Evaluating Broiler Chickens' Acceptance of Dried *Hermetia illucens* Larvae as an Alternative Feed Ingredient

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SUMMARY

The current study was conducted to assess the acceptability of dried Black Soldier Fly (BSF) larvae by broiler chickens. One hundred fifty chickens, 75 from the control group, fed with a free-BSF larvae meal diet, and 75 from the experimental group, fed with a diet containing BSF larvae meal, were used to evaluate their preference for dried BSF larvae offered with corn and compound feed twice daily (at 9 a.m. and 3 p.m.) for 30 minutes during 20 days, from the age of 16 to 35 days, the slaughter age. During the first week of the trial, broilers, both from the control and experimental group, acted as if they were discovering this feed; they only picked it for a few seconds without ingesting it, and moved frequently to corn or the compound feed, more rarely. After one-week adaptation period, the chickens showed higher larval consumption, especially in the morning. The average amount of dried larvae consumed by the experimental group at 9 a.m. and 3 p.m. and by the control group at 9 a.m. and 3 p.m. was 137.42 g, 105.58 g, 131.37 g, and 106.00 g, respectively. There was no significant difference in the quantities of larvae consumed between the control and experimental groups, indicating that the inclusion of larvae meal in the compound feed did not influence the acceptability of dried larvae. However, in both the control and experimental groups, chickens consumed more larvae during the 9 a.m. feeding compared to the 3 p.m. These findings suggest that dried BSF larvae, a viable and sustainable alternative protein source, are well accepted and may promote animal health through the improvement of litter quality when distributed on the litter, and welfare by allowing chickens to express their natural foraging behavior.

KEY WORDS

Dried *H. illucens* larvae, Acceptability, Feed choice test, Feeding behavior, Broiler chickens.

INTRODUCTION

The use of insect larvae as an alternative protein source in poultry nutrition has gained increasing interest due to their high nutritional value and sustainability (1). Among the most promising insect species, *Hermetia illucens* (black soldier fly, BSF) larvae have been extensively studied for their rich protein (40-43%) and lipid (47%) content, making them a sustainable substitute for conventional protein sources such as soybean meal and fishmeal (2-6).
In addition to their nutritional value, BSF larvae may also provide behavioral and welfare benefits by stimulating natural foraging and exploratory behaviors in poultry. For instance, the provision oflive larvae as environmental enrichemenet has been shown to increase foraging behavior, activity levels and overall bird welfare, while reducing frustration related behaviors (7). Similarly, Ipema et al. (8) reported that broilers accepted both live and dried BSF larvae, although live larvae were more attractive due to their movement, which was associated with better litter quality and a reduction in footpad dermatitis and hocks burns. Although these studies highlight the welfare potential of larvae when used as an enrichment strategy, the present research focuses specifically on evaluating broilers' acceptance of dried larvae when offered as a feed ingredient.
In fact, compared to the meal form, which has been shown to improve poultry growth performance, gut health and overall health (6, 9-13) or to the inclusion of whole BSF in chicken diets (14-16), less is known about the acceptability of whole dried *Hermetia illucens* larvae in broiler chickens diets. In this regard, the successful use of dried insect larvae as a protein supplement in poultry production depends not only on their nutritional composition but also on their acceptability by birds. Therefore, feed preference tests are essential to assess whether broilers willingly consume larvae when given a choice between conventional feedstuffs, such as maize and commercial concentrates (17,18). Understanding feed preferences is crucial for optimizing feeding strategies and ensuring the effective utilization of insect-based protein sources in poultry farming, as factors such as palatability, texture and visual characteristics can influence intake (19). Therefore, evaluating broilers' acceptance of dried

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Hermetia illucens larvae provides key information on their suitability and practical applicability in commercial feeding systems.

