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# Feed Preference, Daily Intake, and Laying Performance of Captive-Born Sardinian Partridges (*Alectoris barbara barbara* Bonnaterre, 1790) Offered Whole Defrosted Mealworms (*Tenebrio molitor* L., 1758) as Raw Feed Material with Diet

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**Abstract:** The competence to locate natural feeding sources is one of the main limiting factors for survival in the wild, especially for captive-born birds. Therefore, environmental enrichment through the diet can be strategic before their release into nature. In this research, a feeding trial was undertaken to evaluate the potential use of yellow mealworm (*Tenebrio molitor* L.) larvae (TM) provisions to captive bred couples of Sardinian partridges (*Alectoris barbara barbara* Bonaterre, 1790) during the laying period. Twenty-four couple-caged Sardinian breeding partridges were enrolled during the laying period (April–May 2019) and randomly allotted to two feeding groups of 12 couples each: (a) the control (CON) group was fed a conventional complete pelleted diet for laying quails; (b) the yellow mealworm enriched group was additionally fed 5% whole, defrosted TM larvae (TM5%) on top of the same amount of the control diet. As a prerequisite, partridges were unaccustomed to eating mealworms before the start of the trial. Daily feed intake (DFI), bodyweight (BW), and number of laid eggs (LE) were monitored over five weeks of experimental feeding. Partridges fed the TM5% diet displayed a higher preference for whole mealworms (first choice and complete consumption) than expressed for the CON pelleted feed. Differences in daily dry matter intake ($p = 0.028$) between CON and TM5% groups were observed (DMI: 42.6 ± 1.73 vs. 43.4 ± 1.62 g, respectively); the final body weights (BW) ($p = 0.098$) of birds in the CON group was higher than those in the TM5% group (435 ± 36.9 vs. 416 ± 36.3 g, respectively). Differences in daily energy intake relative to BW ($p < 0.001$) as well as relative to metabolic weight ($BW^{0.75}$) ($p < 0.001$) were observed between groups, but this was not followed by higher BW, probably due to the absence of grit and inaccessible nutrients and energy (larval exoskeleton). No difference in the average LE per week and egg weight was observed between CON and the enriched TM5% groups, though in the last weeks, a statistically lower number of eggs was laid in TM5% group. Our results suggest that whole yellow mealworms can be a promising feed material to broaden the spectrum of competence for natural feeding sources with similar physical form and nutritional characteristics available in the environment.

**Keywords:** partridges; survival; feeding behavior; yellow mealworm; environmental fitness

## 1. Introduction

Sedentary wild birds can be reared in captivity for a variable period of time and for different reasons. In some cases, permanent rearing is necessary, leading to captive-born and bred birds staying in rescue centers for life. In general, the release of individuals into nature follows a detailed evaluation of several factors and aims to attain different purposes, including restocking for the preservation of animal biodiversity in a given habitat, among others [1]. However, low survival rates of captive-born birds after release is a major concern, for which loss of antipredatory behavior can be one of the main reasons [2]. Rearing and feeding conditions have profound effects on captive birds, specifically during early life stages [3,4], as birds are unable to undergo developmental plasticity [5], resulting in decreased adult survival and reproduction once released [6]. Captive-bred birds are typically accustomed to confined conditions and are often habituated to humans [7]. As a result, animals may lack the behavioral patterns necessary for survival in the wild, such as the ability to identify predators, show avoidance responses [8,9], and locate and select food [10], along with flight muscle development [11]. Although released birds generally show reduced survival and breeding success, their offspring have similar odds of survival as their wild peers [12], demonstrating that conditioned rearing experiences influence physical and behavioral features [13].

Individual feeding preferences and dietary patterns are innate determinants of foraging ecology [14]. Inadequate dietary diversity may preclude critical opportunities for experience and learning. Such developmental issues originating from captive breeding lead to a high mortality rate [15,16].

