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Effects of Protein Extracted from CO-3 Grass on Weight Gain of Broiler Chicken

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Abstract – This study evaluated the effects of protein extracted from CO-3 grass on broiler growth in addition to comparing the protein extract from Super Napier, Tridax daisy, and Guinea grass types. Protein extraction was conducted using a green biorefinery approach. Kjeldahl method was used to analyze crude protein content in the extract. A feeding trial was conducted where the control group fed with commercial feed and the treatment group additionally supplemented with 10% CO-3 protein extract dissolved in the drinking water to ensure uniform intake. Each group consisted of 4 replications, with 5 chicks per replicate, monitored over 30 days. In the first week, both groups were fed equally. In the treatment group, the daily commercial feed quantity was reduced by 10%, and this reduction was replaced with 10% CO-3 green protein extract to maintain the total daily feed intake for rest of the three weeks. Weight gain was assessed weekly, and the data was analyzed using an independent t-test with SPSS. It was found that CO-3 grass had the highest crude protein content (22.20 ± 1.34%) compared to other tested grasses. Further, the weekly weight gain from the second week onwards not significantly different between control and treatment groups. These findings suggest that protein extract from CO-3 grass may have potential as a supplemental protein source for broiler chickens.

Keywords- Biorefinery, Grass protein, Protein extraction, Sustainable poultry feed, Weight gain

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Introduction

The increase of consumer demand for affordable animal protein has resulted further growth of the global poultry industry. This is however challenged by the rising feed prices which is over 70% of total production expenditure (Ravindran et al., 2021). The main protein source in the broiler feed is soybean meal which is usually imported to developing countries including Sri Lanka leading to high prices, fluctuation in supply, and has serious environmental impacts such as deforestation and land-use conversion. These restrictions show the necessity to seek alternative options of sustainable, locally sourced and cost-effective protein sources that will decrease reliance on imported feed materials (Santamaria et al., 2020).

Studies have demonstrated that most plant-based supplements would be able to improve growth performance, immune functioning, and gut health in broiler chickens (Daramola., 2019; John et al., 2024) . Particularly, the set of Leaf Protein Concentrates (LPCs) produced by green biorefinery technologies have attracted attention because of its high digestibility and amino acid composition appropriate to monogastric animals (Santamaria et al., 2020). According to Abdelnour et al. (2018), the opportunities of underused plant biomass as good sources of protein in the manufacturing of poultry feed needs to be explored.

Sri Lanka has a variety of tropical forages such as CO-3 grass, Super Napier, Tridax daisy and Guinea grass that are well-known because of their rapid growth, high biomass generation as well as the ability to adjust to the local climatic conditions. Although they are known to provide nutritional benefits in the ruminant diet, little is known regarding their potential use as a protein source to monogastric animals, particularly broilers. Very few studies have examined the extraction of green protein of these grasses or their suitability as dietary supplements to poultry. Consequently, there is a great knowledge gap concerning the nutritional value of local tropical grasses and their usability in broiler feed.

Thus, the need to study the protein composition of these commercially available grasses and analyze their suitability as dietary supplements in the production of poultry has been timely and essential. The use of such resources would help make the poultry industry in Sri Lanka more sustainable, economical as well as environment friendly. This research is focused on extracting protein in selected grasses and compare them regarding their nutritional profile and evaluate the growth performance of broiler chicken fed with CO-3 grass protein extract. The results will offer meaningful information on local feed resource usage and help to work out other protein approaches to the poultry industry.

Materials and Methods

Study Location

The experimental phase of this study was conducted within the laboratory facilities of the Department of Biosystems Technology, Faculty of Technology, South Eastern University of Sri Lanka, located in Oluvil, Ampara District.

Ethical Approval

Ethical clearance for this research was obtained from the Ethics Committee of the Faculty of Technology, Southeastern University of Sri Lanka. The committee granted approval at the meeting held on 27th February 2025, after reviewing the application submitted for ethical consideration. The approval was granted under ERC number ERC/FT/2025/11. All procedures in this study were conducted in accordance with the ethical guidelines and standards set by the committee.