Given the above-mentioned background, this study aims to evaluate the feeding behaviour and acceptance of dried BSF larvae in broiler chickens through a choice-feeding test, in which the larvae were offered with corn and compound feed in linear feeders. The goal was to assess the chickens' acceptance and interest in dried BSF larvae. Demonstrating the acceptability of dried larvae would support their potential future use not only as sustainable protein source in broiler diets but also pending further investigation as an enrichment material when distributed in the litter.

ANIMALS AND MANAGEMENT

Ethical approuvement

The experimental trials were approved by the Official Animal Care and Use Committee of the National Institute of Agronomy of Tunisia (Protocol No. 05/15).

Experimental desig

The present study utilized animals from a previously conducted trial investigating the impact of the incorporation of Black Soldier Fly (BSF) larvae meal into broiler diets on growth performance, carcass quality, meat quality, and cecal microbiota, where the experimental design was described in detail (20). Briefly, a total of three hundred unsexed one-day-old Arbor Acres broiler chicks were randomly distributed into two isonitrogenous and isoenergetic dietary treatments, designed to meet age-specific nutrient requirements of broilers in three phases, that is, starter (d1-14), grower (d15-28), and finisher (d29-35) diets. Each of the 2 treatment groups consisted of 6 pens as replicated with 25 chicks per pen. The control diet was based on maize and soybean meal, while in the experimental diet, soybean meal was partially substituted with 5% BSF larvae meal in the starter diet and with 10% in the grower diet, while the finisher diet was a BSF-free diet. The chicks were raised according to the husbandry guidelines provided by Aviagen, Inc. (Experiment I).

The feeding behavior trial was conducted on a sub-population of 150 chicks from Experiment I, using three pens (n = 75) from the experimental group and three pens (n = 75) from the control group in order to evaluate if a prior exposure to BSF meal did affect the chickens' later acceptance or consumption of whole dried larvae. Such an experimental approach, where behavioral observations are conducted on a representative subset of animals from a nutritional trial, has been previously applied in poultry research (21).

The chicken's feed preference was studied through a feed choice test, which consisted of distributing the same quantities of three feeds simultaneously placed in a linear feeder divided into 3 equal compartments for 30 minutes twice a day, at the same time each day (by 9 a.m. and 3 p.m.), in addition to the experimental and control diets distributed *ad libitum*.

Birds from the experimental group received whole dried BSF larvae, experimental feed, and cracked corn, and birds from the control group received whole dried BSF larvae, control feed, and cracked corn. The start time was recorded, and the 6 pens were regularly observed. The time spent eating each feed, along with any transitions from one feed to another, was also noted. Throughout the study, larvae provisioning and time records were kept by the same persons.

The trial was conducted using birds from the control and the experimental groups to determine whether a potential preference for the taste of BSF, incorporated as a meal in the experimental diets, could influence the acceptability of the larvae by chickens. While it was conducted twice a day (at 9 a.m. and 3 p.m.), to compare dried larvae consumption between morning and afternoon. The trial was conducted for 20 days, from the age of 16 days, when the chicken's beak had sufficiently developed to hold whole dried larvae until they reached the 35-day slaughter age. The amount of each feed to be fed to the broilers was determined based on the feed intake on the previous days. Otherwise, it was gradually increased when at least one of the three feeds was finished and the 30-minute trial period had not yet ended. Hence, the distributed quantities were 150 g/pen of each feed during the first 11 days, then gradually increased to 250 g/pen for 3 days, then to 350 g/pen during the last 6 days of the trial. At the end of the 30 minutes, the feeders were removed, and the refusals were weighed to calculate the feed intake of each feed.

Whole dried *H. illucens* larvae were provided by Next-Protein company, Tunisia, and all the dried larvae used in this experiment originated from the same rearing batch, and they were harvested at instar stages 4-5 (13-18 days old). The dried larvae of *Hermetia illucens* were stored in a dry, dark environment at a temperature between 15 and 20°C to prevent moisture absorption and oxidative degradation.

