



Partial Substitution of Soybean Meal with *Azolla pinnata* L. (1758) Meal in Diets for F3 Australorp Chicks during the Growth Phase in Kisangani, DRC

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Abstract

The present study aimed to determine the growth rate and feed conversion ratio of F3 hybrid chickens of the Black Australorp breed during the growth stage and to assess the effect of partial substitution of soybean meal with *Azolla pinnata* meal at this stage. The experiment was conducted at PK 7 on the former Buta Road, Avenue Forescom, Bloc Motumbe, Plateau Boyoma district, Makiso commune, Kisangani city, DR Congo, located at 0° 33' 44.76492"N and 25° 13' 27.23628"E, at an altitude of 395.9 m. The growing birds (F3 hybrid Black Australorp) were used in a completely randomized experimental design with four treatments (T0 or control group, T1, T2, and T3). *Azolla pinnata* meal partially replaced soybean meal at replacement levels corresponding to 5% (T1), 10% (T2), and 15% (T3) of the soybean meal fraction in the experimental diets. After a 14-week experimental period, average feed intake was highest in T1 (10790.5 ± 7937.6 g) and lowest in T2 (9060.7 ± 7572.4 g). Mean body weight was greatest in T1 (1049.7 ± 742.1 g), followed by T0 (1012.2 ± 727.8 g), T2 (1003.4 ± 711.5 g),



and T3 (987.2 ± 702.8 g). The highest feed conversion ratio was observed in T0 (9.9 ± 2.82), followed by T1 (9.80 ± 2.79) and T2 (7.91 ± 2.29). The inclusion of *Azolla pinnata* meal in the diet of Black Australorp chickens did not cause any depressive effect on zootechnical performance compared with roasted soybean meal. *Azolla pinnata* meal can be used in poultry diets as a substitute for soybean meal up to 10% during the growth stage studied in this trial. Its effects during the laying stage and on egg production were not evaluated in the present study.

Subject Areas

Animal Husbandry

Keywords

Substitution, Soybean, *Azolla pinnata*, Australorp, Kisangani, Democratic Republic of Congo

1. Introduction

Poultry farming is a strategic alternative for reducing the deficit in animal protein. Poultry meat, valued for its white meat, and eggs contribute to food security and face fewer cultural or religious constraints than other meats [1]-[3].

In sub-Saharan Africa, the availability of quality, low-cost feed remains a major constraint for traditional poultry production, where feeding largely depends on the scavenging environment [4] [5]. Conventional protein-rich ingredients such as soybeans are becoming scarce and expensive, intensifying competition between human and animal nutrition.

In this context, using non-conventional, locally available ingredients appears to be a sustainable solution. Several studies have highlighted the value of local plants and by-products in animal feeding [6]-[8]. Among these, *Azolla pinnata*, a protein-rich aquatic plant, stands out because it does not compete with human food [9] [10].

Little known to poultry farmers in Kisangani, *Azolla pinnata* could help address limited access to conventional plant protein sources. This study aimed to assess the effect of incorporating *Azolla pinnata* meal on growth and feed conversion ratio in F3 hybrid Black Australorp chickens during the growth phase.

2. Materials and Methods

The experiment was conducted in Kisangani (DR Congo; altitude 395.9 m). The biological material consisted of one-week-old F3 hybrid Black Australorp female chicks, a third-generation hybrid chicken, allocated to four treatments in a completely randomized design. The experimental diets were: T0: control (without *Azolla pinnata*); T1: replacement of 5% of the soybean meal with *Azolla pinnata* meal; T2: replacement of 10% of the soybean meal with *Azolla pinnata* meal; and T3:

replacement of 15% of the soybean meal with *Azolla pinnata* meal.

A total of 72 chicks were reared in 12 experimental cages, with six chicks per cage. Chicks were allocated to ensure similar initial body weight and rearing conditions. Each cage was equipped with feeders, drinkers, and rice-hull litter. The experiment lasted 20 weeks, including 6 weeks of brooding and 14 weeks of growth. **Table 1** summarizes the experimental design.

Table 1. Experimental design of the study.

