Final report PhD project of P-Campus Graduate School

Potential of Black Soldier Fly Larvae (BSFL) reared on recycled phosphorus-rich substrates to be included in broiler diets

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1. Summary and conclusions

Phosphorus (P) is essential in broiler diets. However, P utilization in animal farming or agriculture practices is not efficient. Although not currently legally permitted, mineral recycling could, in principle, reintegrate P from sewage sludge into animal diets to better close the nutrient cycle. Supplementing black soldier fly larvae (BSFL; *Hermetia illucens*) with recycled minerals could be a solution to recover essential minerals while reducing heavy metal contaminations from recyclates. We hypothesized that feeding broilers with recycled mineral-enriched BSFL beneficially affects P intake with no adverse effects on growth performance and heavy metal intake.

First experiment: The first experiment was conducted to determine the optimum level of whole BSFL to be given to broilers. Our results show that chickens can consume whole defrosted black soldier fly larvae (BSFL; *Hermetia illucens*) up to 30% of their voluntary FI in a few minutes. Larvae eating time and eating rates of broilers suggest a strong preference for BSFL over regular grain-based feed. Defrosted whole BSFL can be included in broiler rations up to 20% without adverse effects on growth performance and nutrient conversion efficiency, metabolism, slaughter weight and carcass traits, as well as fatty acid (FA) profile in plasma, muscle and fat tissues. However, 30% whole BSFL were associated with lower protein

utilization efficiency and caused alterations in the FA compositions of plasma, breast and abdominal fat resulting in an increased saturated FA (SFA) content at the expense of monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA).

Second experiment: This study was performed to evaluate the accumulation potential of recycled minerals derived from two types of sewage sludge recyclates (Biochar (BCH) and Single Super Phosphate (SSP)) in BSFL. Our results suggested that the concentrations of the minerals found in the larvae confirm that the micronutrient profile of BSFL depends on the mineral concentrations in the feeding substrate. Both BCH and SSP supplements increased calcium (Ca) content in BSFL, whereas only SSP increased P content. Inclusion of SSP was associated with lower heavy metal content in larvae which reflects the lower concentrations of heavy metals in SSP due to the heavy metal reduction technology used and suggest an advantage of SSP over BCH recyclate. The SSP recyclate caused lower accumulation of heavy metals compared to BCH in BSFL. Recycled mineral-enriched larvae exceed the current EU regulations for manganese (Mn) and cadmium (Cd) content. Because of toxicity of excessive Mn and Cd in feed or food, thresholds for its incorporation level in animal diets or certain foodstuffs might be necessary in future depending on the BSFL processing technology or feeding source.

Third experiment: The third trial was conducted to evaluate performance and mineral metabolism in broilers fed recycled mineral-enriched BSFL. We concluded that 15% of mineral enriched whole BSFL can be included in broiler rations without adverse effects on growth performance, nutrient intakes, nutrient conversion efficiency, blood metabolites and immunoglobulins. Although birds in three different BSFL supply groups had a higher serum Ca than birds with no access to BSFL, feeding broilers with BSFL reared on feeding substrate with Biochar-supplement reduced serum P. These differences in serum Ca and P did not affect the bone characteristics and tibia mineral status of the birds.

2. Introduction and objectives of the PhD project

Until 2050 the human world population is projected to increase by nearly 2.5 billion (Walker, 2016, van Dijk et al., 2021). To prevent global hunger, FAO suggested that world food production needs to increases by 70% (FAO, 2009). To increase the food availability, there would be a higher demand for increased and more efficient use of fertilisers. With rapid population growth and urbanization, waste generation is expected to increase, making the adaptation of bio-waste recovery and recycling technologies critical to improving global sustainability. In all animal species, P is critical in cellular metabolism, as a component of the energy reservoir of the cell, in cellular regulatory mechanisms, and in bone development and mineralization (Bolan et al., 2010). However, P is one of the most limiting nutrient in agriculture and farmed animal production in terms of both price and amount of rock P (Roy et al., 2006, Magnone et al., 2022). The main source of rock P is in Morocco, the US, China, South Africa and Jordan, which is non-renewable and becoming more expensive (Cieślik and Konieczka, 2017). In addition to the problem of limited P resources, the intensive use of P results in a variety of environmental impacts. Phosphorus use in agriculture activities, livestock production, and urbanization can lead to P accumulation in soil particles, which further could enter water systems through subsurface flows (Ruark et al., 2014). This causes P environmental pollution and eutrophication of water bodies (Amann et al., 2018), which in turn might directly or indirectly impact human societies (Bol et al., 2018). Phosphorus recycling from sewage sludge could be an alternative P source for agriculture practices and animal feeding. However, there is the problem of P bioavailability and contamination of the sludge and possibly the P products by heavy metals (Herzel et al., 2016). Using BSFL raised on

organic waste, side streams and food industry by-products as animal feed could offer a sustainable way to recycle untapped nutrient resources (Schiavone et al., 2017). Nevertheless, current EU feed and food laws prohibit the use of waste products in animal feeds. It has been shown that BSFL accumulates most minerals present in the feeding substrate into its body (Shumo et al., 2019). However, the degree of accumulation seems to be dependent on the feeding substrate.