Statistical analysis

Data obtained from this study were analyzed using the SAS software (SAS Institute Inc., SAS® 9.4) (22). Data were tested for normality and homogeneity of variance. All values were grouped, and the mean and standard error were calculated. A one-way analysis of variance (ANOVA) in a fully randomized design using the general linear model (GLM) was performed for all parameters to examine differences between groups. Tukey-Kramer's test assessed differences between least square means (LS means). P <0.05 was considered a significant value. A student's t-test was used to compare two means. The statistical model included treatment effect, hour, feed, interactions, and random error. Only significant effects were used in the model as follows:

$$Yijklmno= \; + Ti + Hi+ Fk+T*Hl+ T*Fm+ H*Fn + \varepsilon ijklmno$$

Where: Yijklmno = measured variable; μ = overall mean; Ti = effect of treatment (C, E), Hi; effect of Hour (9 and 15); Fk; effect of feed (CC, DM, and CM); T*Hl; the interaction T*H; T*Fm; the interaction T*F; H*Fn; the interaction H*F, and εijklmno is the residual error.

RESULTS AND DISCUSSION

Broilers in the control and experimental groups showed the same feeding behavior during the 20-day feed choice test. Throughout this test, two phases of feeding behavior were observed.

Phase 1: The first week of the trial

During the first week phase of the trial, dried larvae, experi-

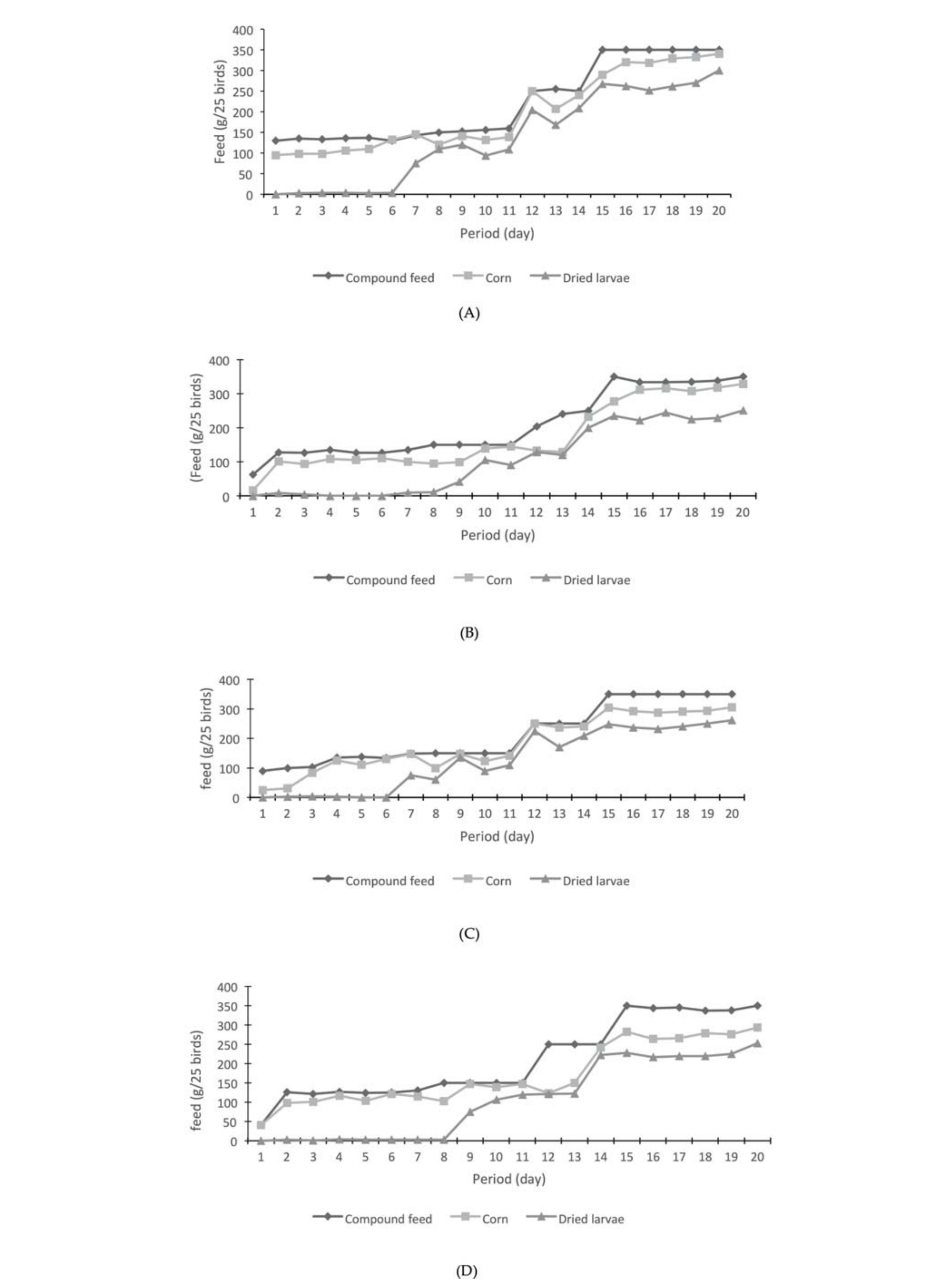