Selection and Collection of Plant Samples

Four forage grass species were selected based on their agronomic significance, protein content potential, availability in local farming systems, and adaptability to Sri Lankan climatic conditions. The selected species included CO-3 grass (*Pennisetum purpureum* × *Pennisetum americanum* hybrid), Super Napier grass (*Pennisetum purpureum* hybrid), Tridax daisy (*Tridax procumbens*), and Guinea grass (*Panicum maximum*) in same growth stage. Fresh samples of CO-3 and Super Napier were harvested from the experimental plots at AgroTech Park Malwaththa, affiliated with the Southeastern University of Sri Lanka. Tridax daisy and Guinea grass samples were collected from the wild growth in the Oluvil village area.

Protein Extraction Procedure from Grasses

Sample Preparation

Upon arrival in the laboratory, plant materials were washed under running tap water followed by 1 % chlorex (Sodium hypochlorite) rinse to remove soil particles, insect residues, and microbial contaminants. Samples were chopped into uniform segments of approximately 2–3 cm in length using sterilized stainless-steel scissors. Each sample was then weighed using a precision digital balance (±0.01 g accuracy) to ensure standardization across treatments. The homogenization process involved blending 100 g of each sample into 200 ml of water using a laboratory blender. The homogenized mixture was filtered through a triple-layer muslin cloth and collected to the beakers. This yielded a green juice rich in soluble proteins, carbohydrates, and micronutrients.

pH Adjustment and Protein Precipitation

To facilitate protein precipitation, the pH of the green juice was gradually adjusted to 4.5 isoelectric pH using a 4% (w/v) citric acid solution, while continuously stirring with a magnetic stirrer (Biologix, MS-H280-Pro) at 250 rpm (Santamaria et al., 2020). The pH level was monitored using a digital pH meter (Hanna, HI98190). This acidic environment promoted isoelectric precipitation.

Thermal Coagulation

Following pH adjustment, the acidified juice was subjected to heat coagulation. The solution was heated in a thermostatically controlled water bath at 70°C for 15 minutes, which denatured and coagulated the soluble proteins. Gentle stirring was maintained during heating to avoid localized overheating and protein degradation (Santamaría-Fernández and Lübeck, 2020).

Filtration and Drying of Protein Extract

The coagulated proteins were collected by vacuum filtration using 0.45 µm Whatman GF/C glass microfiber filter papers mounted on a Buchner funnel system. The collected protein paste was spread thinly on stainless steel trays and dried in a hot air oven at 45° C for 24 hours. The dried protein extracts were stored in air-tight, opaque polyethylene containers at <10° C to prevent oxidation and microbial contamination (Domokos-Szabolcsy et al., 2023).

Protein Content Analysis

Quantitative estimation of crude protein content in the dried extracts was performed using the Kjeldahl method, a gold standard for nitrogen determination in organic materials using AOAC Official Method 979.09 (AOAC, 2016).

Digestion Phase

1.0 g portion of the dried sample was digested using 20 ml of concentrated sulfuric acid and one Kjeldahl catalyst tablet, which contained the required catalyst mixture for efficient digestion. All components were placed together in a digestion tube and the digestion was carried out at approximately 400° C using a Kjeldahl digestion unit. The process was continued for about 4 to 5 hours until a clear solution was obtained, indicating that the organic nitrogen had been completely converted to ammonium sulfate.

Distillation and Titration

Following digestion, the sample was subjected to distillation in the presence of 40% NaOH using a Kjeldahl distillation apparatus. The liberated ammonia was trapped in a 4% boric acid solution and titrated with 0.1 N HCl. The crude protein content was calculated using the conversion factor (N × 6.25), accounting for the average nitrogen content of proteins. Each sample was analyzed in triplicate to ensure statistical reliability. The data was expressed on a percentage dry matter basis (DM%).

Feeding Trial

Experimental Animals and Housing

A total of 40 one-day-old broiler chicks (Cobb 500 strain) were randomly assigned to two treatment groups. The first group (T1) received a commercial feed supplemented with 10% CO-3 protein extract, while the second group (T2) served as the control and was fed only the commercial feed.

Each treatment group was divided into 4 replicates, with 5 chicks per replicate (Table 1). The feeding trial was conducted at the South Eastern University of Sri Lanka, and the experimental pen was provided by the Agrotech Park, Malwaththa Farm. The pen was constructed with two levels: an upper and a lower part. Each of these levels were subdivided into 4 equal sections to accommodate the replicates. Each replicate housed 5 chicks, which were individually tagged on the leg for identification. All compartments were equipped with a separate feed bucket and water drinker. The pens were cleaned and disinfected before the trial commenced. Temperature, lighting, and humidity were maintained according to standard broiler management guidelines throughout the 30-day trial period.

