Scientific Research Publishing

Open Journal of Animal Sciences, 2026, 16(1), 47-59
https://www.scirp.org/journal/ojas
ISSN Online: 2161-7627
ISSN Print: 2161-7597

Azolla pinnata: Composition and Effect by Substitution of Cottonseed Cake on Growth Performance of Crossbred Piglets (Large White × Landrace) in Karusi, Burundi

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How to cite this paper: Constantin, N., Albert, I., Anitha, D., Melance, N., Ntirandekura, J.-B., Napoléon, M., Adrien, K., Joseph, B. and Felix, K. (2026) *Azolla pinnata*: Composition and Effect by Substitution of Cottonseed Cake on Growth Performance of Crossbred Piglets (Large White × Landrace) in Karusi, Burundi. *Open Journal of Animal Sciences*, **16**, 47-59.
https://doi.org/10.4236/ojas.2026.161004

Received: August 17, 2025
Accepted: December 16, 2025
Published: December 19, 2025

Abstract

The main objective of this study was to assess the nutritional composition of *Azolla pinnata* and evaluate its effect on growth performance in piglets when used as a partial substitute for cottonseed cake under practical farm conditions in Karusi, Burundi. A total of 16 weaned crossbred piglets (Large White × Landrace), aged two months, and were randomly allocated into four treatment groups. The piglets in the control group were fed with concentrate feed alone; piglets in Treatment 1 were supplemented with 3% *Azolla*, Treatment 2 with 6% *Azolla* supplement, and Treatment 3 with 9% *Azolla*. The trial was conducted for 45 days. Piglets were weighed at regular intervals of 3 days and the last 4 days throughout the 45-day experimental period. The analysis of crude protein (CP), fat (F), and crude fiber (CF) was conducted at the Laboratory for Soil and Agro-Food Products Analysis (LASPA) of ISABU while the analyses of moisture (M), dry matter (DM), organic matter (OM), and ash were carried out at the National Veterinary Laboratory (LNV) in Bujumbura. A one-way analysis of variance (ANOVA) was performed to test whether different levels of *Azolla* inclusion significantly influenced measured parameters

such as weight gain, feed intake, and feed conversion ratio. A linear mixed-effects model was created. The nutrient compositions were Dry Matter 85%, Crude Protein 31.6%, fat 9.55%, CF 15.3%, Organic Matter 87.4, Moisture 15% and Ash 12.6%. The difference in feed intake between treatments was not statistically significant at the 5% level. The Average Daily Gain and FCR showed a significant difference ($p < 0.05$) among the groups. Treatment 2 with 6% *Azolla* supplement showed superior performance compared to the other treatments. Mixed-model analysis (random effect on piglet) showed that body weight increased significantly over time, with a clear effect from Day 22 onwards ($p < 0.001$). Treatment T3 resulted in a significant reduction in average body weight compared to the control (T0), while Treatment T2 showed a positive interaction with time, suggesting improved growth at certain days. No significant effect was observed for Treatment T1. No overall significant Day × Treatment interaction was detected.

Keywords

Azolla pinnata, Nutritional Composition, Weaned Piglets, Growth Performance, Burundi

1. Introduction

Azolla pinnata is a fast-growing, free-floating aquatic fern belonging to the family *Azolla*ceae, known for its symbiotic relationship with the nitrogen-fixing cyanobacterium *Anabaena azollae*. This association enables *Azolla* to fix atmospheric nitrogen, making it highly productive in low-input environments and an attractive bioresource for integrated farming systems [1] [2].

Nutritionally, *Azolla pinnata* contains 20% - 30% crude protein (on a dry matter basis), alongside essential amino acids (lysine, methionine), vitamins (notably vitamin A and B12), and minerals such as calcium, phosphorus, iron, and potassium [3]-[9]. It also provides dietary fiber (15.49% - 16%) and has low levels of anti-nutritional factors, which makes it suitable for various dietary [4] [9]. Also, the *Azolla* meal had no deleterious effect on the palatability of broiler [4].

