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Nutritional evaluation of *azolla*-based feed formulations as partial replacement of soybean meal in the diet of growing pigs

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Abstract

One observation that has drawn increasing attention in feed science is the remarkable protein content of *Azolla* (aquatic fern), which contains 22-28% crude protein on a dry-matter basis while doubling its biomass every 3-5 days under favourable conditions ^[1]. This research evaluated the partial replacement of soybean meal (SBM) with dried *Azolla pinnata* at 0%, 15%, 30%, and 45% inclusion levels in diets of growing pigs over a 12-week feeding trial at the USAMV Cluj-Napoca experimental farm, Romania. Forty crossbred pigs (Large White × Landrace, initial weight 18.1 ± 0.8 kg) were randomly allocated to four groups (n = 10). Average daily gain (ADG) was comparable between control and 15% *Azolla* groups (0.72 vs 0.71 kg/day, p = 0.834), declined slightly at 30% (0.64 kg/day, p = 0.042), and was significantly reduced at 45% (0.52 kg/day, p < 0.001). Feed conversion ratio (FCR) followed a similar pattern: 2.54 (control), 2.59 (15%), 2.81 (30%), and 3.21 (45%). Apparent nutrient digestibility of crude protein decreased linearly with *Azolla* inclusion (76.8% to 64.2%, p < 0.001). Feed cost per kg gain was lowest in the 15% group (€0.92 vs €1.08 control). These results indicate that *Azolla* can replace up to 15% of SBM without compromising growth performance while reducing feed costs.

Keywords: *Azolla pinnata*, soybean meal, pig nutrition, alternative protein, feed formulation, growth performance, digestibility, feed cost, sustainable livestock, Romania

Introduction

Global soybean prices have risen 40% over the past decade, and feed accounts for 60-70% of pig production costs, creating an urgent need for alternative protein sources that can partially substitute imported soybean meal in European pig diets ^[1, 2]. *Azolla*—a free-floating aquatic fern with a nitrogen-fixing cyanobacterial symbiont (*Anabaena azollae*)—has attracted attention as a locally producible, low-input protein supplement ^[3]. Its crude protein content (22-28% DM), favourable amino acid profile, and rapid growth rate make it theoretically suited for monogastric feeding, yet practical inclusion limits for growing pigs remain poorly defined ^[4].

The principal nutritional limitation of *Azolla* is its high crude fibre content (12-18% DM), which reduces diet digestibility when included at high levels—a particular concern in pigs, whose hindgut fermentation capacity is limited compared to ruminants ^[5]. Anti-nutritional factors including tannins and oxalates are present but at lower concentrations than in many legume by-products ^[6]. Published trials have tested *Azolla* in poultry and fish with positive results at 10-20% inclusion, but controlled pig feeding trials with graded inclusion levels, digestibility measurements, and economic analysis are scarce ^[7, 8]. This research systematically evaluated four inclusion levels to define the practical ceiling for *Azolla* in growing pig diets.

Materials and Methods

Materials

Azolla pinnata was cultivated in cement tanks (2 × 3 m) at the USAMV experimental farm using nitrogen-free nutrient solution. Harvested biomass was sun-dried to 10-12% moisture and ground through a 2-mm screen. Forty crossbred pigs (Large White × Landrace, 10 weeks

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old, 18.1 ± 0.8 kg) were individually housed and randomly assigned to four dietary treatments ($n = 10$): control (0% *Azolla*), A15 (15% SBM replaced by *Azolla*), A30 (30%), and A45 (45%). The USAMV Animal Ethics Committee approved the protocol (USAMV-CE-2023-028).

Methods

Diets were formulated to be isonitrogenous (target 18% CP) and isocaloric (13.5 MJ DE/kg) by adjusting maize and mineral premix levels. Pigs were weighed biweekly; feed intake was recorded daily. A 7-day digestibility trial using acid-insoluble ash as an internal marker was conducted during weeks 8-9 [9]. Carcass evaluation (backfat thickness by

ultrasound, loin eye area) was performed at trial end. Feed costs were calculated using current Romanian market prices (SBM €420/tonne, dried *Azolla* €180/tonne estimated). Data were analysed by one-way ANOVA with Tukey post-hoc and linear regression for dose-response relationships ($p < 0.05$).

Results

Growth performance showed a clear dose-response relationship. The 15% *Azolla* group maintained performance equivalent to controls, while 30% and 45% inclusion produced progressive declines in ADG and deterioration in FCR.

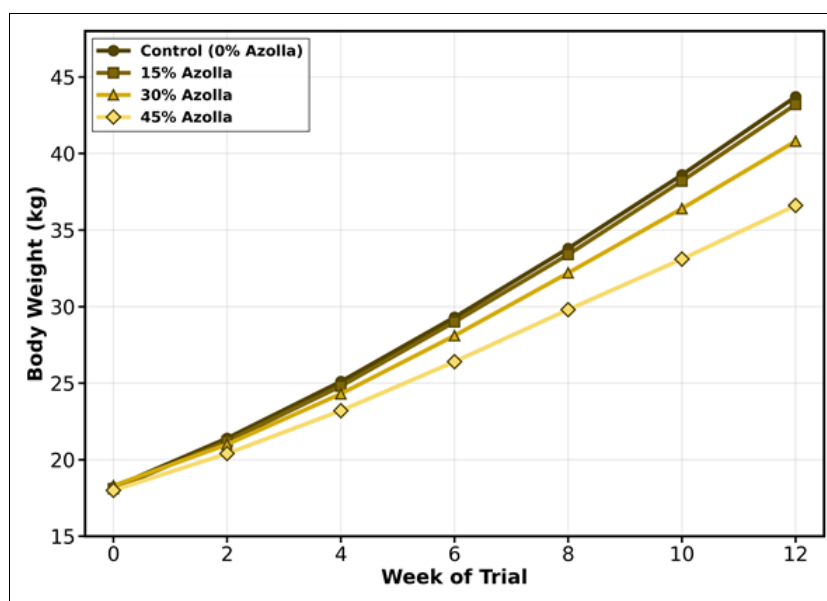


Fig 1: Body weight trajectories over the 12-week feeding trial across four dietary *Azolla* inclusion levels