Treatment	Substitution level	Replicate 1	Replicate 2	Replicate 3
T0	0%	EU1	EU2	EU3
T1	5%	EU4	EU5	EU6
T2	10%	EU7	EU8	EU9
T3	15%	EU10	EU11	EU12

Abbreviation: EU, Experimental Unit.

Iso-protein rations were formulated according to poultry nutritional requirements. Each day at 7:00 AM and 5:00 PM, each diet was weighed and distributed. The next day at the same times, unconsumed feed was collected and weighed, and cages were cleaned. Drinking water was provided ad libitum. An additional 15 g of feed per bird was provided during the first month [11]. Thereafter, the feed quantity was increased according to birds' needs.

Birds were weighed individually at the beginning of the study and then weekly. Feed intake was first measured daily at the cage level as feed offered minus refusals, then summed up to obtain weekly cage intake. Weekly cage intake was divided by the number of birds present in that cage to express intake on a per-bird basis. Body weight was recorded each week individually, and body weight gain was calculated for each bird as the difference between the current week's body weight and the previous week's body weight; cumulative gain corresponded to the difference between body weight at the relevant week and initial body weight at the start of the trial. Average daily gain was calculated from weekly body weight gain divided by 7. Feed conversion ratio (FCR) was calculated as feed intake divided by body weight gain for the same period; thus, weekly FCR used weekly feed intake and weekly weight gain, whereas overall FCR used cumulative feed intake and cumulative body weight gain.

To reduce the incidence of infectious diseases responsible for animal and financial losses, we implemented a biosecurity system: regular cleaning of feeders and drinkers, clean feed and water, physical separation of the experimental units from other poultry houses and reduced external visits.

Animal ethics statement

The study was conducted with attention to animal welfare and routine good husbandry practices. Birds were monitored daily, supplied with feed and water according to the experimental protocol, and maintained under hygiene and bio-

curity measures intended to reduce stress and disease risk. Handling for weighing and routine management was limited to what was necessary for the conduct of the trial and data collection.

Figure 1 and **Figure 2** below show freshly harvested *Azolla pinnata* spread in open air and dried *Azolla pinnata* in storage, respectively.

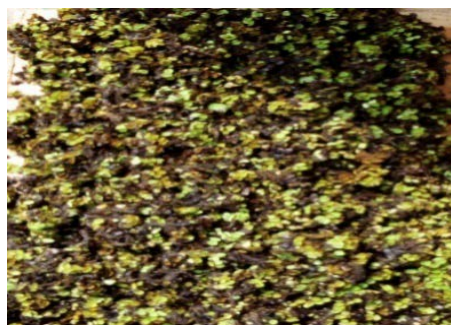


Figure 1. Fresh *Azolla pinnata* spread in open air.



Figure 2. Dried *Azolla pinnata* in storage.

The different diets are presented in **Table 2**, and their nutritional values after laboratory analysis at the Faculty Institute of Agricultural Sciences of Yangambi (IFA-Yangambi) are presented in **Table 3** below.

Table 2. Formulation of starter and grower diets (g/100g).

Ingredient (g)	Starter				Grower			
	T0	T1	T2	T3	T0	T1	T2	T3
Maize grain	46.5	46.5	46.5	46.5	56.5	56.5	56.5	56.5
Rice bran	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7
Palm kernel cake	10.43	10.43	10.43	10.43	19.43	19.43	19.43	19.43
Fish meal	7.8	7.8	7.8	7.8	6.8	6.8	6.8	6.8
Soybean	27.8	26.41	25.02	23.63	9.8	9.31	8.82	8.33
<i>Azolla pinnata</i>	0.00	1.39	2.78	4.17	0.00	0.49	0.98	1.47
NaCl	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Bone meal	1	1	1	1	1	1	1	1

Continued

Snail shell	0.51	0.51	0.51	0.51	0.51	0.51	0.51	0.51
Mineral-vitamin premix	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Total	100	100	100	100	100	100	100	100

T0: control diet; T1: diet containing *Azolla pinnata* at a level equivalent to replacement of 5% of the soybean meal; T2: diet containing *Azolla pinnata* at a level equivalent to replacement of 10% of the soybean meal; T3: diet containing *Azolla pinnata* at a level equivalent to replacement of 15% of the soybean meal; Mineral-vitamin premix: mineral and vitamin supplement.