Figure 1 - Evolution of the consumption of the compound feed, corn, and dried larvae according to service hours, 9 a.m. or 3 p.m., and groups, experimental or control, throughout the trial. **(A)** Consumption of the three feeds by the experimental group at 9 a.m.; **(B)** Consumption of the three feeds by the experimental group at 3 p.m.; **(C)** Consumption of the three feeds by the control group at 9 a.m.; **(D)** Consumption of the three feeds by the control group at 3 p.m.

mental or control compound feed, and corn were distributed. Then, between 32% (8/25) and 44% (11/25) of the chickens started with the compound feed, whereas 20 (5/25) to 36% (9/25) preferred corn first. Consequently, the compound feed was the most consumed feed, followed by corn. It is also interesting to note that, in most cases, broilers that started with either compound feed or corn did not change their preferred feed for at least 15 minutes. After that, they either drank water or rested. The few alternations between feeds occurred after they had consumed the preferred feed for at least 15 minutes. At this early age, chickens did not consume dried BSF larvae (Figure 1). Chickens behaved as if they were discovering this feed; they rarely approached the dried larvae and only picked it for a few seconds without ingesting it, and then more often switched to corn and less frequently to the compound feed, more rarely. We also observed that the chickens' beaks struggled to easily grasp the dried larvae during this phase. Thus, the quantities of dried larvae consumed daily during the first 6 days did not exceed 6 g per group.

