GUT-LOADING DIET EVALUATION FOR CRICKETS (*ACHETA DOMESTICUS*), MEALWORMS (*TENEBRIO MOLITOR*), AND SUPERWORMS (*ZOPHOBAS MORIO*) FOR THE PURPOSES OF OPTIMIZING INSTITUTIONAL PROTOCOLS

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Introduction

Several papers over recent years have looked at the practice of gut loading feeder insects for the purposes of improving nutrient composition of these diet items for an array of insectivores; most important being amphibians, reptiles, and birds (Attard, 2011; Coslik et al., 2009; Hunt et al., 2001). These particular species are especially prone to nutritional maladies including metabolic bone disease, rickets, osteomalacia, and hypovitaminosis A (McWilliams & Leeson, 2001; Pessier & Rodriguez, 2015). The Oregon Zoo recently experienced multiple cases in our herpetology and bird departments that necessitated an evaluation of gut loading protocols throughout the zoo. These include presentation of hypovitaminosis A in a rough-skinned newt, a ruddy duck, and hammerkop chicks, and possible metabolic bone disease in a Meller's chameleon and red-billed hornbill chick.

After evaluating the protocols, it was determined that the departments were using different methods for gut loading their feeder insects. To help with consistency and quality control of the feeder insects, and to evaluate the differences between newer and established gut loading diets, a series of trials were conducted to address the animal health concerns and keeper concerns. One of the chief concerns brought by the keeper staff was the increased insect mortality on a newer gut loading diet compared to our previous established gut loading diet. Second, was the concern regarding vitamin A content of the gut loading diet, and third was the overall effectiveness of a gut loading versus the time and effort required manage multiple insect containers in an area.

The first objective of this study was to determine the effect on mortality of how the gut loading diet is presented. The second objective was to determine the efficacy of different gut loading diets on increasing the Ca:P ratio in feeder insects. The third objective was to determine the efficacy of different gut loading diets on increasing vitamin A content on feeder insects.

Materials & Methods

Insect Receiving

All insects were obtained via a commercial vendor (Redline Food, Inc.) and shipped overnight to the Oregon Zoo. Once received, all insects were put into polycarbonate containers and provided with Purina Game Bird + Turkey Startena Diet (PS, Table 1) for 24 hours before being put into any of the trials. For mealworms and superworms, PS diet was provided as a bedding substrate with fresh collard greens provided as a source of moisture. For crickets, PS diet was provided in a dish with a separate dish of water provided. All trials were conducted in a climate controlled room in the Oregon Zoo Veterinary Medical Center, with the temperatures kept between 23-28°C throughout the trial.

Pre-trial mortality assessment

Prior to the gut loading trials, 1000 mealworms and 1000 superworms were divided into 3 treatment groups. Treatment groups consisted of PS offered as a bedding substrate, Mazuri® Better Bug® Gut Loading Diet (MBB, Table 1) offered as a bedding substrate, and MBB offered in a food dish with no bedding substrate. Insects were counted into the containers, and live and dead insects were counted out of the containers after 48 hrs.

Gut loading trial 1

The 3 species of insects were each divided into 4 treatment groups with 3 replicates per group with 250 Crickets or Superworms per replicate and 500 mealworms per replicate. Treatment groups consisted of PS, Mazuri® Hi Calcium Gut Loading Diet (MHC), MBB, and our in-house Oregon Zoo Tortoise Chop (OZTC, Table 1). The OZTC consisted of 16% Bok Choy, 16% Broccoli, 12% Collard Greens, 19% Kale, 16% Shredded Carrot, and 21% Ground Mazuri® Tortoise LS Diet (all on a DM Basis; Table 1). All insects were provided the treatment diets *ad libitum* in a polycarbonate dish in each container for 48 hrs. Crickets were also provided a separate dish water for moisture. At the end of 48 hrs, the number of dead insects was recorded and removed. All remaining live insects were euthanized via CO_2 by our veterinary medical staff and stored at -80°C before shipping. Samples of all treatment diets were also taken during the study. At the completion of the trial, all samples were packed on dry ice and sent to Dairy One Forage Laboratory (730 Warren Rd., Ithaca, NY 14850 USA) for proximate and mineral analysis.

Gut loading trail 2

The 3 species of insects were each divided into 3 treatment groups with 3 replicates per group with 250 Crickets or Superworms per replicate and 500 mealworms per replicate. Treatment groups consisted of PS, MHC, and MBB. Cricket feeding and care was consistent with the methods used in Trail 1 (above). At the completion of the trial, all samples, including diet samples of PS, MBB, and MHC, were packed on dry ice and sent to Nestle Purina Analytical Laboratories (824 Gratiot, St. Louis, MO 63102 USA) for vitamin A analysis.