The **hypotheses** of the PhD project were: 1) BSFL can be used in broiler diets with no adverse effects on P homeostasis, animal performance, bone quality and metabolism. 2) Using recycled P-rich substrates in BSFL feeding substrate causes P accumulation in their bodies. 3) Supplementation of broiler diets with BSFL mineral-enriched with recycled P beneficially affects P intake with no adverse effects on heavy metal intake. The **objectives** of the experiment included: 1) to explore the supplementation of sewage sludge recyclates to feeding substrates on performance and mineral accumulation in BSFL; 2) to investigate the use of BSFL raised on organic materials containing sewage sludge recyclate with high P concentration in broiler diets on performance, heavy metal accumulation, metabolism, and bone status of broilers at different ages;.

3. Material and methods

3.1. Experiment-1: What is the optimum level of whole BSFL to be given to broilers?

A total of 252 1-day (d)-old broiler birds (Ross-308) was used to determine the optimum level of BSFL in broiler diets. The birds were allocated to 4 groups and fed age-specific commercial broiler diets (starter, d0-d14; grower, d15-28; and finisher d29-42). Broilers in the control group (CON) received the age-specific basal diet, and had no access to BSFL. Birds in the remaining 18 pens received whole frozen and defrosted BSFL in addition to the basal diet at increasing levels, i.e. 10%, 20% or 30% of the feed intake (FI) of CON birds (hereafter referred to as groups L10 (n = 63), L20 (n = 63), and L30 (n = 63), respectively). Pen based feed intake was determined daily. During the experiment, temperature and humidity were monitored daily at room level to meet relevant recommendations (Aviagen, 2018). The birds were slaughtered at an age of 4 and 6 weeks and samples of blood, tissues and bones were collected. In the FBN-slaughterhouse carcass characteristics including wings, legs, breast, and abdominal fat weight, pH values and color parameters (L* (brightness), a* (red-green) and b* (yellow-blue)) of the breast muscle samples were assessed. Chroma and hue were calculated as chroma = (a*2+b*2)1/2 and hue = arctan (b*/a*) (Kongsup et al., 2022). Water holding capacity was determined according to Grosse et al. (1975). In addition, plasma and breast meat FA profiles were determined using capillary gas chromatography. Furthermore, FA profiles in broiler feeds (starter, grower and finisher) and BSFL were determined.

3.2. Experiment-2: What is the level of accumulation of recycled minerals in BSFL?

This experiment was conducted to produce BSFL with increased P content, which subsequently was used in broiler feed (Experiment-3). For this, recycled P from different sewage sludge recyclates were used. Based on the standard fly/insect diet 'Gainesville', we generated a P-reduced BSFL feeding substrate in order to investigate the effects of a P enrichment via sewage sludge recyclates. This was achieved by reducing the wheat bran content and the inclusion of sugar beet pulp (modified Gainesville fly diet (FD)). For the test substrates the content of wheat bran and corn meal content was further reduced to include 4% of the supplement Biochar (BCH; recyclate from pyrolysis of sewage sludge) or Single-Super Phosphate (SSP; recyclate of sewage sludge using a different production method with enriched phosphate compared to BCH but reduced heavy metal content). Therefore, 3 different diets were fed to BSFL with 12 repetitions included in two runs from which 3

repetitions per run were sampled. Dietary groups were: 1- BSFL raised on modified P-reduced Gainesville fly diet (FD), 2- BSFL raised on modified Gainesville fly diet + SSP (FD+SSP; 4%), 3-BSFL raised on modified Gainesville fly diet + BCH (FD+BCH; 4%). The larvae were harvested, weighed, and individually frozen in liquid nitrogen for supplementing broilers in Experiment-3. The larvae and frass were analysed for nutrient and mineral composition. An analysis of heavy metal were performed. The bioaccumulation factor (BAF) of minerals and heavy metals was calculated according to Eq. 1 (Walker, 1990): mineral concentration [g/kg dry matter (DM)] in larvae at harvest / mineral concentration in the feeding substrates [g/kg DM]. The BAF values > 1 suggest accumulation, while BAF < 1 suggest depletion of minerals from larval body.

3.3. Experiment-3: How does supplementation with mineral-enriched BSFL affect performance and mineral metabolism in broilers?