The Sardinian partridge (*Alectoris barbara barbara* Bonnaterre, 1790) is a monogamous wild bird belonging to the *Phasianidae* family (Order *Galliformes*) that is widely distributed in different countries of the Mediterranean basin, ranging from northwestern Africa (Morocco, Algeria, and Tunisia) to Spain and Sardinia (Italy), where they are currently found [1]. Partridges are mostly walking birds, occasionally taking very small flights. Birds prefer sandy and dry areas and exhibit typical nesting and sheltering behavior beneath the bushes of the Mediterranean maquis. Successful captive breeding efforts over the last two decades have led the Sardinian partridge to be categorized as a species of "least concern" by the IUCN [17]. In this regard, the feeding habits of Sardinian partridges in captivity have been given particular attention [18].

Partridges are omnivores, selecting a wide range of food items according to seasonal availability, such as seeds, fibrous plant material, insects, and mollusks [18]. However, the provision of manufactured diets in captivity triggers prolonged physiological and behavioral adaptation, as well as morphological adaptation of the digestive tract [18], with effects on foraging efficiency, dietary breadth, and general fate of the released birds. An increase in dietary breadth appears crucial for captive partridges in the period before release. In view of the dietary preferences and feed selection as broad parts of foraging behavior upon learning [14], the physical form of the diet has a strong impact on the digestive tracts of birds, according to selected feedstuffs [18]: gut development; microflora composition; and, consequently, the digestibility of nutrients [19] are in fact strongly impacted.

It was hypothesized that dietary enrichment through the supply of insects would improve, by contributing to their dietary breadth and competence, the fitness of captive partridges to environment. The use of insects and their derivatives in the feed and diets of poultry and similar species in captivity is a practice that allows to integrate those feed components in the diet that normally the same species would seek in the wild. Among the many insect species used as feed around the world, the yellow mealworm (*Tenebrio molitor L.*) is one of the most widely used due to the ease of mass rearing, even on an industrial scale [20]. Usually, mature larvae are used as feed and can be offered alive or dead and more or less processed (vacuum-packed, dehydrated). Therefore, a feeding trial was set up with the aim to assess the effects of whole yellow mealworm supplementation of the conventional pelleted diet offered to captive-bred Sardinian partridges on feed preference, feed intake, and laying performance.

## 2. Materials and Methods

### 2.1. Ethics Statement

The research was conducted following the Directive of European Union 2010/63/EU on animal welfare and protection. The experiment followed standard agricultural practices, involving no invasive procedures in the handling and manipulation of animals.

### 2.2. Partridges Diet and Management

A total of 24 breeding couples (individual body weight (BW) range at start between 435 and 441 g) of Sardinian partridges were enrolled in the experimental feeding trial for five weeks during the laying period (April to May 2019). Couples were housed in cages (dimensions: W—30 cm, H—30 cm, L—40 cm) with metal wire flooring (1 × 2 cm) in an open environment with shelters, without artificial ventilation or heating system. All couples were allotted randomly to one of the two experimental feeding groups, consisting of 12 couples each. One experimental group (CON) was fed a conventional complete diet in the form of pelleted feed for laying quails and acted as control; the other experimental group (group TM5%) was fed the same conventional complete diet, with the addition of whole defrosted yellow mealworm larvae at an amount of 5% of the daily offer of the pelleted feed. The nutrient composition of the two diets are reported in Table 1. As a pre-requisite for inclusion in the trial, partridges had never been fed mealworms prior to the initiation of the study. Throughout the experiment, feed was provided ad libitum, with a daily feed offer of 100 g of pellets per couple, plus 5 g of defrosted mealworms (for TM5% group), and free access to clean drinking water.

The larvae in the trial were provided by the section of Sassari (Italy) of the Institute of BioEconomy of the Italian National Research Council (CNR), where a standard stock colony of yellow mealworm is currently being reared. The larvae stock was exclusively fed durum wheat bran and fresh vegetables as water source. Mature larvae were harvested from the feed substrate and then starved for 24 h to eliminate the residual frass contained in the gut before being deep frozen and stored at −20 °C until use, allowing a rearing protocol for their use in animal feeding, as recently authorized for domestic pigs and poultry as food-producing species [21].