Statistics

All samples were compared only within species. All statistical analyses were performed by single-factor ANOVA. When the F-test was significant ($P \leq 0.05$), means separation was performed using Tukey HSD.

Results

Pre-trial mortality

The pre-trial mortality assessment (Table 2) showed that feeding MBB as a bedding substrate instead of in a separate dish for the larval insects caused a drastic increase in the mortality for both the mealworms and superworms over the normal 48 hour gut loading time frame (30.7% and 100%, respectively).

Gut-loading trial 1

Crickets

All results from the cricket portion of this trial can be found in Table 3. As the focus of this trial was to look at the gut-loading for the purposes of Ca and P, those results will be highlighted will be highlighted in the text. Crickets in the MBB group showed greater Ca levels (0.88%, $P < 0.001$) than any of the other groups. The proportion of Ca in the MHC was almost half of the proportion found in the MBB group (0.48%), and the amounts in each of the PS and OZTC groups (0.17% and 0.12%, respectively) were less than a quarter of the amount in the MBB group. Although the amount of P was greater in the MBB group than the PS or MHC groups, with the OZTC being similar to all values ($P = 0.01$), the range of difference was not physiologically significant (0.85-0.90%). However, the Ca:P ratio was greater in the crickets on the MBB diet (0.98, $P < 0.001$) than any other diets, followed by the MHC diet (0.57), and then the similar levels seen in the PS and OZTC diets (0.20 and 0.13, respectively).

Mealworms

The Ca levels in the mealworms (Table 4) were greater in the ones fed the MHC diet (1.05%, $P < 0.001$, Table 4), with the MBB mealworms containing half the level (0.55%) of the MHC mealworms, and the PS and OZTC mealworms being similarly lower than both other groups (0.15% and 0.14%). The P levels were not different between mealworms fed any of the gut-loading diets ($P = 0.09$), but the Ca:P ratio was greatest in the MHC mealworms (1.31, $P < 0.001$), followed by the MBB group (0.70), and the PS and OZTC groups (0.19 and 0.18, respectively).

Superworms

The Ca levels in the superworms fed the MHC and MBB diets (0.23% and 0.33%, respectively; Table 5) were greater ($P < 0.001$) than the levels seen in the superworms fed the PS (0.06%) and OZTC (0.07%) diets (Table 5). The P measured in the superworms fed the PS diet were greater (0.66%, $P < 0.001$) than those measured in any of the other diets (0.57-0.58%). The Ca:P ratio was similarly greater ($P <0.001$) in the MHC and MBB diets (0.41-0.57) than the PS and OZTC diets (0.09-0.13). Also, as seen in the pre-trial mortality study, there was a high death loss of superworms fed the MBB diet during this trial (16.34%), whereas there was no death loss seen when mealworms were fed any of the other diets in the trial.

Gut-loading trial 2

For many samples, vitamin A, measured as retinol and the esters, was below detectable levels (1.66 IU A/g DM; Table 6). None of the superworm samples had detectable vitamin A, and no vitamin

A was detected in the crickets fed MHC or MBB. Crickets fed the PS diet had 1.99 IU A/g DM. Mealworms all showed low levels of vitamin A (1.74-2.14 IU A/g DM), but there was no difference due to effect of diet.

Discussion

For captive insectivorous birds, reptiles, and amphibians, gut-loading has become an essential part of a balanced diet when feeding commercial feeder insects. The captive bred crickets, mealworms, and superworms being fed have inadequate Ca and vitamin A to meet the nutritional needs of these animals. The trials conducted for this study were to determine which gut-loading diet would be the best option for our collection. The PS diet was included as the control diet. When insects are not being gut-loaded, this is the diet on which the insects are maintained. This diet is not meant to have a gut-loading level of Ca or vitamin A, as shown in Table 1. The OZTC diet was included as part of our trial diets because our herpetology department wanted a diet that contained vegetable items that were naturally high in vitamin A and its precursor, beta-carotene. There is evidence that this type of diet would increase retinol levels in toads fed crickets gut-loaded in this manner (Odum *et al.*, 2015) without an additional gut-loading diet. This diet was designed to be fed to herbivorous reptiles in our collection, and thus the Ca:P ratio was 2.02 by design. The last two diet options, MHC and MBB, were both commercially available gut-loading diets with similar Ca and vitamin A concentrations. The difference between the two comes down to protein and fat concentration and ingredient list, with the MBB containing more CP and fat coming from fishmeal and spirulina instead of the corn, soybean meal, and porcine meal in the MHC.