This experiment performed over 42 days evaluated the effects of feeding larvae reared on diets containing sewage sludge recyclate materials (SSP or BCH) using a total of 80 (n=6 pens; 2-4 birds/pen) broiler birds (Ross-308). The 1-d-old birds were allocated to 4 groups that were fed age-specific commercial broiler diets. Broilers in the control group (CON) received the agespecific basal diet, and had no access to BSFL. Birds in the remaining 18 pens received 15% of the feed intake (FI) of CON birds as defrosted whole BSFL (that were produced in Experiment 2) in addition to the basal diet, namely larvae grown on FD (L-FD), on FD+BCH (L-BCH) or on FD+SSP (L-SSP). All of the chickens were slaughtered at the end of the experiment on d 42. Pen-based feed intake and body weight were recorded at daily and weekly intervals, respectively. Feed conversion ratio (FCR) was calculated on a weekly basis per pen. Mortality was recorded daily. During the experiment, temperature and humidity at room level were monitored daily to meet relevant recommendations (Aviagen, 2018). Feed samples (grainbased and BSFL) were taken during the experiment for nutrient analysis. Nutrient analysed in the diets include DM, ash, crude fat, crude fibre, crude protein, carbohydrates, NDF, ADF, Ca, total P, magnesium (Mg), potassium (K), sodium (Na). Heavy metals concentration in feeds was also measured. On d 42 blood samples were taken from slaughtered birds for blood serum and plasma. The serum samples were used to determine the serum biochemical parameters. After slaughter, the tibiotarsus (tibia) were removed, singly packed in polyethylene bags and stored at -20°C. The tibias were combusted and Ca and P were determined.

4. Results

4.1. Experiment-1: What is the optimum level of whole BSFL to be given to broilers?

Our results show that all broilers were able to consume whole BSFL within a short time. The calculated larvae eating rate (LER; representing the speed of intake), was higher in L10 group than those in L20 and L30 (P < 0.05), which suggest a high competition among the birds to consume a limited amount of BSFL. The ratio of LER to feed intake rate (FER) was higher than the 50-fold change difference (FCD) which indicated a high preference (at least 50-fold) of broilers for eating BSFL over regular feed. Inclusion of up to 30% whole BSFL in broiler diets did not compromise growth of the birds (P > 0.1), however, we found some trade-offs in nutrient and energy intakes induced by inclusion of BSFL. The maximum inclusion rate of whole BSFL (i.e. 30%) in broiler diets reduced DM and metabolizable energy intake (ME), although total fat intake was higher in L30 than in CON (P < 0.05). In addition, L30 birds had a higher FCR (based on fresh matter intake) compare to the other groups (P < 0.05). Moreover, 30% BSFL in broiler diets increased plasma uric acid (UA) and serum alkaline phosphatase concentration (P < 0.05). Carcass traits including; slaughter weight, dressing percentage, percentages of breast and wings, pH and color traits of breast meat as well as water holding

capacity were not affected by the factor group (P > 0.1). Thus, dry matter and crude protein contents of breast muscle were higher in L30 than in L10 and CON birds (P < 0.05), which suggests that even high inclusion rates of whole BSFL in broiler diets do not compromise the meat quality of broiler meat for human consumption. In contrast, the inclusion of 30% whole BSFL in broiler diets increased SFA concentration in plasma, breast muscle and adipose tissue of broilers (P < 0.05), while MUFA proportion in adipose tissue was reduced (P < 0.05). The L30 group had a lower PUFA concentration in plasma and adipose tissue than CON (P < 0.05). In addition, conjugated linoleic acid (isomer C18:2cis-9, trans-11) in plasma, muscle and abdominal fat was higher in L30 birds followed by L20 and L10 compared with CON (P < 0.05).

4.2. Experiment-2: What is the level of accumulation of recycled minerals in BSFL?

All BSFL feeding substrates had similar nutrients and ME contents, but different mineral and heavy metal contents, which was due to the different mineral and heavy metal contents in the BCH and SSP recyclates. The SSP recyclate had a lower heavy metal content (Mn, iron (Fe), zinc (Zn), cupper (Cu), arsenic (As), Cd, lead (Pb) and mercury (Hg)) than BCH, which correspondingly caused differences in mineral and heavy metal contents in the feeding substrates. The results show that larvae reared on the diets containing FD+SSP substrate had a lower body mass (P < 0.1), and specific growth rate (P < 0.05) compared to FD. Larval crude protein (P < 0.1) and crude fat (P < 0.05) were lower in FD+SSP compared to FD. The FD+SSP larvae had a higher Ca and P content but the BAF for Ca was lowest. Both Ca and P accumulated in larval body (BAF > 1). Concentration of Mn in larvae did not differ among the groups (P < 0.05), but the Mn BAF was lower in FD+BCH larvae than in those fed on FD+SSP and FD which was possibly linked to the highest Mn concentration found in FD+BCH feeding substrate. The BAF results showed accumulation of Mn in all larval groups (BAF > 1). Arsenic concentration in FD+SSP larvae was higher than in FD+BCH and FD larvae (P < 0.05). The As BAF indicates that As was not accumulated in the larval body (BAF < 1) of any substrate group. However, FD+SSP larvae showed a relatively higher As BAF compared to FD+BCH and FD larvae (P < 0.05). Moreover, larvae of the FD+BCH group had a higher Cd concentration than those in the FD group (P < 0.05). Cadmium had the highest BAF among all measured heavy metals with a tendency for a higher BAF in FD+SSP compared to the FD larvae (P = 0.080). The Mn and Cd contents of BSFL were above the EU authorized maximum concentration.