Table 3. Nutritional values of the starter and grower diets.

Nutritional value	Starter				Grower			
	T0	T1	T2	T3	T0	T1	T2	T3
MS (%)	94.80	93.22	94.20	92.22	96.22	94.15	93.20	93.25
CB (%)	5.1	6.1	6.6	6.9	6.6	6.9	7.1	7.7
P.B (%)	23.39	23.10	22.90	22.88	17.40	17.10	17.88	17.56
M.G (%)	6.80	5.20	4.20	4.00	6.80	5.20	4.20	4.0
Glucide (%)	59.51	58.82	60.5	58.44	65.42	64.95	64.02	63.99
Mm (%)	3.3	2.8	3.0	3.0	2.9	3.1	2.8	3.2
Ca (mg/kg)	0.099	0.079	0.022	0.025	0.07	0.09	0.05	0.08
P (mg/kg)	0.35	0.25	0.25	0.27	0.31	0.29	0.36	0.38
ENA (%)	56.21	56.02	57.5	55.44	62.52	61.85	61.22	60.79

MS: dry matter; H: moisture; P.B: crude protein; C.B: crude ash; M.G: crude fat; Ca: calcium; P: phosphorus.

Statistical analysis

Data were entered in Microsoft Office Excel 2010 and analyzed at a 5% significance level. Descriptive statistics (mean, standard deviation, and coefficient of variation) were calculated for all outcomes. Mean body weight measured repeatedly over weeks was analyzed using repeated-measures ANOVA to test the main effects of treatment, week, and treatment \times week interaction. Mean weekly feed intake was analyzed using the Friedman test as a non-parametric repeated-measures comparison across weeks; when the global Friedman test was significant, Wilcoxon pairwise comparisons with Bonferroni adjustment were used to identify which treatment pairs differed. Average daily gain and overall feed conversion ratio were compared among treatments using the Kruskal-Wallis test. In addition, mixed-effects analyses were used for longitudinal relationships involving repeated weekly observations and covariates, particularly to model mean body weight as a function of feed intake, week, treatment, and their interactions while accommodating for intra-cage variability. The association between feed intake and mean body weight, and the association between feed conversion ratio and week, were also described

by correlation/regression analysis for interpretation of direction and magnitude of trends.

3. Results

The study results, including feed intake, weight gain, and feed conversion ratio (FCR), are presented in the tables and figures below.

3.1. Feed Intake

Total and mean feed intake during the study period are presented in **Table 4**.

Table 4. Total and mean feed intake by treatment (g).

	Treatment			
	T0	T1	T2	T3
Total	208,987.5	215,809.5	181,213.0	199,630.5
Mean	10,449.4	10,790.5	9060.7	9981.5
SD	7777.7	7937.6	7572.4	8553.8
CV (%)	74.4	73.6	83.6	85.7

Total denotes cumulative feed intake across all cages within each treatment; mean denotes feed intake per bird within each treatment. Abbreviations: SD, standard deviation; CV, coefficient of variation.

As shown in **Table 4**, mean feed intake was $10,449.4 \pm 7777.7$ g in T0, $10,790.5 \pm 7937.6$ g in T1, 9060.7 ± 7572.4 g in T2, and 9981.5 ± 8553.8 g in T3. Coefficients of variation greater than 30% indicate marked heterogeneity in feed intake across treatments.

The Friedman test showed a significant overall difference in mean weekly feed intake across the study period ($p < 0.0001$), but no significant pairwise differences were detected after Bonferroni correction. The week-by-treatment interaction was not significant ($p > 0.05$; adjusted $R^2 \approx 0.9494$).

Overall, feed intake increased by approximately 1285 g per week, with no significant difference in the rate of increase among treatments (**Figure 3**).

Figure 4 shows a positive association between mean feed intake and mean bird body weight during the study period, independent of treatment (coefficient ≈ 0.092 , $p < 2 \times 10^{-16}$; treatment effect, $p > 0.05$). The treatment–mean weight interaction was not significant ($R^2 \approx 0.94$), indicating that feed intake predicted mean body weight independently of treatment.