The results from Trial 1 (Tables 2, 3, and 4) show that both the PS and OZTC diets produced insects with very low Ca:P ratios. This showed fairly conclusively that insects produced from feeding these two diets would lead the consuming animals to Ca and P imbalances that would be detrimental to their health. The results from the MHC and MBB fed insects were species specific. In crickets, the MBB diet produced a Ca:P ratio of approximately 1, while the MHC was significantly lower at about half of the MBB levels. Conversely, the mealworms did better on the MHC diet with a more desirable Ca:P ratio of 1.31 and the MBB diet producing a ratio half the level of the MHC. The superworms Ca:P ratios were similarly about 0.5 for both MBB and MHC fed crickets, which is well below a desired 1.0 or higher. This allowed us to make the decision that our final choice would be between the MBB and MHC diets, because the OZTC and PS diets would never be able to produce the Ca:P levels we need to have of 1.0 or higher. Also notable was the fact that, although there were some significant differences seen within some of the species with regards to CP and fat, these differences were not likely biologically significant and were similar to the values seen by Baker *et al.* (1998). This shows that even large differences in protein and fat concentrations in the feeds do not greatly alter the protein and fat concentrations in the insects, thus choosing a gut-loading diet is not dependent on these two measures as a factor.

With this information, the second trial eliminated the OZTC and focused on the vitamin A differences in insects fed the control diet (PS) and the MBB and MHC diets. Previously measured vitamin A values in non-gut-loaded adult crickets and mealworms has been shown to be 0.8 IU/g, and superworms had measured values of about 0.95 IU/g (Baker *et al*., 1998). However, gut-loaded crickets have been shown to reach levels as high as 14.59 IU/g when measuring retinol and β-carotene (Li *et al.*, 2009). The level of accurate detection of vitamin A at the lab we sent our samples was 1.66 IU/g. Therefore, several of the samples tested showed below detectable levels. Surprisingly, the only cricket group to have detectable concentration of vitamin A was the PS diet (1.99 IU/g). There was no detectable vitamin A concentration in any of the superworm diets, and although the mealworms all had measurable levels of vitamin A (1.74-2.14 IU/g), there was no significant difference between the diets fed. When making our decision regarding which diet to choose for our protocols, this information removed vitamin A as a deciding factor.

Considering all of this information, we were left with Ca:P concentration and mortality as our two main deciding factors. The mortality with the MBB diet did not have a clear explanation. When the problem was first seen, samples of the diet were sent back to the manufacture to ensure that it met specification on nutrient composition and particle size. They confirmed that the product met all specifications. It is possible that the higher fat content of the diet could have caused a problem with the worms on a physical level. As the worms would crawl through the product, they could be getting coated with the oils, which may be making it harder for them to respire due to clogged trachea tubules. However, there may be ingredient items which could be affecting the worms in a different manner.

Conclusion

From the data, our current protocols were amended so that we will now go back to using the MHC gut-loader diet when gut-loading our crickets. We will continue to use the PS as our receiving and sustaining diet for non-gut-loaded insects, and we will be able to use both the PS and MHC as substrate for the mealworms and superworms, as there has never been a history of increased mortality on these diets. In the future, we will be exploring the use of different insect dusting products to help elevate Ca and vitamin A provided to our collection animals via our feeder insects.

Acknowledgements

The authors would like to thank the keepers in our Bird and Herp/Fish department for their assistance in the set up and the daily care of the insects of the study. We would also like to thank our Veterinary Staff for their services in providing the humane euthanasia of all of the insects used in the study.

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Table 1. Nutritional analysis of various feeder insect gut loading diets

Parameter	PS[1]	MHC[2]	MBB[3]	OZTC[4]
DM %	89.6	90.5	93.8	11.0
CP %	33.2	19.7	33.1	21.8
Crude Fat %	3.4	3.9	18.1	3.30
Ash %	7.64	25.70	26.67	11.84
Ca %	1.22	9.33	8.80	1.29
P %	0.89	0.58	0.89	0.64
Ca:P Ratio	1.37	16.09	9.89	2.02
Mg %	0.24	0.24	0.25	0.28
K %	1.23	0.90	0.72	3.27
Na %	0.17	0.21	0.38	0.36
Fe mg/kg	169	314	376	211
Zn mg/kg	104	79	83	60
Cu mg/kg	17	15	12	7
Mn mg/kg	87	76	75	55
Vitamin A IU/g	7.06	16.32	14.04	-