While *Azolla* is primarily explored in animal nutrition, emerging research supports its potential in human nutrition, especially in combating protein and micronutrient deficiencies. It has been proposed as an ingredient in fortified biscuits, soups, and health supplements due to its high iron and β-carotene content [10] [11]. *Azolla caroliniana* is a high-yielding plant with great potential for cultivation and domestication [11]. However, the use of *Azolla* for human consumption is limited by its high total polyphenolic content (TPC) [10] [12]. It requires further processing and formulation research [10] [11] [13]-[15].

In animal feeding, *Azolla* has been tested as a substitute for conventional protein sources across several species. Several studies showed that *Azolla* can be fed to animals like cow, buffalo, sheep, goat and rabbit due to its easy digestibility (due to its high protein and low lignin content), increases feed efficiency, average daily

gain of animals, and milk production by 15% - 20% [16]-[18]. In poultry, *Azolla* can replace 5% - 15% of standard concentrate diets without adverse effects on growth or egg production [19] [20]. The inclusion of *Azolla* meal up to 7.5% in the diet of laying hens can enhance laying performance and egg quality traits while lowering feed costs [7] [21] [22].

In fish farming, *Azolla* is used fresh or dried at 10% - 50% inclusion in fish meal replacement with positive effects on weight gain and feed conversion [3] [23]-[25]. *Azolla* can be used as a partial substitute for plant meals (25%) as the growth [25]. Feeding tilapia with *Azolla* resulted in normal hematological and biochemical functions, with insignificant differences for the measured parameters except for the red blood cell count, which significantly ($P < 0.05$) increased in fish fed *Azolla* at 20% compared to the control, with no differences from those fed at 20% and 30% [24]. Phosphorus from *Azolla* could be used in diet to sustain Nile tilapia growth and as "environmentally-friendly" ingredient to limit P loss, while providing N to the field, beneficially in tropical marshland pond where this nutrient is already limiting [3]. Others authors suggested raising Nile tilapia with *Azolla*-diets in ponds to improve growth and produce fish with a better FA composition, for the well-being of consumers [23].

In rabbit diets, substitution levels of 10% - 30% have improved growth and reduced feeding costs. It was concluded that ensiled *azolla* with agricultural roughages can be economically included in rabbits diets up to 30% without adverse effects on rabbit's performance under the local condition [26]. In ruminants, *Azolla* is typically used as a supplement rather than a direct replacement, often at 1 - 2 kgDM/day/head in fresh form to improve milk yield and animal health [27]. *Azolla pinnata* can be used as a novel initiative for protein replacement in Sahiwal female calves [5]. *Azolla* meal upto 20% level may be incorporated in goat ration for better performances and cost-effective production under Bihar condition without any adverse effect [28]. For pigs, incorporation of *Azolla* at 15% - 30% of the total diet has shown improvements in feed conversion ratio (FCR) and daily weight gain, especially when replacing costly protein sources like cottonseed cake or soybean meal [29]. *Azolla* can replace 15% concentrate mixture without affecting body weight gain. Addition of *Azolla* to the diet of the growing crossbred pigs substantially reduces feed cost as well as recurring cost per kg body weight gain and per kg pork production [30].

The substitution of expensive or environmentally unsustainable ingredients, such as cottonseed cake or soybean meal, with *Azolla pinnata* offers an economically viable and ecologically sustainable strategy for smallholder livestock producers. This is particularly important in developing countries where protein sources are scarce or unaffordable [21].

In Burundi, livestock sector faces persistent challenges related to feed shortages, high costs of conventional ingredients, and low productivity, especially in pig farming, which is commonly practiced by smallholder families. Cottonseed cake, widely used as a main source of protein, is increasingly expensive and not always

locally available. Meanwhile, *Azolla pinnata*, which thrives in Burundi's climate, remains underutilized and poorly studied despite its high potential as a low-cost alternative.