This relationship indicates that each additional gram of feed intake was associated with an increase of approximately 0.092 g in mean body weight.

Mixed-effects analysis confirmed significant effects of feed intake and week on mean body weight while accounting for repeated observations over time; interaction effects were weak or not significant.

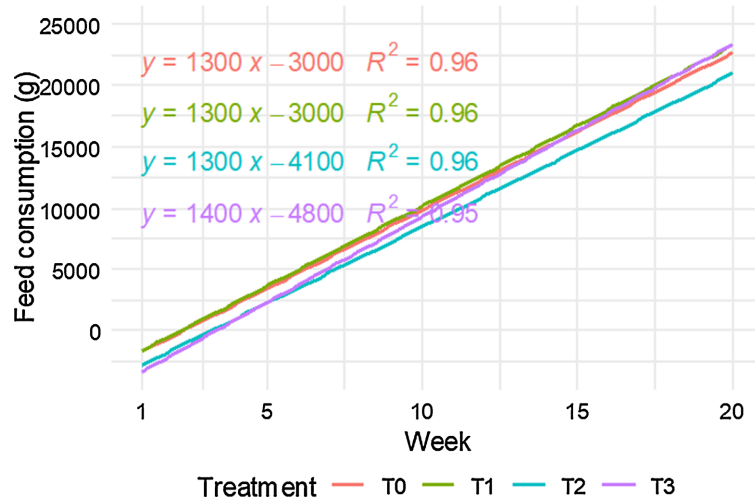


Figure 3. Weekly mean feed intake by treatment during the study period, showing an overall increase over time.

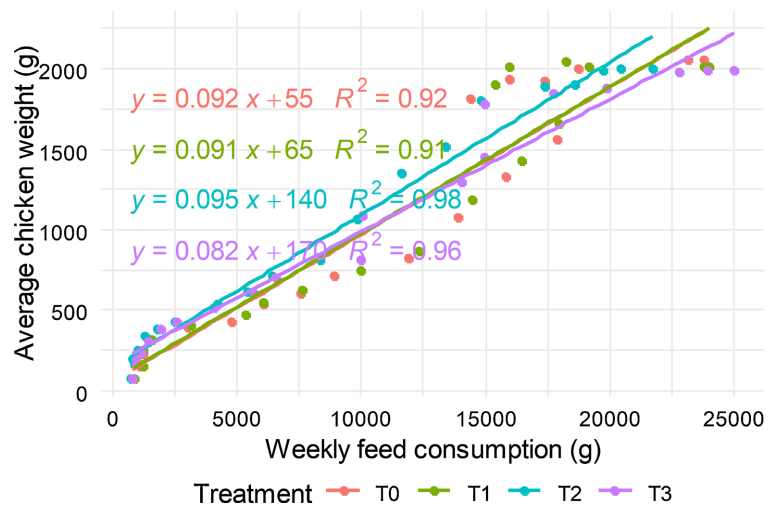


Figure 4. Relationship between mean feed intake and mean body weight during the study period, showing a positive association independent of treatment.

3.2. Growth

The effect of treatment on mean body weight during the study period is presented in **Table 5**.

Table 5. Mean body weight by treatment during the study period (g).

Statistic	Treatment			
	T0	T1	T2	T3
Mean	1012.2	1049.7	1003.4	987.2
SD	727.8	742.1	711.5	702.8
CV (%)	71.9	70.7	70.9	71.2

Mean denotes body weight per bird within each treatment. Abbreviations: SD, standard deviation; CV, coefficient of variation.

As shown in **Table 5**, mean body weight was 1012.2 ± 727.8 g in T0, 1049.7 ± 742.1 g in T1, 1003.4 ± 711.5 g in T2, and 987.2 ± 702.8 g in T3. Coefficients of variation greater than 30% indicate marked heterogeneity across treatments.

Repeated-measures ANOVA showed significant effects of treatment ($p = 2.02 \times 10^{-9}$) and week ($p = 2.43 \times 10^{-125}$) on mean body weight. The treatment-by-week interaction was also significant ($p = 1 \times 10^{-9}$), indicating that treatment effects varied over time.

The significant treatment-by-week interaction ($p = 1 \times 10^{-9}$) indicates that treatment effects on growth varied over time.