4.3. Experiment-3: How does supplementation with mineral-enriched BSFL affect performance and mineral metabolism in broilers?

All the chickens in larvae intake groups (L-BCH, L-SSP) consumed mineral-enriched whole BSFL in a short time. Feeding 15% of mineral-enriched whole BSFL in broiler rations had no adverse effects on growth performance, nutrient intakes, nutrient conversion efficiency, blood metabolites and immunoglobulins (P > 0.1). Also, plasma albumin, cholesterol, glucose, NEFA, triglyceride, and UA were not affected by group (P > 0.1). However, L-FD broilers tended to have higher plasma triglyceride levels compare to L-SSP (P = 0.057). Birds of BSFL intake groups had a higher serum Ca (P < 0.05). In addition, chickens in the L-BCH group had a lower serum P than those in CON (P < 0.05). Tibia characteristics and tibia mineral status of the birds were not affected by factor group (P > 0.05).

5. Discussion

5.1. Experiment-1: What is the optimum level of whole BSFL to be given to broilers?

We assumed that the higher LER could be due to increased larval feeding competition in L10 birds as a result of fewer palatable nutrients such as fat and protein, which are known to be perceived by chickens (Cheled-Shoval et al., 2017). We quantified a strong linear increase in LER with time of more than 200-fold from the first day to the last day of experiment,

indicating a steadily increasing eating rate in response to time, which was considered to be a result of two interrelated factors, social learning and growth, i.e. increasing body weight (Slagsvold and Wiebe, 2011, Tallentire et al., 2018). The high interest, that is, preference of chickens for BSFL compared to regular grain-based feed opens the possibility of including whole BSFL in daily rations for broilers. Insect larvae are rich in various nutrients and are one of the natural feed sources of poultry, which are very motivating for consumption (Bokkers and Koene, 2002), so birds may clearly prefer larvae over regular feed. Ipema et al. (2020b) also observed a strong appetite value of live BSFL for broilers which was associated with a higher activity and increased foraging behaviour. Our results showed that inclusion of up to 30% whole BSFL in broiler diets did not compromise the growth of the birds. Similarly, previous studies showed that inclusion of BSFL meal had no adverse effect or increased growth of broilers (Lee et al., 2018, Moula and Detilleux, 2019, Biasato et al., 2020). In addition, Moula et al. (2018) reported that body weights of chickens fed standard feed supplemented with 8% whole defrosted larvae were higher than those of control chickens.

In our study, L30 birds had a lower total DM intake, which resulted in a lower ME intake that could not be compensated for by the higher fat intake via BSFL consumption. A principal component analysis revealed that the higher fat intake in the L30 group was the main driving force for reducing DM intake. According to Wang et al. (2017), high dietary fat in broilers might alter the response of hypothalamic appetite-related peptides which is associated with circulating insulin (Obrosova et al., 2007). We observed a linear increase of the CP:ME intake ratio in response to feeding increasing levels of BSFL, which was accompanied by a higher plasma UA concentration in L30 birds whereas protein utilization was less efficient in the same birds. Moreover, the higher fat intake which was possibly associated with adverse effects on liver function (Jiang et al., 2013).

Carcass traits of broilers were not compromised by dietary inclusion of BSFL, which was in line with the results of de Souza Vilela et al. (2021a) and de Souza Vilela et al. (2021b) that using up to 20% full-fat BSFL in the diet did not influence key meat characteristics and broiler performance. Despite higher fat intake, we could not find any difference between groups for abdominal fat content in the carcass. Li et al. (2016) suggested that dietary fat with a high proportion of medium chain fatty acids (MCFA) improves energy availability and reduces the deposition of adipose tissue. Therefore, the lack of differences in abdominal fat content among the groups could be explained by the high MCFA content of larvae.

The FA analysis of BSFL revealed a high SFA (71%) and low PUFA content (i.e. approximately 12%). We found a higher SFA level in plasma, breast muscle and adipose tissue and a reduced PUFA level in plasma and adipose tissue of the broilers fed different levels of BSFL which could be due to the carry-over effect from BSFL fat. Moreover, biochemical mechanisms may be involved in the alteration of FA profile. In chickens, C18:3 n-3 and C18:2 n-6 are essential FA and precursors for long chain PUFA synthesis (Cherian, 2015). The lower proportion of PUFA in broiler plasma and adipose tissue might be due to lower dietary intake of C18:3 n-3 and C18:2 n-6.