To date, there is no published data in Burundi evaluating the effects of *Azolla pinnata* as a substitute for cottonseed cake in pig diets. Yet, region such as Karusi provide favorable agro-ecological conditions for the cultivation of *Azolla*, and farmers are in urgent need of affordable, locally available feed solutions.

The present study was therefore conducted to assess the nutritional composition of *Azolla pinnata* and evaluate its effect on growth performance in pigs when used as a partial substitute for cottonseed cake under practical farm conditions in Karusi, Burundi.

2. Materials and Methods

2.1. Study Area

The study was conducted at Research Regional Station of Karusi during four months from 2024 December to 2025 March. The study location was situated at the latitude of 03˚05'3488424"N and longitude of 30˚10'1607628"E and altitude of 1560 meters above sea level (mas l) altitude. It falls under the dry central plateaus agro-ecological zone, which has an average temperature and humidity of 25˚C and 45% respectively.

2.2. Experimental Design

A Randomized Complete Block Design (RCBD) was adopted for this experiment. A total of 16 weaned crossbred piglets (Large White × Landrace), aged two months, were randomly allocated into four treatment groups (n = 4 per group). Selected piglets were dewormed one week prior treatment and supplemented with different levels of *Azolla* in their concentrate feed by partially replacing the cottonseed ingredient (Table 1).

The treatment groups were as follows:

- Control (T0): 0% *Azolla* (no supplementation).
- Treatment 1 (T1): 3% *Azolla.*
- Treatment 2 (T2): 6% *Azolla.*
- Treatment 3 (T3): 9% *Azolla.*

The feeding trial lasted for 45 days. Initial body weights were recorded prior to the start of the experiment. Subsequent weights were taken every three days and during the last four days of the trial. All piglets were fed with a standard commercial feed sourced from the local market, with the *Azolla* supplement incorporated according to treatment. Each experimental animal was identified using an ear notch for proper management and data collection. The proportions used correspond to the ISABU formulation.

2.3. Chemical Analysis of Feed Ingredients

Samples of 500 g each were collected from *Azolla*, cottonseed cake, and the

Table 1. Feed ingredients used in the experimental diet.

	T0 (%)	T1 (%)	T2 (%A)	T3 (%)
Maize flour	35	35	35	35
Wheat bran	27	27	27	27
Rice bran	15	15	15	15
Cottonseed cake	9	6	3	0
Azolla meal	0	3	6	9
Palm kernel cake	10	10	10	10
Vitamin-mineral premix	0.5	0.5	0.5	0.5
Sea salt	1	1	1	1
Bone meal	1	1	1	1
ground limestone	1.5	1.5	1.5	1.5
Total	100	100	100	100

experimental rations (R0, R1, R2, R3). These were submitted to two laboratories for proximate analysis. The analysis of crude protein (CP), fat (F), and crude fiber (CF) was conducted at the Laboratory for Soil and Agro-Food Products Analysis (LASPA) of ISABU, using standard Association of Official Analytical Chemists (AOAC) methods.

Additional analyses of moisture (M), dry matter (DM), organic matter (OM), and ash were carried out at the National Veterinary Laboratory (LNV) in Bujumbura, Burundi. The methods and official references used for each parameter were as follows:

- Crude protein (CP): Kjeldahl method (AOAC 984.13).
- Fat (F): Soxhlet extraction (AOAC 920.39).
- Crude fiber (CF): Acid and alkaline digestion (AOAC 962.09).
- Moisture (M): Oven drying at 105˚C (AOAC 934.01).
- Dry matter (DM): Calculated by subtracting moisture content from total weight.
- Ash: Incineration at 550˚C (AOAC 942.05).
- Organic matter (OM): Determined by subtracting ash content from dry matter.

All analyses were performed according to the Association of Official Analytical Chemists (AOAC) official methods.