By contrast, the treatment-by-mean-weight interaction was not significant ($R^2 \approx 0.94$). Week-specific comparisons showed no significant differences at D1 and D18 ($p > 0.05$), whereas most other weeks (D2 - D17 and D19 - D20) showed significant differences of varying magnitude.

Post-hoc test results (Tukey HSD) showed that:

- 1) At week 2, only T0 and T1 differed significantly.
- 2) From weeks 3 to 7, differences were observed mainly between T0 - T1 and T1 - T3.
- 3) From week 9 onward, differences became more consistent, particularly between T1 and the other treatments.
- 4) Between weeks 13 and 15, T1 differed clearly from T2 and T3, often with strong significance.
- 5) At week 14, all pairwise comparisons were significant.
- 6) At weeks 19 and 20, significant differences persisted, especially between T0 and T3.

Overall, treatment T1 produced the best results, whereas treatment T3 generally ranked among the least effective.

3.3. Calculation of Average Daily Gain

No statistically significant difference in average daily gain ($p > 0.05$) was observed between treatments (data not shown). Averaged daily over 20 weeks, *Azolla pinnata*-based treatments did not differ from each other, although differences appeared during certain specific periods.

Summary:

- Growth was influenced by the type of *Azolla pinnata*-based ration.
- Differences between treatments appeared as early as the second week of treatment, gradually increased, and became very clear between weeks 13 and 15.
- Treatment T1 performed best and appeared most favorable for growth, whereas treatment T3 was often the least effective.
- Overall average daily gain did not show significant differences, indicating that differences were concentrated in specific rearing phases.

3.4. Mean Feed Conversion Ratios

Mean feed conversion ratios over the entire trial period are presented in **Table 6**.

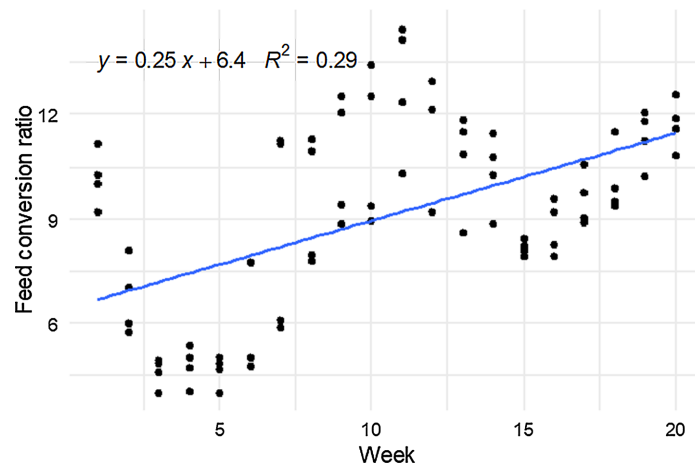
Table 6. Mean feed conversion ratio by treatment during the study period.

Statistic	Treatment			
	T0	T1	T2	T3
Mean	9.79	9.80	7.91	8.78
SD	2.82	2.79	2.29	2.68
CV (%)	28.76	28.46	28.96	30.49

Mean denotes feed conversion ratio per bird within each treatment. Abbreviations: SD, standard deviation; CV, coefficient of variation.

Table 6 shows that the mean feed conversion ratio was 9.79 ± 2.82 in T0, 9.80 ± 2.79 in T1, 7.91 ± 2.29 in T2, and 8.78 ± 2.68 in T3. Coefficients of variation were below 30% in T0 and T1, reflecting relatively homogeneous data, whereas it was above 30% in T3, indicating heterogeneity. The Kruskal-Wallis test did not show a significant difference among treatments ($p = 0.0605$), despite different mean values.

The correlation between feed conversion ratio and week was positive and moderate ($r = 0.51$, $p = 1.33 \times 10^{-06}$). Feed conversion ratios increased slightly over the weeks, but the change was small, as shown in **Figure 5**.

**Figure 5.** Relationship between feed conversion ratio and week during the study period, showing a moderate positive correlation and a slight increase over time.

