Wangchuk K, and Norbu. (2022). Cattle feed resources assessment and coping strategies adopted during feed scarcity in eastern Bhutan. BJAS Special Issue, 27–34.

Wilcoxon F. (1945). Individual comparisons by ranking methods. Biometrics Bulletin, 1(6), 80–83.

Zaroug M. (1985). Feed quality analysis: Practical approaches in the field. FAO Animal Production and Health Paper, Food and Agriculture Organization, Rome.

Zaefarian F, Cowieson AJ, Pontoppidan K, Abdollahi MR, and Ravindran V. (2021). Trends in feed evaluation for poultry with emphasis on in vitro techniques. Animal Nutrition, 7(2), 268–281.