Phase 2: From the second week to the end of the trial

Larvae consumption patterns changed starting on the 7th day of the trial at 9 a.m. for both the control and experimental groups (Figures 1A and 1C) and on the 9th day at 3 p.m. (Figures 1B and 1D). At that time, strong competition for all three types of feed (corn, compound feed, and dried larvae) was observed across all six pens (3 experimental and 3 control). Previously, the competition was mainly for corn and compound feed. In this phase, the percentage of birds that first ate dried larvae ranged from 12% (3/25) to 24% (6/25) in each group, while the distribution of chickens around corn and compound feed stayed similar to the first phase. For example, on day 7 at 9 a.m., 32% (8/25) of the broilers in the first experimental pen started with corn, 32% (8/25) with compound feed, and 20% (5/25) with dried larvae. In the second experimental pen, 36% (9/25) ate corn, 40% (10/25) ate concentrate, and 24% (6/25) ate dried larvae. The third experimental pen showed 34% (8/25) of the chickens consuming corn, 36% (9/25) consuming concentrate, and 22% (5/25) consuming dried larvae. The control group showed a similar feeding behavior. In the first pen, 36% (9/25) of chickens consumed corm, 40% (10/25) consumed compound feed, and 12% (3/25) consumed dried larvae. In the second and the third pens, the respective values were 36 (9/25), and 32% (8/25) for corn, 40 (10/25) and 38% for compound feed, and 16 (4/25), and 12% (3/25) for dried larvae. In both control and experimental groups, chickens consumed dried larvae for at least 3 minutes and at most 10 minutes. For broilers that consumed dried larvae for 3 to 4 minutes, the most frequent changes were from larvae to corn and, less frequently, to the compound feed. In contrast, broilers that ingested dried larvae for 10 minutes tended to drink or rest afterward. Furthermore, some chickens that were resting or pecking in the litter came directly to consume dried larvae, a behaviour not previously observed. This behaviour continued until the end of the trial, with a few notable changes, such as (i) the time spent by chickens consuming dried larvae without switching to another feed initially being around 3 to 4 minutes, with dried larvae consumption around 100 g (Figure 1). Later, the duration of dried larvae consumption gradually increased by around 20 minutes for some birds, which in turn increased the quantities ingested, reaching a maximum of 300 g at 9 a.m. on the 20th day of the trial (the last day) for the experimental group (Figure 1. A), (ii) the number of birds starting with corn exceeded those starting with concentrate, contrary to what was observed during the first 6 days when chickens were more attracted by the compound feed.

Over the 20 days of the trial, the average daily consumption of dried larvae per group was 119 g for the control group and 122 g for the experimental group. For corn and compound feed, the average daily consumption was 180 g and 213 g for the control group, and 186 g and 215 g for the experimental group, respectively. Hence, there were no significant differences between the consumption levels of the three feeds between the control and the experimental groups. Notably, the compound feed was the most consumed feed, with average daily intake values of 213 g for the control group and 215 g for the experimental group. Corn followed closely, with an average daily consumption of 180 g and 186 g for the control and experimental groups, respectively, while dried larvae recorded consumption levels of 119 g for the control group and 122 g for the experimental group (Figure 2). The average quantities of dried larvae ingested by the experimental group at 9 a.m., the Experimental group at 3 p.m., the control group at 9 a.m., and the control group at 3 p.m. were 137 g, 106 g, 131 g, and 106 g, respectively (Figure 3). It was found that consumption levels of dried larvae were higher at 9 a.m. than at 3 p.m. in both control and experimental groups ($P<0.05$).

The results of our observations showed two different phases of feeding behaviours. During the first week, broilers were more attracted to the compound feed, likely due to its adaptation to its form, colour, and taste. In contrast, dried larvae consumption was negligible, possibly due to their dark colour and physical characteristics. Some chickens were observed touching the larvae with their beaks to appreciate their physical characteristics without consuming them, as they were discovering the feed (19). To mitigate this initial aversion, we propose crushing the larvae during the first week to facilitate consumption. Additionally, gradually mixing the crushed larvae with broiler feed may help improve acceptance, allowing birds to adapt progressively to the new feed. Future studies should investigate the effectiveness of these strategies in reducing aversion and enhancing palatability. By the second week, feeding behavior had shifted; birds distributed more evenly between the compound feed and corn feeders, and a marked preference for dried BSF larvae was observed. During the trial, chickens may have been attracted to corn due to its visually appealing yellow colour. In addition, a marked preference for dried larvae was noticed over time, which may be attributed to a gradual adaptation process, as chickens became more accustomed to maggots and learned to accept and appreciate them (23). Overall, the compound feed was the most consumed feed, followed by corn, and lastly, dried larvae. While the consumption of corn and compound feed was relatively similar, dried larvae had the lowest intake. Picard et al. (19) and Bouvarel et al. (23) have noted that feeding habits, which tend to influence the intake levels of different feeds, can explain these consumption differences. Chickens, as granivores, have an instinctive preference for consuming corn and compound feed over dried larvae. An analysis of the consumption trends demonstrated that the intake levels for all feeds converged towards the end of the trial, suggesting that insects could serve as a viable feed source for chickens. Similar results were found by Traore et al. (18), who conducted a comparable trial in Burkina Faso using 18-month-old local