Table 1

Experimental design

Treatment	Replicate	Sample size per replicate	Sample size
T1 (Feed supplement with CO-3 grass protein extract)	4	5 chicks	20
T2 (commercial feed)	4	5 chicks	20

Feed Formulation and Administration

The broiler feeding chart recommended by Aviagen (2016) was followed for all experimental groups. For the T1 group, the daily commercial feed quantity was reduced by 10%, and this reduction was replaced with 10% CO-3 green protein extract in order to maintain the total daily feed intake. The T2 group was maintained solely on 100% commercial feed without any CO-3 supplementation.

The CO-3 protein extract was mixed with water prior to administration. The total daily water intake for the 40 chicks was divided into two portions morning and evening. The required amount of CO-3 protein extract powder was dissolved in the morning water portion and thoroughly stirred to ensure a uniform suspension. This supplemented water was provided to the T1 group using standard poultry drinkers. Once the birds consumed the supplemented morning water fully, the second portion of clean water was provided in the evening. The T2 group received clean water during both periods, without any added supplements. The protein extract was administered in liquid form to ensure uniform mixing and ease of intake during this preliminary evaluation.

Growth Performance Evaluation

Body weights of individual chicks were recorded three times per week using a digital scale with ±0.1 g precision. Weight gain was calculated as the difference between final and initial body weight. Mortality and general health conditions were observed and documented daily. Any birds were not died during the trial period.

Statistical Analysis

All collected data were statistically analyzed using an independent t-test in IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA) to determine significant differences between treatment and control.

Results

Crude Protein Content of Different Forage Grasses

The crude protein content of four selected forage grass species was evaluated to assess their potential as alternative protein sources in broiler feed supplementation. The grasses analyzed included CO-3 grass (*Pennisetum purpureum* × *Pennisetum americanum* hybrid), Super Napier (*Pennisetum purpureum* hybrid), Tridax daisy (*Tridax procumbens*), and Guinea grass (*Panicum maximum*). The results, illustrated in Figure 1, showed a significant variation in protein content among the grass types. CO-3 grass exhibited the highest mean crude protein content at 22.20 ± 1.34%, followed by Tridax daisy at 17.31 ± 5.06%. Super Napier and Guinea grass had relatively lower protein contents of 11.76 ± 0.19% and 11.23 ± 1.47%, respectively.

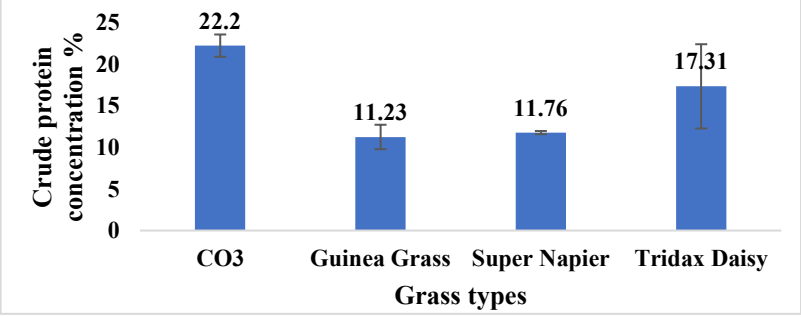

Figure 1. *Crude protein concertation in grass protein extract*

Proximate Composition of CO-3 Grass Protein Extract

Given the superior protein content observed in CO-3 grass, a detailed proximate analysis was conducted to evaluate its overall nutritional profile and suitability as a feed supplement. The analysis, shown in Table 2, included the determination of crude protein, crude fat, ash, fiber, nitrogen, and moisture content. The results indicated that the CO-3 protein extract contained 22.20 ± 1.34% crude protein, 2.81 ± 0.14% fat, 5.60 ± 0.22% ash, 3.15 ± 0.05% fiber, 3.55 ± 0.21% nitrogen, and 21.53 ± 1.01% moisture.