5.2. Experiment-2: What is the level of accumulation of recycled minerals in BSFL?

Exposing larvae of *Drosophila melanogaster* to heavy metals such as Cd has been shown to induce oxidative stress (Yang et al., 2022), which can result in reduced lipid storage and down-regulated expression of lipid metabolism genes (Akhmetova et al., 2021). Therefore, the lower crude fat content in FD+SSP larvae might be associated to the higher accumulation of heavy metals such as Fe, As, Cd and Pb in FD+SSP larvae than in those of FD and FD+BCH larvae. Similarly, Schmitt et al. (2019) discussed that reduced fat content in BSFL might be related to

a high larval accumulation of heavy metals such as Cd, Cu and Zn. Both recyclates (SSP and BCH) are rich in minerals which may be reintegrated into the nutrient cycle. The SSP recyclate contained higher Ca, P and Na levels, but was lower in Mg and K concentration than BCH. The BAF is an estimate of the efficiency of larvae to take up and accumulate certain minerals from the substrate in the body. The FD+SSP larvae had a lower Ca BAF than those of the FD+BCH and FD groups, which suggests that Ca was less efficiently accumulated by larvae in the FD+SSP group than in the other groups. But since Ca BAF values were greater than 1 in all groups this indicates Ca enrichment relative to the Ca content in the substrate. The P concentration was higher in FD+SSP larvae than that in FD+BCH and FD larvae, which was linked to a higher P concentration in the FD+SSP substrate than in FD+BCH and FD. Phosphorus accumulated in all groups (BAF > 1) however, the FD+BCH larvae had a lower P accumulation than those in the FD group, suggesting a lower P accumulation efficiency from the BCH recyclate. Currently the knowledge on the regulation of Ca and P homeostasis in BSFL is limited but the involvement of the Malpighian tubules in homeostasis and storage of minerals in insects such as *Drosophila melanogaster* is likely (Browne and O'Donnell, 2016, Rose et al., 2019).

Joseph et al. (2021) suggested that carbon-coated minerals on the surface of Biochar effectively reduce the bioavailability of heavy metals in soil. Therefore, although the concentration of certain minerals such as Mn was higher in BCH than in SSP recyclate, it is possible that they are hardly available from the substrate, e.g., depending on the pH of the environment. This could be the reason for the lower Mn BAF in FD+BCH larvae.

It is known that BSFL accumulate high amounts of Cd (Proc et al., 2020), which is highly toxic and has serious health risks for animal and humans (Truzzi et al., 2019). The BSFL our study also showed a high Cd content. In general, little information is available on mineral transport mechanisms in BSFL. However, it has been suggested that high BAF for Cd in BSFL could be attributed to the large number of Ca²⁺ channels in their gut, which facilitate the Cd transport by means of heat shock proteins, resulting in high Cd accumulation compared to other heavy metals (Van der Fels-Klerx et al., 2016, Bessa et al., 2021). In summary, in the present study, the Fe, Zn, Cu, As, Pb and Hg contents in BSFL did not exceed the current EFSA recommendations for the maximum allowance limit for animal diets, however, it was exceeded for Mn and Cd content of BSFL. This suggests that when adding BSFL reared on substrates supplemented with sewage sludge recyclates to broiler feed rations, one must observe an upper inclusion level.

5.3. Experiment-3: How does supplementation with mineral-enriched BSFL affect performance and mineral metabolism in broilers?

Although there were differences in the nutrient, mineral and heavy metal content of the larvae in the different groups, we did not observe differences in feed, nutrient, mineral and heavy metal intakes in broilers. Lack of differences in feed, nutrient, mineral and heavy metal intakes could be attributed to the amount of BSFL (15%) offered to the birds. The inclusion level that we used in this experiment did not compromise feed intake in larval intake groups and thus, did not cause negative effects on nutrient intakes. However, the lower fat content in FD+SSP larvae might be associated to the lower plasma triglyceride concentration in L-SSP birds compared to L-FD broilers.

Our results show that tibia characteristics and tibia mineral status of the birds were not changed by the intake of 15% mineral-enriched larvae. In line with our results, Moula et al. (2018) reported that tibia ash content of local poultry breed fed 8% of defrosted whole BSFL was not affected. Also, Ipema et al. (2020a) found that tibia measurements (tibia length, length fluctuating asymmetry, width fluctuating asymmetry and breaking strength) of broilers

fed rations in which 5 or 10% of the total dietary DM was replaced with live BSFL were not affected.