[1] Purina Game Bird + Turkey Startena – Control Diet
[2] Mazuri® Hi Calcium Gut Loading Diet
[3] Mazuri® Better Bug® Gut Loading Diet
[4] Oregon Zoo Tortoise Chop Diet (16% Bok Choy, 16% Broccoli, 12% Collard Greens, 19% Kale, 16% Shredded Carrot, and 21% Ground Mazuri® Tortoise LS Diet; all on a DM Basis)

Table 2. Pre-trial mortality of mealworms (*Tenebrio molitor*) and superworms (*Zophobas morio*) fed Purina Game Bird + Turkey Startena or Mazuri® Better Bug® Gut Loading Diet as a bedding substrate or in a feeding dish for 48 hours.

Insect	PSB[1]	MBBB[2]	MBBD[3]
Mealworm Mortality %[4]	0.30	30.7	0.31
Superworm Mortality %[4]	0.00	100.00	0.83

[1] Purina Game Bird + Turkey Startena – Control Diet as Bedding Substrate
[2] Mazuri® Better Bug® Gut Loading Diet as Bedding Substrate
[3] Mazuri® Better Bug® Gut Loading Diet in Feeding Dish
[4] Mortality: # of dead insects at 48 hr/# of insects at 0 hr = % Mortality

Table 3. Nutritional analysis and mortality of crickets (*Acheta domesticus*) fed various gut loading diets over 48 hours.

Parameter	PS[1]	MHC[2]	MBB[3]	OZTC[4]	*P-values*
DM %	27.27[a]	27.37[a]	26.73[a]	24.90[b]	<0.001
CP %	60.63[ab]	59.90[b]	60.13[ab]	63.57[a]	0.04
Crude Fat %	24.47	26.30	25.83	25.30	0.29
Ash %	8.75[ab]	6.12[b]	6.89[b]	10.16[a]	<0.01
Ca %	0.17[c]	0.48[b]	0.88[a]	0.12[c]	<0.001
P %	0.86[b]	0.85[b]	0.90[a]	0.88[ab]	0.01
Ca:P Ratio	0.20[c]	0.57[b]	0.98[a]	0.13[c]	<0.001
Mg %	0.09[a]	0.09[a]	0.10[a]	0.08[b]	0.02
K %	1.17[ab]	1.15[b]	1.15[b]	1.24[a]	0.01
Na %	0.43[b]	0.42[b]	0.48[a]	0.50[a]	<0.001
Fe mg/kg	89.67[b]	81.67[b]	107.67[a]	66.00[c]	<0.001
Zn mg/kg	218.33[a]	203.00[b]	207.67[b]	225.67[a]	<0.001
Cu mg/kg	23.00	21.67	23.00	22.33	0.10
Mn mg/kg	45.00[a]	39.00[ab]	36.33[b]	34.67[b]	0.01
Mortality %[5]	5.60	5.73	7.73	6.13	0.40

[1] Purina Game Bird + Turkey Startena – Control Diet

[2] Mazuri® Hi Calcium Gut Loading Diet

[3] Mazuri® Better Bug® Gut Loading Diet

[4] Oregon Zoo Tortoise Chop Diet (16% Bok Choy, 16% Broccoli, 12% Collard Greens, 19% Kale, 16% Shredded Carrot, and 21% Ground Mazuri® Tortoise LS Diet; all on a DM Basis)

[5] Mortality: wt of dead crickets at 48 hr/wt of crickets at 0 hr = % Mortality

[abc]Values with similar superscripts within the same row are similar ($P \leq 0.05$)

Table 4. Nutritional analysis and mortality of mealworms (*Tenebrio molitor*) fed various gut loading diets over 48 hours.