Table 2

Mean proximate composition of protein extract of CO-3 grass

Nutrients	Mean ±SD (%)
Crude protein (%)	22.20 ± 1.34
Fat (%)	2.81 ± 0.14
Ash (%)	5.60 ± 0.22
Fiber (%)	3.15 ± 0.05
Nitrogen (%)	3.55 ± 0.21
Moisture (%)	21.53 ± 1.01

Note. Values are mean ± Standard deviation

Weight Gain Performance of Broilers Fed with CO-3 Grass Protein Extract

To evaluate the impact of CO-3 grass protein supplementation on broiler growth performance, weight gain data were collected over a four-week period. Broiler chicks were divided into two treatment groups: T1, group received a modified feed in which 10% of the commercial feed was replaced with CO-3 grass protein extract to maintain the same total daily feed intake, and T2, the control group, receiving only commercial feed.

Table 3 provides the descriptive statistics of broiler weight gain in both treatment groups. In week 1, T1 group showed a minimum weight gain of 130 g, a maximum of 316 g, with an overall mean of 215.23 g. In contrast, T2 group showed a minimum weight gain of 114 g, a maximum of 283 g, with overall mean of 189.22 g. During week 2, T1 broilers gained weight ranging from 301 g to 577g with a mean of 416.53 g, while T2 broilers gained between 251 g and 568g with a mean of 393.17 g. During week 3, T1 had a slightly higher average weight gain (510.42 g) compared to T2 (501.42 g). By week 4, T1 achieved a maximum weight gain of 770 g and a minimum of 329 g, with a mean of 566.58 g, while T2 had a slightly lower mean of 549.20 g, ranging between 350 g and 708 g.

Table 4 provides a statistical comparison of mean weight gain between T1 and T2 groups across the four weeks. A significant difference ($p = 0.004$) was observed in week 1, with T1 outperforming T2. Although T1 continued to show higher average weight gain in weeks 2, 3, and 4, the differences were not statistically significant ($p = 0.088$, 0.39, and 0.721 respectively).

Table 3

Mean weight gain of chickens supplemented with CO-3 grass protein extract

Week	T1 (weight gain with CO-3 grass protein extract supplementation)			T2 (weight gain with the feeding of commercial feed)		
	Minimum	Maximum	Mean	Minimum	Maximum	Mean
1	130.00	316.00	215.23 ± 53.44	114.00	283.00	189.22 ± 44.40
2	301.00	577.00	416.53 ± 69.30	251.00	568.00	393.17 ± 79.26
3	367.00	619.00	510.42 ± 53.94	344.00	651.00	501.42 ± 58.39
4	329.00	770.00	566.58 ± 108.94	350.	708.00	549.20 ± 68.14

Note. Values are mean ± standard deviation

Table 4

Effect of CO-3 grass protein extract on mean weight gain of chickens

Week	T1 (Feed supplemented with CO-3 grass protein extract)	T2 (Commercial Feed)	P value
1	214.64 ± 53.71	189.22 ± 44.40	0.004
2	415.69 ± 69.59	372.69 ± 79.26	0.088
3	512.85 ± 50.98	501.68 ± 58.39	0.39
4	566.58 ± 108.94	549.20 ± 68.14	0.721

Note. Values are mean ± standard deviation

Discussion

The observed differences in protein content of the grass types can be attributed to species-specific genetic variations, environmental factors, and the physiological maturity of the grasses at the time of harvest. CO-3 grass, a hybrid developed for high-yield and nutritional performance, demonstrated superior protein content, making it a promising candidate for nutritional supplementation in poultry diets.

The values of proximate composition underscore the nutritional richness of CO-3 grass. The high crude protein concentration supports its value as a protein source, while the low fiber and moderate fat content suggest good digestibility and energy provision, essential for broiler growth. The ash content reflects the presence of essential minerals, contributing to the overall nutrient balance. The moisture content, although moderate, is within acceptable limits for processed feed ingredients. The combination of these parameters affirms the suitability of CO-3 protein extract as a functional and beneficial component in poultry nutrition.

The protein content of the CO-3 grass protein extract obtained in this study (22.20%) falls within the range of protein content of green biomass protein fractions, with protein yield and purity known to depend on the species and maturity of the plant and the extraction

efficiency used (Domokos-Szabolcsy et al., 2023). Similar to earlier research on plant-based protein supplements in broilers, it was determined that there is an initial positive impact on weight gain and a lack of significant differences in subsequent growth phases, indicating that moderately high levels of inclusion only have short-term nutritional effects and no long-term effects on performance (Stødkilde et al., 2019). The variations among the studies are probably affected by the level of inclusion, digestibility of proteins, and the substitution or supplement of the traditional feed components by the plant protein (Kiggundu et al., 2025).