6. References

- Amann, A., O. Zoboli, J. Krampe, H. Rechberger, M. Zessner, and L. Egle. 2018. Environmental impacts of phosphorus recovery from municipal wastewater. Resources, Conservation and Recycling 130:127-139.
- Aviagen. 2018. Broiler Management Handbook. Pages 1–147 Newbridge, UK.
- Bessa, L. W., E. Pieterse, J. Marais, K. Dhanani, and L. C. Hoffman. 2021. Food Safety of Consuming Black Soldier Fly (Hermetia illucens) Larvae: Microbial, Heavy Metal and Cross-Reactive Allergen Risks. Foods 10(8):1934.
- Biasato, I., I. Ferrocino, S. Dabbou, R. Evangelista, F. Gai, L. Gasco, L. Cocolin, M. T. Capucchio, and A. Schiavone. 2020. Black soldier fly and gut health in broiler chickens: insights into the relationship between cecal microbiota and intestinal mucin composition. J. Anim. Sci. Biotechnol. 11(1):1-12.
- Bokkers, E. A. and P. Koene. 2002. Sex and type of feed effects on motivation and ability to walk for a food reward in fast growing broilers. Applied Animal Behaviour Science 79(3):247-261.
- Bol, R., G. Gruau, P.-E. Mellander, R. Dupas, M. Bechmann, E. Skarbøvik, M. Bieroza, F. Djodjic, M. Glendell, and P. Jordan. 2018. Challenges of reducing phosphorus based water eutrophication in the agricultural landscapes of Northwest Europe. Frontiers in Marine Science 5:276.
- Bolan, N., A. Szogi, B. Seshadri, and T. Chuasavathi. 2010. The management of phosphorus in poultry litter. Nat Res 7.
- Browne, A. and M. J. O'Donnell. 2016. Segment-specific Ca2+ transport by isolated Malpighian tubules of Drosophila melanogaster: A comparison of larval and adult stages. J. Insect Physiol. 87:1-11.
- Cheled-Shoval, S. L., N. Reicher, M. Y. Niv, and Z. Uni. 2017. Detecting thresholds for bitter, umami, and sweet tastants in broiler chicken using a 2-choice test method. Poult. Sci. 96:2206-2218.
- Cherian, G. 2015. Nutrition and metabolism in poultry: role of lipids in early diet. J Anim Sci Biotechnol 6(1):1-9.
- Cieślik, B. and P. Konieczka. 2017. A review of phosphorus recovery methods at various steps of wastewater treatment and sewage sludge management. The concept of "no solid waste generation" and analytical methods. Journal of Cleaner Production 142:1728-1740.
- de Souza Vilela, J., T. I. Alvarenga, N. R. Andrew, M. McPhee, M. Kolakshyapati, D. L. Hopkins, and I. Ruhnke. 2021a. Technological quality, amino acid and fatty acid profile of broiler meat enhanced by dietary inclusion of black soldier fly larvae. Foods 10:297.
- de Souza Vilela, J., N. M. Andronicos, M. Kolakshyapati, M. Hilliar, T. Z. Sibanda, N. R. Andrew, R. A. Swick, S. Wilkinson, and I. Ruhnke. 2021b. Black Soldier Fly larvae in broiler diets improve broiler performance and modulate the immune system. Anim Nutr 7(3):695-706.
- FAO, O. 2009. How to feed the world in 2050, high-level expert forum. Available at: fao. org/fileadmin/templates/wsfs/docs/Issues_papers/HLEF2050_Global_Agriculture. pdf [accessed on 10 November 2014].
- Grosse, F., U. Bretschneider, and G. Saß. 1975. Eine Methode zur direkten Bestimmung des Preßsaftes von Fleisch. Fleisch.
- Herzel, H., O. Krüger, L. Hermann, and C. Adam. 2016. Sewage sludge ash—A promising secondary phosphorus source for fertilizer production. Science of the Total Environment 542:1136-1143.
- Ipema, A. F., E. A. Bokkers, W. J. Gerrits, B. Kemp, and J. E. Bolhuis. 2020a. Long-term access to live black soldier fly larvae (Hermetia illucens) stimulates activity and reduces fearfulness of broilers, without affecting health. Sci Rep 10:1-13.
- Ipema, A. F., W. J. Gerrits, E. A. Bokkers, B. Kemp, and J. E. Bolhuis. 2020b. Provisioning of live black soldier fly larvae (Hermetia illucens) benefits broiler activity and leg health in a frequency-and dose-dependent manner. Appl. Anim. Behav. Sci. 230:105082.
- Jiang, S., H. W. Cheng, L. Cui, Z. Zhou, and J. Hou. 2013. Changes of blood parameters associated with bone remodeling following experimentally induced fatty liver disorder in laying hens. Poult. Sci. 92(6):1443-1453.
- Joseph, S., A. L. Cowie, L. Van Zwieten, N. Bolan, A. Budai, W. Buss, M. L. Cayuela, E. R. Graber, J. A. Ippolito, and Y. Kuzyakov. 2021. How biochar works, and when it doesn't: A review of mechanisms controlling soil and plant responses to biochar. GCB Bioenergy.
- Kongsup, P., S. Lertjirakul, B. Chotimanothum, P. Chundang, and A. Kovitvadhi. 2022. Effects of eri silkworm (Samia ricini) pupae inclusion in broiler diets on growth performances, health, carcass characteristics and meat quality. Anim Biosci 35(5):711.
- Lee, J.-A., Y.-M. Kim, Y.-K. Park, Y.-C. Yang, B.-G. Jung, and B.-J. Lee. 2018. Black soldier fly (Hermetia illucens) larvae enhances immune activities and increases survivability of broiler chicks against experimental infection of Salmonella Gallinarum. J. Vet. Med. Sci.:17-0236.