2.4. Data Collection

Piglets were weighed at regular intervals of 3 days and the last 4 days throughout the 45-day experimental period. Similarly, the quantities of feed distributed and refusals were systematically weighed and recorded. These measurements enabled the calculation of the following zootechnical performance parameters:

- Average Daily Gain (ADG) = (Final weight – Initial weight)/Number of days.
- Total Feed Intake (TFI) = ∑ (Feed offered – Refusals).
- Feed Conversion Ratio (FCR) = TFI/ADG.

- Mortality Rate (MR) = (Number of deaths/Initial number of piglets) × 100.

2.5. Statistical Analysis

Data was analyzed using R software (version 4.5). Statistical treatment focused on the zootechnical performance indicators according to the level of *Azolla* meal inclusion in piglet diets.

- A one-way analysis of variance (ANOVA) was performed to test whether different levels of *Azolla* inclusion significantly influenced measured parameters such as weight gain, feed intake, and feed conversion ratio. This test assessed whether there was an overall significant difference among the experimental groups.
- In cases where the ANOVA revealed a significant effect ($p < 0.05$), a Tukey's post-hoc test was applied to perform multiple comparisons between group means. This test identified the specific inclusion levels at which significant differences occurred between two treatment groups.
- A linear regression analysis was conducted to evaluate the relationship between *Azolla* inclusion rate and final body weight gain. This model quantified the effect of increasing *Azolla* levels on piglet growth, by estimating the slope of the regression line (*i.e.*, the effect per percentage point of *Azolla* inclusion).
- A linear mixed-effects model was also used to account for repeated measurements on the same subjects over time. This model included both fixed effects (Day, Diet, and their interaction) and random effects (individual variability modeled as a random effect on piglet ID). The model formula was: "Weight ~ Day * Diet + (1|ID)".

All statistical analyses were performed at a 5% significance level ($p < 0.05$).

3. Result and Discussion

3.1. Nutrient Composition of Feed

The values of proximate composition of *Azolla pinnata* used in the trial period are presented in Table 2.

Table 2. Nutrient compositions of commercial feed and *Azolla pinnata*.

Nature	CP (%)	F (%)	CF (%)	M (%)	Ash (%)	OM (%)	DM (%)
100% commercial feed (Control)	11.3	4.13	6.59	10	18	82	90
3% Azola (T1)	10.4	4.08	4.52	10	31.6	69	90
6% Azola (T2)	11.5	4.28	7.84	10	34	65.4	90
9% Azola (T3)	10.8	3.96	5.27	10	16	84	90
Coton	23.5	4.39	6.95	8	20	80	92
Azolla	31.6	9.55	15.3	15	12.6	87.4	85

CP: Crude protein; F: fat; CF: Crude Fiber; M: Moisture; OM: Organic Matter; DM: Dry matter.

The nutrient compositions such as DM%, CP%, Fat%, CF%, OM, Moisture and Ash were taken into consideration for the study.

The chemical composition of *A. pinnata* sample revealed that the dry matter content was 85% which was in close agreement with the results of current study [31]. In contrast, the dry matter content was lower than the value (DM = 90.00% ± 0.77%) obtained by some authors [18] and [32].

Given the findings of many authors, the CP content of *A. pinnata* was 31.6%, higher than the CP content of microphylla and A. filiculoides [31] [33] and [16] [34]-[39]. The high crude protein was obtained by [40] was 40.83%; and ranged from 18.4% to 31.7% DM for [41].

The total ash content of *A. pinnata* (12.6%) was lower than the findings of [18] [31] (14.67%); [42] (16.2%); [41] (9.8% - 14.5%); [43] (16.20%); [32] (18.94% ± 0.31%); [36] (20.31%); [40] (17%). But higher than what was found by [44] (2%); [31] (9.20%).

The Organic Matter was 87.4%, higher than the findings of [45] (82.66%); [35] (86.93%); [32] (81.05% ± 0.44%)

The crude fiber was 15.3%, closed with [4] (15.71%); [34] (15.7%); [46] (15%); [47] (15%) but higher than the findings [42] (12.7%); [38] (13.67%); [39] (11.77%); [37] (12.5%); [36] (12.02%); [35] (2025) (14.5%); [40] (4.63%).