### 2.3. Performance Parameters

The health status of partridges was monitored on a daily basis for the whole duration of the study. The body weight (BW) of birds was recorded at the beginning and end of the feeding trial to calculate BW variation over the experimental period. In brief, birds were weighed individually using a digital scale (precision 0.01 g, OHAUS ® PA512C) and by means of a plastic basket for which the bare weight had already been determined. For both dietary groups, CON and TM5%, the daily feed intake (DFI) was calculated by recording the difference in grams of feed offered and refused per day, as collected from the feeder. On such a basis, the daily average DM intake (DMI) and the relative DM intake to BW ($DMIBW^{-1}d^{-1}$), and the relative DM intake to metabolic weight ($BW^{0.75}$), for each couple in both groups were calculated.

### 2.4. Laying Performance

Throughout the trial, production performance was monitored on the basis of number and frequency of laid eggs per week. Couples were monitored twice a day, in the morning and evening, to collect and record egg production. The number of laid eggs (LE) was calculated by using the number of eggs produced on each day per group, divided by the number of birds, multiplied by 100. Each egg was weighed on a digital scale (precision 0.01 g, OHAUS ® PA512C) and fresh weight was recorded. Eggs were then dried at room temperature and weighed once more. Egg shells were separated from the internal content and weighed (OHAUS ® PA512C). The dry weight of the egg content was obtained by subtracting the weight of the shell from the total weight of dry eggs.

### 2.5. Proximate Analyses of the Diets

The crude fat (CFat), crude protein (CP), ash content, and minerals of the pelleted diet were determined according to the standards established by the Association of Official Agricultural Chemists (AOAC) [22]. The proximate composition of yellow mealworms was obtained from Melis et al. [23,24], in which the same batch of larvae reared with the same protocol share overall proximate composition. The chemical composition of experimental diets is shown in Table 1.

**Table 1.** Feeding plan and diets fed to partridge couples according to experimental groups (CON vs. TM5%). Nutrient composition is reported on an as-fed basis (g/kg feed).

| [2] Item | [1] Experimental Feeding | |
|---|---|---|
| | **CON** | **TM5%** |
| Ingredients (g/day per couple, as fed) | | |
| [3] CON diet (g) | 100 | 100 |
| [4] TM larvae (g) | 0 | 5 |
| Nutrient composition (g $kg^{-1}$ as fed) | | |
| DM | 890 | 865 |
| CP | 160 | 161 |
| Cfat | 25.0 | 29.4 |
| Ash | 11.0 | 11.1 |
| Ca | 8.00 | 7.70 |
| ME (MJ $kg^{-1}$ as fed) | 11.8 | 11.7 |

[1] Lasting period: 5 weeks during laying period. [2] Item: DM, Dry matter; CP, Crude protein; Cfat, Crude fat; Ca, Calcium; ME ($MJkg^{-1}$ as fed), Metabolizable energy expressed as Mega Joules per kilogram. [3] Ingredient composition of CON (g $kg^{-1}$ as fed): Corn meal = 480; Soyabean meal = 230; Barley = 150; Wheat bran = 60; Alfalfa meal = 35; Nut hull meal = 14; Limestone = 14; Dicalcium phosphate = 12; Sodium chloride = 3; Premix = 2. [4] Nutrient composition of TM larvae (g $kg^{-1}$as fed): DM = 369 *; CP = 171; Cfat = 118 *; Ash = 13 *; Ca = 1 *; ME ($MJkg^{-1}$ as fed) = 9.05 **. * (Data adapted from Melis et al. [23,24] adjusted. ** Derived from domestic poultry calculations, differences may occur for partridges.