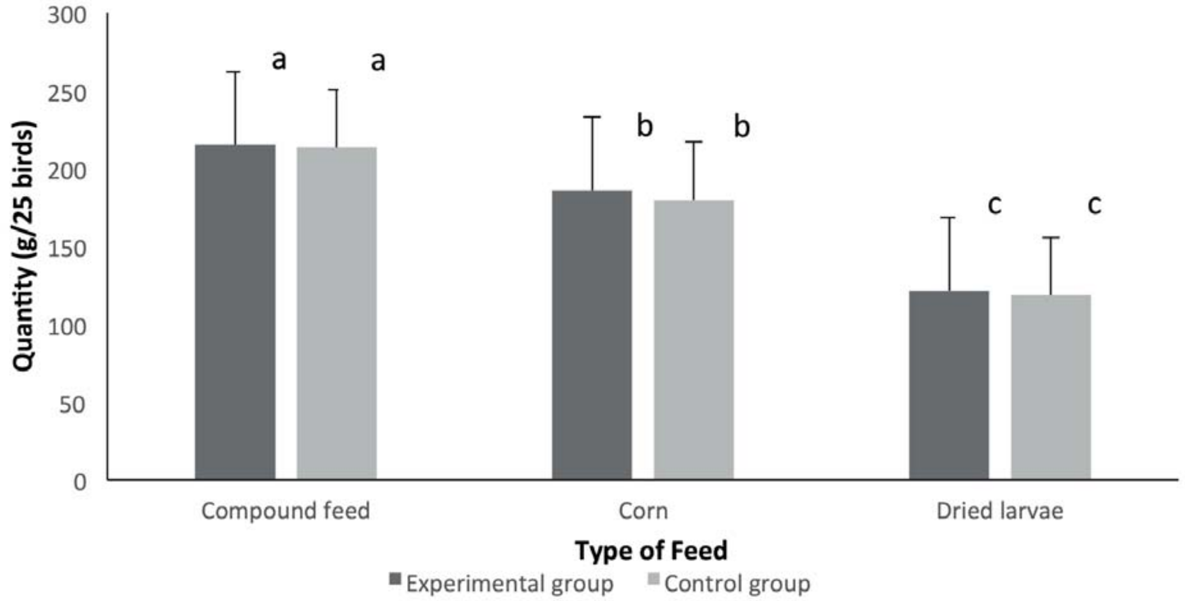

Figure 2 - Mean consumption of compound feed, corn, and dried *Hermetia illucens* larvae by broiler chickens from the experimental and control groups during the feed choice test. Bars represent mean values ± standard deviation (SD). Bars sharing the same letter are not significantly different ($p > 0.05$), whereas different letters indicate significant differences among feed types ($p < 0.05$).

chickens and dried *Musca domestica* larvae.

In both the control and experimental groups, chickens consumed more larvae during the 9 a.m. feeding compared to the 3 p.m. feeding, likely reflecting the variation in feed requirements throughout the day, which may be influenced by the circadian rhythm of broilers (7). Studies have shown that broilers exhibit daily feeding patterns and metabolic cycles that align with their circadian rhythms, affecting their feeding behavior and nutrient utilization across different times of the day (7, 24, 25). However, there was no significant difference in the quantities of larvae consumed between the control and experimental groups, indicating that the inclusion of larvae meal in the compound feed did not influence the intake of dried larvae. Therefore, this study suggests that the optimal time for supplementing dried maggots is around 9 a.m.