The moisture content was 15% lower than the findings [47] (91.25 ± 0.87 - 91.81 ± 0.03%) in *Azolla Pinnata* and carolinniana; [44] (91.77% - 92.25%) in four species of *Azolla*, namely *A. microphylla*, *A. filiculoides*, Wrong finger, and a TNAU hybrid.

The fat content was 9.55% higher than the findings [44] (0.6% - 1.8%).

The nutrient contents of commercial feed were 90% Dry matter, 11.3% crude protein, 6.59%crude fiber, 10% Moisture, 4.13% fat, 82 Organic matter and 18% Total ash.

3.2. Feed Intake, Body Weight Gain and Feed Conversion Ration

Table 3 summarizes the zootechnical performance of piglets under different dietary treatments.

Table 3. Feed intake, weight gain, final weight and feed conversion ratio by treatment group.

Treatment	Feed intake (kg/day) Mean (sd)	ADG (kg/day) Mean (sd)	FW (kg) Mean (sd)	FCR Mean (sd)
T0	0.96 ± 0.19	0.11 ± 0.01	17.6 ± 1.11	8.7 ± 0.38
T1	0.94 ± 0.20	0.10 ± 0.02	16 ± 1.41	9.4 ± 0.38
T2	0.97 ± 0.22	0.15 ± 0.04	20 ± 1.83	6.4 ± 0.53
T3	0.87 ± 0.18	0.09 ± 0.02	13.8 ± 0.10	9.6 ± 0.20

ADG = Average Daily Gain; FCR = Feed Conversion Ratio; FW = Final Weight.

Over the entire experimental period (Day 1 to Day 45), statistical analysis showed that T0 and T2 diets were the most consumed, with average daily feed intakes of 0.96 ± 0.19 kg and 0.97 ± 0.22 kg per animal, respectively. However, the

difference in feed intake between treatments was not statistically significant at the 5% level.

Indeed, the inclusion of *Azolla pinnata* meal resulted in a non-significant decrease in feed intake for piglets in Treatment 3, whereas feed consumption in Treatment 1 and Treatment 2 remained like the control group (T0).

Table 3 presents the results for Average Daily Gain (ADG) of piglets fed different diets. The integration of *Azolla pinnata* meal into the feed significantly improved ($p < 0.05$) the average daily gain of weaned piglets throughout the experimental period, particularly in Treatment 2, which showed superior performance compared to the other treatments.

The highest ADG was in Treatment 2 and the lowest was in treatment3. This result was in contrast with the findings of [31] [48] who reported that the lowest ADG was observed in Control group. It is also in contrast with the findings of [49]. This could be due to differences in the feeding rate, types of feed consumed, breeds, and temperature and health conditions of the piglets [31].

The Average Daily Gain showed a significant difference ($p < 0.05$) among the groups which closed with the results of [31]. The piglets in Treatment 2 had significantly higher growth performance than the other groups. [49] also reported that there was increase in milk yield of cows by 20.96% when supplemented with *A. pinnata*.

The low and high FCR were observed respectively in treatment and control by [31] [50] but it was in contrast with the findings of [51] who reported that the FCR was low in Control group (100% commercial feed). This could be due to difference in the type of commercial feed fed to the piglets and the breed of piglets used for the experiment [31] [50].

3.3. Effect of *Azolla pinnata* on the Whole Zootechnical Parameters

FCR decreases when the increase in growth rate (ADG) is proportionally greater than or equal to feed intake, indicating better feed utilization. A low feed conversion ratio (FCR) reflects an efficient balance between growth performance and feed consumption.

The 6% *Azolla* inclusion group recorded the highest average daily gain (0.15 ± 0.04 kg/day), with a feed intake ($\approx 0.97 \pm 0.22$ kg/day) comparable to the other groups. This resulted in a lower FCR, indicating greater feed efficiency.