### 2.6. Statistical Analysis

Data were analyzed using one-way ANOVA with diet as a fixed factor (CON vs. TM5%). Selected traits of bird performance were used as dependent variables. Some of those were directly determined (BW, DFI, and LE), whereas others were derived by calculation (DMI $BW^{-1}$, ME $BW^{-1}$, and same variables relative to metabolic weight $BW^{-0.75}$). Differences between means were considered significant for a $p$-value < 0.05. Minitab® (18.1.0) software was used to perform the statistical analysis. Confidence intervals and groupings were adjusted according to Tukey's method.

Selected parameters of eggs were correlated with each other to depict laying activity. Pearson's test was used for the assessment of potential correlation ($\sigma < 0.300$ = weak correlation; $0.300 < \sigma < 0.600$ = mild correlation; $0.600 < \sigma < 1.000$ = strong correlation; $+\sigma$ or $-\sigma$, positively or negatively correlated, respectively; significance for $p$-value < 0.05).

In addition, a general linear model was developed to assess the effect of the diet on laying efficiency:

$$y = \mu + D_{j,k} + G_{l,m,n,o,p} + D * G + \varepsilon$$

where $y$ is the dependent variable, $\mu$ is the overall mean, $D$ is the fixed effect of the diet (two levels, CON vs. TM5%), $G$ is the effect of time (five levels per week of experiment), $D * G$ is the interaction of factors, and $\varepsilon$ is the random error.

## 3. Results

All birds in both groups appeared healthy during the whole duration of the study. Average values for final BW ($p = 0.098$) varied between the two feeding groups, showing decreased BW in partridges fed the TM5% diet at the end of the trial in a nonsignificant way (Table 2). Expectedly, the females of each couple turned out to be lighter than males at start. However, a significant difference between genders ($p < 0.0001$) revealed females to be lighter than males (Δ = −8% of BW at the end of the experiment) in both groups. Differences in daily dry matter intake ($p$ = 0.028) between CON vs. TM5% groups were observed (DMI: 42.6 ± 1.73 vs. 43.4 ± 1.62 g, respectively). A significantly higher relative daily dry matter intake per BW ($p < 0.001$) in the TM5% fed group was recorded. Calculated indexes of relative ME energy intake (ME in feed $BW^{-1}d^{-1}$) to BW as well as to metabolic weight ($BW^{0.75}$) pointed to significant differences ($p < 0.001$) between groups. The intake of energy from the diet was higher in partridges in the TM5% group. However, higher energy intake per day led to lower average BW in partridges of the same group.

**Table 2.** Birds' performance in the two feeding groups (CON vs. TM5%).

| [2] Item | [1] Diets | | Pooled SE | *p*-Value |
|---|---|---|---|---|
| | CON | TM5% | | |
| Initial BW (g) | 441 | 438 | 4.000 | 0.887 |
| Final BW (g) | 435 | 416 | 12.00 | 0.098 |
| DFI ($gd^{-1}$) | 46.5 | 49.5 | 0.408 | 0.061 |
| DMI ($gd^{-1}$) | 42.6 | 43.4 | 0.486 | 0.028 |
| DMI $BW^{-1}d^{-1}$ (g) | 0.10 | 0.11 | 0.002 | 0.001 |
| ME $BW^{-1}d^{-1}$ (kJ) | 1.32 | 2.41 | 0.129 | <0.001 |
| ME $(BW^{0.75})^{-1}d^{-1}$ (kJ) | 5.97 | 6.36 | 0.465 | <0.001 |
| Average LE (no. $week^{-1}$) | 2.20 | 2.10 | 0.617 | 0.276 |
| Egg weight (g) | 19.0 | 19.6 | 0.010 | 0.872 |
| Egg shell weight (g) | 2.24 | 1.75 | 0.236 | <0.001 |
| Dry weight egg content (g) | 3.87 | 4.54 | 0.768 | 0.004 |

[1] Diets: CON = conventional pelleted diet; TM5% Enriched diet = 5% defrosted whole mealworms + pelleted diet; [2] Items: Initial BW = Initial body weight; Final BW = Final body weight; DFI = Daily feed intake; DMI = Daily dry matter intake; DMI $BW^{-1}$ $d^{-1}$ = Dry matter intake per body weight per day; ME $BW^{-1}d^{-1}$ = Metabolizable energy expressed as kilojoules per bodyweight per day; ME $BW^{0.75-1}$ $d^{-1}$ = Metabolizable energy expressed as kilojoules per metabolic weight per day; Average LE (no. $week^{-1}$) = Average number of laid eggs per week.