In the present trial, we demonstrated that broiler chickens read-

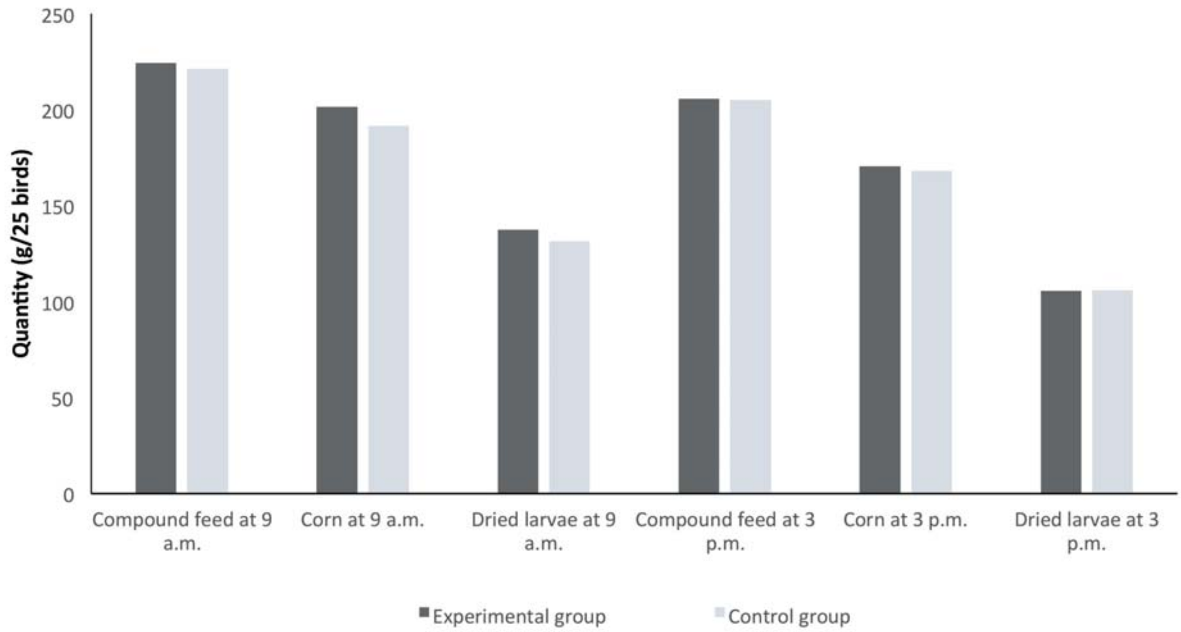

Figure 3 - Mean consumption (g/25 birds) of of compound feed, corn, and dried *Hermetia illucens* larvae by broiler chickens from the experimental and control groups during the feed choice test conducted at 9 a.m. and 3 p.m. Bars represent the mean quantity consumed, with dark gray indicating the experimental group and light gray indicating the control group. Each time point shows the preference of birds for the three feed components offered simultaneously.

ily accepted dried *Hermetia illucens* larvae when offered alongside the basal diet, indicating that such larvae can be considered a viable option for on-farm enrichment. This acceptance underpins the potential to use dried BSF larvae as a litter-scattered enrichment strategy to stimulate natural foraging behaviour. Previous research has shown that the provision of live BSF larvae scattered in the pen significantly increased foraging, standing time and general activity in broilers, thereby improving leg-health indicators such as gait score and hock burn severity (26). Given these behavioural and welfare benefits, integrating dried larvae into the litter environment may not only encourage active behaviour but also contribute to improved litter condition, reduced contact-dermatitis risk and overall bird welfare. Future studies should investigate the longitudinal effects of regular larvae provisioning in commercial-scale settings, specifically addressing broiler performance, health parameters, litter quality and the cost-benefit of enrichment deployment.

CONCLUSION

To conclude, after a one-week adaptation period, the chickens displayed a pronounced preference for dried BSF larvae, with increased consumption observed specially during morning hours. This behavior suggests that BSF larvae are both palatable and acceptable to broiler chickens when offered in addition to the basal diet. As this study focused primarily on acceptability, further trials are needed to evaluate the broader effects of dried larvae distribution on performance, health parameters, and litter quality.

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