- Li, S., H. Ji, B. Zhang, J. Tian, J. Zhou, and H. Yu. 2016. Influence of black soldier fly (Hermetia illucens) larvae oil on growth performance, body composition, tissue fatty acid composition and lipid deposition in juvenile Jian carp (Cyprinus carpio var. Jian). Aquaculture 465:43-52.
- Magnone, D., V. J. Niasar, A. F. Bouwman, A. H. Beusen, S. E. van der Zee, and S. Z. Sattari. 2022. The impact of phosphorus on projected Sub-Saharan Africa food security futures. Nature communications 13(1):1-10.
- Moula, N. and J. Detilleux. 2019. A meta-analysis of the effects of insects in feed on poultry growth performances. Animals 9(5):201.
- Moula, N., M.-L. Scippo, C. Douny, G. Degand, E. Dawans, J.-F. Cabaraux, J.-L. Hornick, R. C. Medigo, P. Leroy, and F. Francis. 2018. Performances of local poultry breed fed black soldier fly larvae reared on horse manure. Anim Nutr 4:73-78.
- Obrosova, I. G., O. Ilnytska, V. V. Lyzogubov, I. A. Pavlov, N. Mashtalir, J. L. Nadler, and V. R. Drel. 2007. High-fat diet–induced neuropathy of pre-diabetes and obesity: effects of "healthy" diet and aldose reductase inhibition. Diabetes 56:2598-2608.
- Proc, K., P. Bulak, D. Wiącek, and A. Bieganowski. 2020. Hermetia illucens exhibits bioaccumulative potential for 15 different elements–Implications for feed and food production. Sci. Total Environ. 723:138125.
- Rose, E., D. Lee, E. Xiao, W. Zhao, M. Wee, J. Cohen, and C. Bergwitz. 2019. Endocrine regulation of MFS2 by branchless controls phosphate excretion and stone formation in Drosophila renal tubules. Sci. Rep. 9(1):1-12.
- Roy, R. N., A. Finck, G. Blair, and H. Tandon. 2006. Plant nutrition for food security. A guide for integrated nutrient management. FAO Fertilizer and Plant Nutrition Bulletin 16:368.
- Ruark, M. D., K. A. Kelling, and L. W. Good. 2014. Environmental concerns of phosphorus management in potato production. American Journal of Potato Research 91(2):132-144.
- Schiavone, A., M. De Marco, S. Martínez, S. Dabbou, M. Renna, J. Madrid, F. Hernandez, L. Rotolo, P. Costa, and F. Gai. 2017. Nutritional value of a partially defatted and a highly defatted black soldier fly larvae (Hermetia illucens L.) meal for broiler chickens: apparent nutrient digestibility, apparent metabolizable energy and apparent ileal amino acid digestibility. Journal of animal science and biotechnology 8(1):1-9.
- Shumo, M., I. M. Osuga, F. M. Khamis, C. M. Tanga, K. K. Fiaboe, S. Subramanian, S. Ekesi, A. van Huis, and C. Borgemeister. 2019. The nutritive value of black soldier fly larvae reared on common organic waste streams in Kenya. Sci. Rep. 9(1):1-13.
- Slagsvold, T. and K. L. Wiebe. 2011. Social learning in birds and its role in shaping a foraging niche. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 366:969-977.
- Tallentire, C., I. Leinonen, and I. Kyriazakis. 2018. Artificial selection for improved energy efficiency is reaching its limits in broiler chickens. Sci. Rep. 8:1-10.
- Truzzi, C., S. Illuminati, F. Girolametti, M. Antonucci, G. Scarponi, S. Ruschioni, P. Riolo, and A. Annibaldi. 2019. Influence of feeding substrates on the presence of toxic metals (Cd, pb, ni, as, hg) in larvae of Tenebrio molitor: Risk assessment for human consumption. Int. J. Environ. Res. Public Health 16(23):4815.
- Van der Fels-Klerx, H., L. Camenzuli, M. Van Der Lee, and D. Oonincx. 2016. Uptake of cadmium, lead and arsenic by Tenebrio molitor and Hermetia illucens from contaminated substrates. PLoS One 11(11):e0166186.
- van Dijk, M., T. Morley, M. L. Rau, and Y. Saghai. 2021. A meta-analysis of projected global food demand and population at risk of hunger for the period 2010–2050. Nature Food 2(7):494-501.
- Walker, C. 1990. Kinetic models to predict bioaccumulation of pollutants. Funct. Ecol.: 295-301.
- Walker, R. J. 2016. Population growth and its implications for global security. American Journal of Economics and Sociology 75(4):980-1004.
- Wang, X. J., S. H. Xu, L. Liu, Z. G. Song, H. C. Jiao, and H. Lin. 2017. Dietary fat alters the response of hypothalamic neuropeptide Y to subsequent energy intake in broiler chickens. J Exp Biol 220:607-614.