Laying activity was unaffected by the diet and no differences in the average number of eggs laid per couple per week ($p$ = 0.276) were observed throughout the experiment (Table 2). A significant reduction in egg laying between the CON and TM5% groups was observed during the last week of investigation ($p$ = 0.016), being lower for the TM5% group. The laying frequency of each couple in both groups (CON vs. TM5%) for the last week is reported in Figure 1. The average weight of eggs laid turned out to be higher in the TM5% group than in the CON group. Pearson's correlation test indicated eggshell weight to be significantly ($p$ = 0.002) and negatively correlated (σ = −0.439) with dry egg content in a moderate way (Table 3). The correlation analysis between the number of laid eggs per week and the weight of laid egg revealed a negative Pearson's coefficient (σ = −0.182) with a weak, nonsignificant, negative correlation ($p$ = 0.215).

**Table 3.** Pearson's correlation coefficients (σ) and significance in brackets ($p$-value) between laid eggs and egg weight for the two groups of partridges across the experimental period.

| | No of Eggs/Week | Egg Weight (g) | Shell Weight (g) |
|---|---|---|---|
| Egg weight (g) | −0.182<br>(0.215) | | |
| Shell weight (g) | 0.069<br>(0.643) | −0.032<br>(0.829) | |
| Dry weight content (g) | 0.059<br>(0.692) | −0.141<br>(0.339) | −0.439<br>−0.439 |

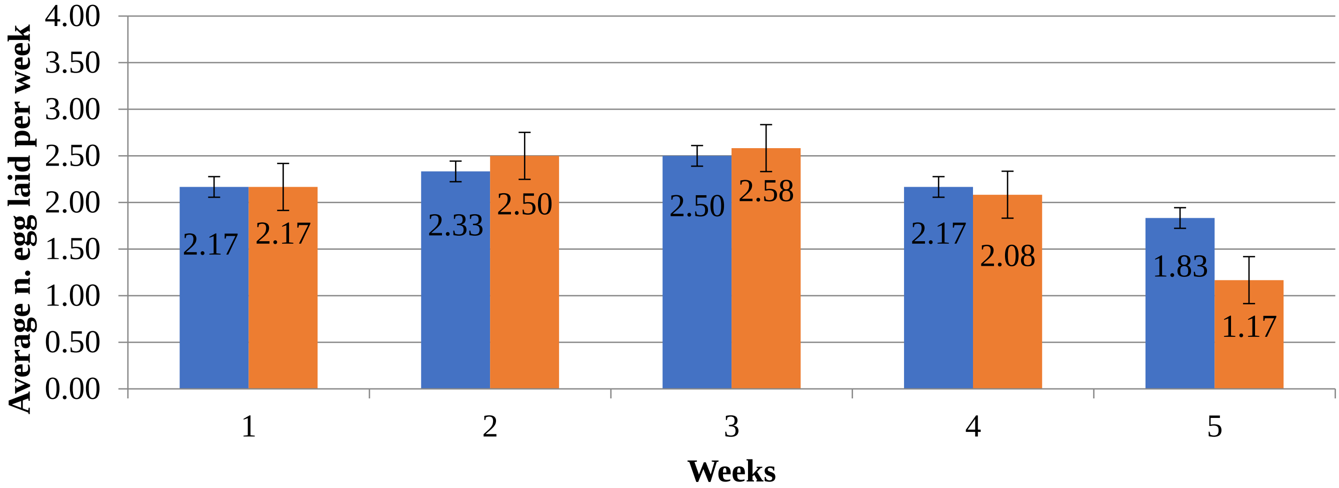


**Figure 1.** Histogram of laying efficiency in partridges of the two experimental groups (CON vs. TM5%) across the experimental period (5 weeks) Blue bars for CON and orange bars for TM5%. Average number of laid eggs is reported from 24 couples (CON = 12 vs. TM5% = 12).