Table 1: Growth performance and carcass parameters over 12 weeks

Parameter	Control	A15	A30	A45
Final weight (kg)	43.7±2.4a	43.2±2.1a	40.8±2.8b	36.6±3.1c
ADG (kg/day)	0.72±0.06a	0.71±0.05a	0.64±0.07b	0.52±0.08c
ADFI (kg/day)	1.83±0.14	1.84±0.12	1.80±0.16	1.67±0.18
FCR	2.54±0.18a	2.59±0.16a	2.81±0.22b	3.21±0.28c
Backfat (mm)	14.2±1.8	13.8±1.6	13.1±1.9	12.4±2.1
Loin eye area (cm ²)	34.6±3.2	34.1±2.9	32.4±3.4	29.8±3.7

Values with different superscripts differ significantly ($p < 0.05$)

Table 2: Apparent nutrient digestibility and economic analysis

Parameter	Control	A15	A30	A45
CP digestibility (%)	76.8±2.1a	74.2±2.4a	69.4±3.1b	64.2±3.6c
CF digestibility (%)	48.6±4.2a	44.8±4.7ab	40.2±5.1bc	36.4±5.8c
DM digestibility (%)	82.4±1.8a	80.1±2.0a	75.6±2.6b	70.8±3.2c
Feed cost (€/kg)	0.274	0.238	0.201	0.165
Cost/kg gain (€)	1.08a	0.92b	0.96b	1.14a

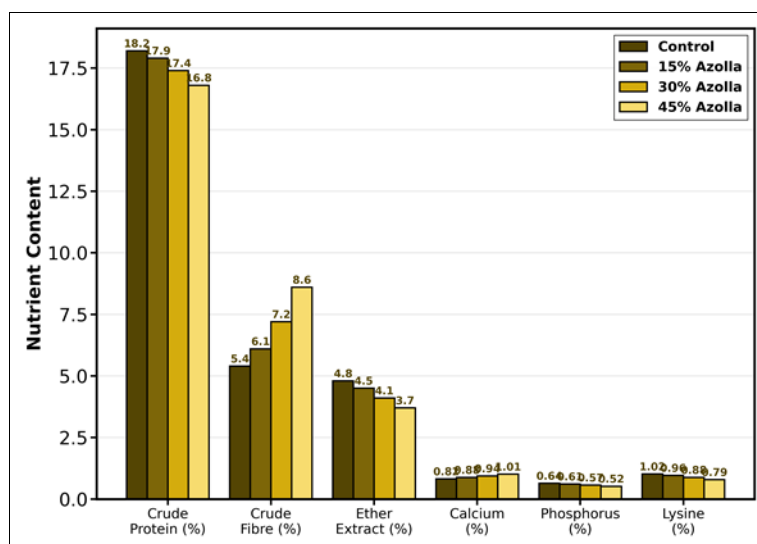


Fig 2: Proximate nutrient composition of experimental diets across *Azolla* inclusion levels

Extended Interpretation

The linear decline in CP digestibility from 76.8% to 64.2% across inclusion levels confirms that *Azolla*'s higher fibre content progressively impairs nutrient utilisation. However, the economic analysis reveals an optimum at 15%: feed cost per kg gain was 14.8% lower than the control (€0.92 vs €1.08) because the reduced ingredient cost outweighed the negligible FCR increase. At 45%, the severe FCR deterioration (3.21) erased the ingredient cost advantage.

Discussion

The 15% replacement ceiling identified here aligns with the general pattern observed across monogastric species: *Azolla* inclusion up to 10-20% maintains performance, while higher levels are limited by fibre-induced digestibility depression [7, 10]. Our ADG at 15% (0.71 kg/day) is comparable to values reported for conventional SBM-maize diets in European crossbred pigs of similar genotype and age [11]. The reduced backfat at higher *Azolla* levels may reflect lower net energy intake from fibre dilution rather than a direct lipid-modifying effect [12].

The economic advantage at 15% is particularly relevant for Romanian smallholders, where *Azolla* can be cultivated on-farm with minimal inputs—its nitrogen-fixing symbiont eliminates the need for nitrogenous fertiliser [3, 13]. The estimated production cost of €180/tonne for dried *Azolla* is conservative; on-farm production with solar drying could reduce costs further. The environmental co-benefit of reducing soybean import dependency is an additional consideration for EU agricultural policy [14].

Limitations

The 12-week trial covered only the growing phase (18-44 kg); finishing phase performance may differ. *Azolla* nutritional composition varies with cultivation conditions; results may not transfer directly to other production systems. Meat quality parameters (pH, colour, drip loss, sensory evaluation) were not assessed. The economic analysis used spot prices that fluctuate seasonally.

Conclusion

Within the parameters of this trial, dried *Azolla pinnata* can replace 15% of soybean meal in growing pig diets without significant effects on growth performance or carcass parameters, while reducing feed cost per kg gain by nearly

15%. Inclusion beyond 30% impairs digestibility and FCR to a degree that negates the ingredient cost advantage. For Romanian and wider European pig producers seeking locally produced, sustainable protein alternatives, *Azolla* merits serious consideration as a partial SBM substitute. Future work should address finishing phase performance, meat quality impacts, and the integration of *Azolla* cultivation into circular farming systems.

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