7. Attachment

7.1. List of Publications

- <u>Seyedalmoosavi, M. M.</u>, Mielenz, M., Veldkamp, T., Daş, G., & Metges, C. C. (2022). Growth efficiency, intestinal biology, and nutrient utilization and requirements of black soldier fly (Hermetia illucens) larvae compared to monogastric livestock species: a review. Journal of Animal Science and Biotechnology, 13(1), 1-20. https://doi.org/10.1186/s40104-022-00682-7.
- Seyedalmoosavi, M. M., Mielenz, M., Görs, S., Wolf, P., Daş, G., & Metges, C. C. (2022). Effects of increasing levels of whole Black Soldier Fly (Hermetia illucens) larvae in broiler rations on acceptance, nutrient and energy intakes and utilization, and growth performance of broilers. Poultry Science, 101 (12) 1-15. https://doi.org/10.1016/j.psj.2022.102202

- <u>Seyedalmoosavi, M. M.,</u> Dannenberger, D., Pfuhl, R., Görs, S., Mielenz, M., Maak, S., Wolf, P., Daş, G.,
 & Metges, C. C. (2022). Lipid metabolism, fatty acid composition and meat quality in broilers supplemented with increasing levels of defrosted black soldier fly larvae. Journal of Insects as Food and Feed. https://doi.org/10.3920/JIFF2022.0125.
- <u>Seyedalmoosavi, M. M.</u>, Mielenz, M., Schleifer, K., Görs, S., Wolf, P., Tränckner, J., Hüther, L., Dänicke, S., Daş, G., Metges, C.C. Upcycling of recycled minerals from sewage sludge through black soldier fly larvae (Hermetia Illucens): impact on growth and mineral accumulation. (In preparation).
- <u>Seyedalmoosavi, M. M.,</u> Daş, G., Mielenz, M., Wolf, P., Metges, C.C. Recycled-mineral enriched whole black soldier fly larvae in broiler diets: growth performance, nutrient intakes, blood metabolites and bone characteristics. (In preparation).

7.2. Abstracts in conferences

- <u>Seyedalmoosavi S.M.M.</u>, Daş G., Mielenz M., Wolf, P., Metges C.C. (2020). Potentiality of Black Soldier Fly Larvae (BSFL) reared on recycled phosphorus-rich substrates to be included in broiler diets. Beginner level. Day of the Doctoral Student. Leibniz Institute for Farm Animal Biology. (Oral presentation).
- <u>Seyedalmoosavi S.M.M.</u>, Daş G., Metges C.C. (2020). Potentiality of Black Soldier Fly Larvae (BSFL) reared on recycled phosphorus-rich substrates to be included in broiler diets. P-campus symposium. (Online presentation).
- <u>Seyedalmoosavi S.M.M.</u>, Daş G., Mielenz M., Wolf, P., Tränckner, J., Metges C.C. (2021). Black Soldier Fly Larvae reared on recycled phosphorus-rich substrates as a feed component for broilers. Lecture for the online presentation of the P-campus Ringvorlesung.
- <u>Seyedalmoosavi S.M.M.</u>, Daş G., Metges C.C. (2021). Influence of different amounts of black soldier fly larvae (BSFL) in the ration on nutrient and energy utilization and growth of broilers. 75th Digital Conference of the Society of Nutrition Physiology (GfE). (Oral presentation).
- <u>Seyedalmoosavi S.M.M.</u>, Daş G., Mielenz M., Metges C.C. (2021). Black Soldier Fly larvae reared on feed mixed with recycled sewage sludge accumulate Ca and P. 4th Phosphorus in Europe Research Meeting (PERM). (Presented as poster).
- <u>Seyedalmoosavi S.M.M.</u>, Daş G., Mielenz M., Schleifer, K., Wolf, P., Tränckner, J., Metges C.C. (2021). Growth performance, body composition and mineral bio-accumulation of black soldier fly larvae reared on a fly diet supplemented with sewage sludge recyclates. Day of the Doctoral Student. Leibniz Institute for Farm Animal Biology. (Oral presentation).
- <u>Seyedalmoosavi S.M.M.</u>, Daş G., Maak S., Mielenz M., Metges C.C., Wolf, P. (2022). Effects of different levels of whole black soldier fly larvae in broiler rations on bone characteristics. 76th Conference of the Society of Nutrition Physiology (GfE). (Oral presentation).
- Seyedalmoosavi S.M.M., Daş G., Dannenberger D., Maak S., Mielenz M., Wolf, P., Metges C.C. (2022). Whole black soldier fly larvae in broiler rations: impact on carcass characteristics, blood metabolites and fatty acids profiles in plasma, muscle and fat tissues. 76th Conference of the Society of Nutrition Physiology (GFE). (Oral presentation).
- <u>Seyedalmoosavi S.M.M.</u>, Mielenz M., Daş G., Metges C.C. (2022). Broiler eating rate suggests preference for black soldier fly larvae (BSFL) over regular feed. 73rd European Federation of Animal Science (EAAP). (Oral presentation).

7.3. Workshops

- Basic P-analytics-workshops at the Biological Station Zingst, Germany. Date: 26-30 November, 2019.
- Statistics course for the PhD students at the Institute of Genetics and Biometry (FBN). 07-11 December, 2020.