## 4. Discussion

Results of the present study indicate that partridges of the TM5% dietary group readily accept whole yellow mealworms as a feeding source, even though they were previously untrained and solely accustomed to a pelleted diet. Feed preference led to immediate and complete consumption of defrosted larvae from the feeder before initiating consumption of the pelleted feed in 100% of couples, pointing to whole mealworms as being the first choice and part of the bird's innate feeding behavior. These results are consistent with the findings of Whiteside et al. [13], who revealed pheasants reared on a diet containing mealworms, fruits, and seeds show a preference for these feed items over the supplemented commercial diet. Authors also reported pheasants fed on this enriched diet survived five times longer after release than captive conspecific adults solely fed a commercial diet.

Despite finding a lower final weight in breeding partridges of the TM5% group compared with birds in the control group, the number of eggs laid per week and the egg weight produced by each couple did not differ according to the dietary treatment throughout the whole experimental feeding (5 weeks). The inclusion of TM did not affect the BW, as it remain consistent with the live BW of wild birds [25]. It is worthy to point out that the partridges from the TM5% group showed a markedly reduced laying activity with a significant decrease in the numbers of eggs laid in the last week. This dietary effect must be considered in the light of BW loss, rather than dietary energy intake, both relative to BW and metabolic weight ($BW^{0.75}$). Concomitantly, the eggs laid in the last week showed a heavier dry content with a thinner eggshell in the TM5% group. This being said, partridges of the CON group started to reduce their laying frequency around this time as well, likely due to the approaching end of the laying season. The laying frequency turned out to be anticipated and marked in the TM5% group, probably due to the difference in energy levels of female birds. The provision of whole mealworms only with the pelleted diet does not appear to be optimal for the birds in this trial and is likely related to the lack of digestive efficiency. Indeed, the chitinous exoskeleton of whole mealworms may represent a mechanical hurdle for the digestive function of these partridges, especially if present above a certain amount. As birds in this trial were reared in cages, they had no access to grit nor alternative mineralized material, which is voluntarily ingested by their wild counterparts in order to help the mechanical comminuting of feedstuffs in the gizzard. Indeed, intake of grit and clasts of certain size and hardness, stored in the gizzard, allows mechanical grinding of seeds, insects, and lengthy fibrous material. Hence, one of the reasons behind the observed weight loss of birds fed the TM5% diet can be attributed to the lack of mechanical grinding in the gizzard leading to limited enzymatic access to the nutrient content inside the larvae.

However, this condition did not prevent partridges from selecting larvae above the pelleted diet, which was consistently consumed as a second choice. In view of these findings, a recommendable provision of grit to caged birds fed or supplemented with natural dietary components could support mechanical digestion in the gizzard.

The breadth of offered stuffs is recognized as proper nutrient and energy sources likely increase the potential for appropriate selection and composition of the diet, resulting in increased foraging efficiency and cognitive development across a variety of taxa [26]. Furthermore, birds reared on a simulated natural diet tend to exploit a wider range of feed options, only seldom relying on monotonous feed (which usually consists of thermally and physically processed feed), in view of the modulation for functional and efficient digestive systems [18]. Altogether, several factors can therefore have an impact on foraging efficiency, which consequently influences fitness and reproductive success upon release [26]. While wasting less time foraging could result in a reduction in energy costs associated with feed consumption [27], limiting consumption time also reduces the likelihood of encountering predators [28,29]. Galliforms are vulnerable to predation in particular because adult birds forage by keeping their faces and eyes lowered, facing the ground, reducing their vigilance and predator detection [30]. Indeed, a major cause of grey partridge release failure comes from the fact that these captive-bred birds exhibit developmental deficiencies in terms of habitat selection, predator vigilance, and flight response [9,31,32]. Similarly, wild pheasants experience considerably lower predation rates in comparison with captive-reared individuals, indicating predator stimuli during the early stages of development to aid in the acquisition of vigilance [26]. Partridges in the present study actively selected mealworms, implying animals may have developed spatial memory that would presumably benefit them in detecting similar feeding sources in nature, thereby making the transition from artificial diet easier. These findings would open the debate on how simple modifications in feeding practices during periods of captivity could have long-lasting beneficial effects after release [33,34].