Parameter	PS[1]	MHC[2]	MBB[3]	OZTC[4]	*P-values*
DM %	41.37[a]	41.50[a]	40.00[b]	31.90[c]	<0.001
CP %	53.00[b]	53.57[b]	56.60[a]	56.33[a]	<0.001
Crude Fat %	32.87[a]	31.80[ab]	31.57[ab]	30.47[b]	0.03
Ash %	4.32	5.74	4.69	5.07	0.06
Ca %	0.15[c]	1.05[a]	0.55[b]	0.14[c]	<0.001
P %	0.79	0.80	0.79	0.78	0.09
Ca:P Ratio	0.19[c]	1.31[a]	0.70[b]	0.18[c]	<0.001
Mg %	0.22[a]	0.22[a]	0.20[b]	0.19[b]	<0.001
K %	1.01[a]	1.00[a]	0.94[b]	1.00[a]	0.02
Na %	0.15[ab]	0.13[b]	0.15[ab]	0.18[a]	<0.01
Fe mg/kg	79.00	86.33	75.33	67.00	0.10
Zn mg/kg	176.33	127.33	136.67	138.33	0.14
Cu mg/kg	23.00[a]	20.00[bc]	21.00[b]	19.33[c]	<0.001
Mn mg/kg	24.33[a]	18.67[b]	13.67[c]	13.67[c]	<0.001
Mortality %[5]	1.22	1.90	2.04	1.67	0.77

[1] Purina Game Bird + Turkey Startena – Control Diet

[2] Mazuri® Hi Calcium Gut Loading Diet

[3] Mazuri® Better Bug® Gut Loading Diet

[4] Oregon Zoo Tortoise Chop Diet (16% Bok Choy, 16% Broccoli, 12% Collard Greens, 19% Kale, 16% Shredded Carrot, and 21% Ground Mazuri® Tortoise LS Diet; all on a DM Basis)

[5] Mortality: wt of dead mealworms at 48 hr/wt of mealworms at 0 hr = % Mortality

[abc]Values with similar superscripts within the same row are similar ($P \leq 0.05$)

Table 5. Nutritional analysis and mortality of superworms (*Zophobas morio*) fed various gut loading diets over 48 hours.

Parameter	PS[1]	MHC[2]	MBB[3]	OZTC[4]	*P-values*
DM %	42.03^{b}	42.77^{b}	47.37^{a}	37.27^{c}	<0.001
CP %	48.53	48.80	49.30	48.83	0.4
Crude Fat %	44.07^{ab}	43.87^{b}	45.67^{a}	44.63^{ab}	0.05
Ash %	6.95^{a}	4.65^{b}	3.76^{b}	6.47^{a}	<0.001
Ca %	0.06^{b}	0.23^{a}	0.33^{a}	0.07^{b}	<0.001
P %	0.66^{a}	0.57^{b}	0.58^{b}	0.57^{b}	<0.001
Ca:P Ratio	0.09^{b}	0.41^{a}	0.57^{a}	0.13^{b}	<0.001
Mg %	0.10	0.11	0.11	0.10	0.36
K %	0.65	0.70	0.66	0.67	0.12
Na %	0.09	0.12	0.10	0.11	0.08
Fe mg/kg	34.67^{b}	39.00^{ab}	44.33^{a}	33.67^{b}	<0.01
Zn mg/kg	77.00	76.33	76.67	75.67	0.79
Cu mg/kg	9.67	9.00	9.67	9.00	0.12
Mn mg/kg	8.67	9.33	9.00	8.00	0.15
Mortality %[5]	0.00^{b}	0.00^{b}	16.34^{a}	0.00^{b}	<0.001

[1] Purina Game Bird + Turkey Startena – Control Diet

[2] Mazuri® Hi Calcium Gut Loading Diet

[3] Mazuri® Better Bug® Gut Loading Diet

[4] Oregon Zoo Tortoise Chop Diet (16% Bok Choy, 16% Broccoli, 12% Collard Greens, 19% Kale, 16% Shredded Carrot, and 21% Ground Mazuri® Tortoise LS Diet; all on a DM Basis)

[5] Mortality: wt of dead superworms at 48 hr/wt of superworms at 0 hr = % Mortality

[abc]Values with similar superscripts within the same row are similar ($P \leq 0.05$)

Table 6. Vitamin A[1] analysis of crickets (*Acheta domesticus*), mealworms (*Tenebrio molitor*), and superworms (*Zophobas morio*) fed various gut loading diets over 48 hours.

	Vitamin A (IU/g DM)			
Species	**PS[1]**	**MHC[2]**	**MBB[3]**	***P-values***
Cricket	1.99	ND[5]	ND[5]	-[6]
Mealworm	1.74	2.14	1.77	0.44
Superworm	ND[5]	ND[5]	ND[5]	-[6]

[1] Vitamin A assay measured as retinol and esters

[2] Purina Game Bird + Turkey Startena – Control Diet

[3] Mazuri® Hi Calcium Gut Loading Diet

[4] Mazuri® Better Bug® Gut Loading Diet

[5] Values that registered as present but below the level of detection (1.66 IU/g DM) are indicated as ND.

[6] Statistical analysis not done due to ND values.