Regarding weight gain of the chickens, a steeper initial growth trajectory was observed in the T1 group during week 1, followed by relatively parallel growth trends between T1 and T2 in subsequent weeks. The data suggest a temporary advantage provided by the CO-3 protein extract, particularly in early developmental stages of broiler growth. This trend indicates that while CO-3 protein extract had a marked positive impact on early-stage growth, the effect appeared to diminish over time. These findings suggest that CO-3 protein extract has potential as a supplemental protein source for poultry. The results of this study demonstrate that CO-3 grass, due to its high protein content and favorable proximate composition, it holds promise as a main protein source in broiler diets. Its inclusion at a level of 10% in place of a commercial feed substitute enhanced early-stage weight gain in broiler chickens, suggesting improved nutrient utilization. While the continuing effects on growth were not statistically significant, the overall performance trends indicate that CO-3 protein extract may contribute positively to poultry production systems, especially in resource-limited settings seeking cost-effective and locally available feed alternatives.

Conclusions

This study evaluated the nutritional potential and performance impact of CO-3 grass protein extract as a dietary supplement in broiler production, with a particular focus on growth performance in broiler chicks. Among the four forage grasses examined, CO-3 grass exhibited the highest crude protein content justifying its selection for supplementation trials. When incorporated at a 10% level into commercial feed, CO-3 supplementation significantly enhanced early weight gain in broilers during the first week with no changes in the rest of the weeks. These findings conclude that CO-3 protein extract might be a sustainable protein supplement for poultry production in regions like Sri Lanka. Since it is a preliminary study, it is recommended that future studies explore optimal inclusion levels, conduct long-term feeding trials with higher sample size, and assess additional parameters such as feed conversion efficiency, immune function, carcass yield, and economic returns. Future studies should be also focused on incorporating the protein into solid feed formulations to improve standardization and dosing accuracy.

References

Abdelnour, S. A., Camacho, D., & Díaz, F. (2018). Protein quality and amino acid profiles of tropical forage species and their potential application in monogastric animal diets. *Journal of Tropical Agriculture and Food Science*, *46*(3), 205–217.

Association of Official Analytical Chemists (AOAC). (2016). *Official methods of analysis of AOAC International* (20th ed.). AOAC International, Gaithersburg, MD, USA

Aviagen. (2016). Ross 308 European Parent stock: Nutrition Specifications, 1–8.

Daramola, J. A. (2019). Role of medicinal plant leaf meals in poultry nutrition: Impact on serum metabolism and antioxidant status. *African Journal of Plant Science*, *13*(7), 128–136.

Domokos-Szabolcsy, É., Yavuz, S.R., Picoli, E., Fári, M.G., Kovács, Z., Tóth, C., Kaszás, L., Alshaal, T. & Elhawat, N. (2023). Green biomass-based protein for sustainable feed and food supply: an overview of current and future prospective. *Life*, *13*(2), 307.

John, O. O., Adeoye, O. O., & Taiwo, V. O. (2024). Effects of *Uvaria chamae* leaf meal on growth performance, immune response, and gut microbiota in broiler chickens. *Poultry Science Journal*, *44*(2), 123–131.

Kiggundu, M., Nampijja, Z., Kigozi, A., Mugerwa, S., Molly, A., Namazzi, C. & Bombom, A. (2025). Substituting fishmeal with Napier grass protein: effects on intake, growth performance and carcass characteristics of broiler chickens. *European Journal of Agriculture and Food Sciences*, *7*(1), 13-21.

Ravindran, V., Singh, U. P., & Duchen, S. (2021). Sustainability challenges of soybean use in poultry feed: Environmental and economic considerations. *Sustainable Agriculture Review*, *37*, 223–245.

Santamaria, P., Camargo, M., & Diaz, F. (2020). Leaf protein concentrates from tropical biomass: Nutritional and sustainability perspectives. *Global Food Security*, *25*, 100353.

Santamaría-Fernández, M. & Lübeck, M. (2020). Production of leaf protein concentrates in green biorefineries as alternative feed for monogastric animals. *Animal Feed Science and Technology*, *268*, 114605.

Stødkilde, L., Damborg, V.K., Jørgensen, H., Lærke, H.N. & Jensen, S.K. (2019). Digestibility of fractionated green biomass as protein source for monogastric animals. *Animal*, *13*(9), 1817-1825.