In nature, feeding preferences and dietary selection behavior are not innate but rather shaped by the environment and learning ability during the early life stages [13]. This ability to efficiently distinguish and select natural food resources is a critical mechanism for reducing stress, mortality, and developing efficient vigilance [33]. Feeding preferences can be learnt through social interaction. For instance, in the domestic hen (*Gallus gallus*) or white-tailed ptarmigan (*Lagopus leucurus*), parental individuals utter a peculiar sound to attract small chicks to favorable feed items [35,36]. Moreover, releasing captive parent-reared adults in family groups or fostering captive parent-reared birds in wild barren pairs increased grey partridge survival rates compared with solitary captive-reared adult partridges [37]. Food handling skills, particularly of live prey, are generally acquired through interaction with and handling of a variety of feed sources and contribute to the sustenance and environmental fitness of animals [38]. This type of learning behavior has been observed in a variety of animal species. For example, captive *Mustela nigripes* (black-footed ferrets) were found to be more effective hunters when exposed to live prey than when fed on dead prey alone [39,40]. Furthermore, captive-held bank voles lack the ability to open nuts, contrary to their wild conspecifics, which readily open them [41].

The partridges in our study were fed defrosted larvae of *T. molitor*; hence, no interaction between predator and living prey could be developed. However, the physical form and nutrient composition of frozen larvae (exoskeleton made of chitin and succulent content, lipid and protein bases) is somewhat comparable to that of live individuals [23]. In fact, selection based not only on the physical form but also on the specific nutrient composition of the larvae agrees with the findings by Cullere et al. [42]. Authors demonstrated that broiler quails in a feed-choice test tended to select the diet containing *Hermetia illucens* meal. The observed preference of partridges for yellow mealworm larvae is also consistent with the findings of other studies reporting poultry to display a marked preference for scattered mealworms [43] or *H. illucens* larvae [44]. Given that, birds raised in open-air backyard poultry systems are evolutionarily more inclined towards insects [45].

## 5. Conclusions

Provision of whole defrosted yellow mealworm larvae in combination with a commercial complete pelleted diet pointed to the marked preference of mealworms as a first dietary choice for unaccustomed captive-bred Sardinian partridges. The supplementation of whole mealworms potentially increased the bird's competence regarding the recognition of new feeding sources, having a similar physical form and composition as natural feeding sources in the environment, possibly increasing their chance of survival upon release. Laying performance of breeding partridges in both experimental groups was not affected by diet, though the use of whole mealworms as feed material led to a nonsignificant decrease in body weight at the end of the trial. This decrease in body weight was hypothesized to be the result of insufficient mechanical breakdown of the chitinous exoskeleton of the provided larvae in combination with the marked preference of this feed, resulting in inefficient nutrient exploitation by the birds. Similarly, reduced nutritive intake by birds likely resulted in the earlier onset of laying reduction in the TM5% group, which produced a significantly lower number of eggs, showing thinner eggshell with heavier dry egg content towards the end of the experiment. Although, feeding captive-bred couples of Sardinian partridge with defrosted larvae appears to be a promising practice, especially if the birds are destined to be released into the wild. Further trials are needed to evaluate the addition of grit material that should be provided alongside whole larvae in order to allow optimal mechanical breakdown and nutrient accessibility for the birds.

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