4. Discussion

Incorporating different proportions of *Azolla pinnata* to replace soybean meal at levels of 5%, 10%, and 15% of the soybean meal fraction into the diets of F3 hybrid Black Australorp chickens produced variable effects depending on the rearing phase. Treatment T1 (5%) showed better performance, particularly between weeks 13 and 15, which corresponds to an active growth period. Feed intake increased significantly with age (≈ 1285 g/week), reflecting a normal physiological change in requirements.

Compared with many *Azolla pinnata* feeding trials conducted in commercial

broilers or standard layer strains, our experiment evaluated F3 hybrid Black Australorp birds under Kisangani (DRC) conditions, addressing a local feed-cost constraint and potentially different baseline growth dynamics [12] [13]. In addition, we tested *Azolla pinnata* as a true soybean-meal substitute (not merely a supplement) at graded inclusion levels representing replacement of 5%, 10%, and 15% of the soybean meal fraction, which allows a direct comparison with soybean-replacement approaches reported elsewhere [14]-[16]. The trial also covered a longer window (20 weeks: 6 weeks brooding + 14 weeks growth) than typical broiler studies, enabling us to identify phase-specific responses (notably clearer treatment separation around weeks 13 - 15) [12] [17]. Finally, by analyzing weekly trajectories and treatment-by-week effects rather than relying only on end-point means, we show that *Azolla pinnata* responses can be time-dependent, and our overall interpretation (benefits at low-to-moderate inclusion and potential efficiency penalties at higher inclusion, likely linked to fiber) aligns with broader recent syntheses [10] [18] [19].

Although T1 had the highest mean intake, differences among treatments were not significant after statistical correction, suggesting high variability in the data (CV > 30%). A positive correlation between intake and weight gain confirms that *Azolla pinnata* may improve palatability and/or digestibility of the diets [10] [17] [15].

These results are consistent with those of Ouedraogo *et al.* [14] and Alalade and Iyayi [20], showing that *Azolla pinnata* can partially replace soybean meal (up to 10%) without negative effects on growth [15] [18]. However, high inclusion levels may reduce digestibility because of crude fiber content [10]. Other studies [12] [17] [18] also confirm that gradual incorporation of *Azolla pinnata*, rich in protein (20% - 30%), can significantly improve chicken body weight.

Regarding feed conversion ratio (FCR), the trend was slightly higher in T1 (9.80 ± 2.79), with no significant differences between treatments ($p = 0.0605$). The gradual increase in FCR with age ($r = 0.51$; $p < 0.001$) reflects declining feed efficiency toward the end of the cycle. These observations are consistent with those of Desole [21], who emphasized that moderate *Azolla pinnata* supplementation improves feed efficiency, while an excess can reduce it [10] [18]. FCR obtained in our study are comparable to those reported for local chickens in growth studies in Burkina Faso [22], but higher than those reported for local chickens in Cameroun [9].

Overall, the effect of *Azolla pinnata* appears to depend on the physiological stage, with a more pronounced impact during the active growth phase.

This study has several limitations that should be considered when interpreting the results. First, the trial was conducted at a single site, which may limit the generalizability of the findings to other production environments. Second, only one genetic line of F3 hybrid Black Australorp chickens was evaluated, so the response to *Azolla pinnata* meal may differ in other genotypes. Third, the high within-group variability observed for several traits reduces the precision of treatment comparisons. Finally, the study was restricted to the growth phase and did not assess

laying performance or economic outcomes; therefore, the practical value of *Azolla pinnata* substitution under production conditions should be confirmed in broader and longer-term studies.

5. Conclusions and Recommendation

This study aimed to evaluate the effect of *Azolla pinnata* meal on the zootechnical performance of F3 hybrid Black Australorp chickens during the growth stage. The results show that including *Azolla pinnata* up to 15% does not have a depressive effect on growth or feed conversion ratio, although the best performance was observed at an inclusion rate of 5%.

Thus, *Azolla pinnata* can be considered an alternative protein source to soybean meal in poultry feeding, provided that its inclusion is limited to moderate levels (<10%) [10] [15] [18] during the growth phase.

We suggest that further research be conducted over longer periods, including the laying phase [13] [16], since the present work was limited to the growth stage.

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Conflicts of Interest

The authors declare no conflicts of interest.

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