In contrast, the control and T1 groups had lower average daily gains despite similar feed intake levels, leading to higher FCR values, reflecting lower efficiency.

The T3 group showed a slightly different pattern, with the lowest feed intake ($\sim 0.87 \pm 0.18$ kg/day) and a moderately reduced ADG ($\sim 0.09 \pm 0.02$ kg/day), resulting in an intermediate FCR.

3.4. Modeling the Impact of Dietary Treatments on the Body Weight Growth of Piglets

The mixed-model analysis (random effect on piglet) shows that body weight in-

creases significantly over time ($p < 0.001$ from day 22 onwards) (Figure 1). Treatment T3 leads to a significant reduction in the average body weight of piglets compared to the control (T0), while Treatment T2 shows positive interactions with time, suggesting improved growth at certain days. No significant effect is observed for Treatment T1.

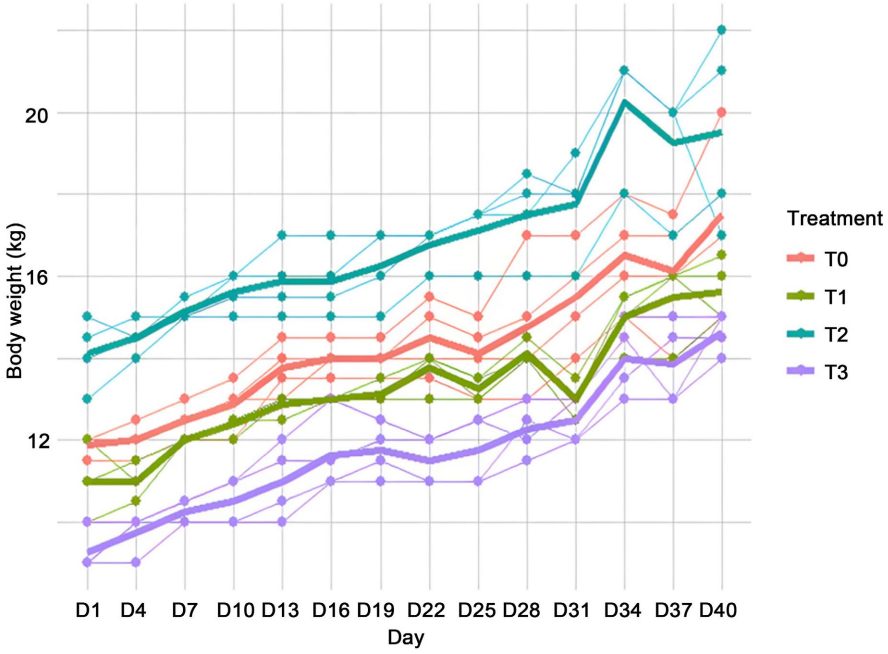

Figure 1. Evolution of the body weight growth by treatment.

3.5. Effect of Dietary Treatments on Piglet Mortality Rate

A total of 16 crossebred piglets were used in the experiment over a period of 45 days. The same number of animals was maintained until the end of the study, and no mortality was recorded in any of the four treatment groups throughout the experimental period.

4. Conclusions

This study evaluated the nutritional composition of *Azolla pinnata* and its effect as a substitute for cottonseed cake on the growth performance of weaned piglets in Burundi. Sixteen piglets were allocated to four dietary groups containing 0%, 3%, 6%, or 9% *Azolla* and monitored over 45 days for feed intake, weight gain, body weight, and feed conversion. The interaction between days and dietary treatments on growth performance was also assessed.

The findings show that *Azolla pinnata*, owing to its protein content, can be incorporated into piglet diets. In particular, the 6% inclusion level significantly improved average daily gain and feed conversion ratio compared to the control. These results suggest that *Azolla pinnata* represents a promising alternative feed ingredient for pig production in Burundi, with potential for broader application if its local production is scaled up.

A limitation of this study is the relatively small sample size (n = 4 per group), which may reduce the statistical power of the conclusions. Future studies with larger cohorts are recommended to confirm and extend these findings.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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