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SPECIAL ISSUE: *Insects on the monogastric menu*

Utilising yellow mealworm larvae reared on deoxynivalenol-contaminated wheat as a feed ingredient for poultry diets

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RESEARCH ARTICLE

Abstract

This study determined if yellow mealworm larvae (YML) grown on deoxynivalenol (DON) contaminated wheat would affect broiler chicken performance. The YML were reared on wheat with low (LDW; 630 µg/kg) or high (HDW; 30,730 µg/kg) DON concentrations. The DON concentrations in the dried insect meals were 0 or 17.5 µg/kg for YML grown on LDW and HDW, respectively. Seventy-five male Ross 708 broilers were randomly placed into 15 cages and reared on one of three diets from day 1-35 (five replications/treatment). On day 14, broiler numbers were reduced to four per cage. The diets consisted of a control containing no YML meal (CD) and two diets containing 5% YML meal produced on either LDW (LMD) or HDW (HMD). Feed intake and body weight (BW) were measured over the duration of the experiment to calculate feed to gain ratio (F:G). On day 35, all birds were slaughtered and dissected to collect weights of the breasts, thighs, drums, wings, abdominal fat pads, and organs. Crude protein retention was higher in birds fed the LMD and HMD treatments compared to CD (P=0.0091). Dry matter retention was higher in the HMD diet compared to the CD and LMD diets (P=0.0046). Feed intake was lower in birds fed HMD compared to CD and LMD (P=0.0031) although final BW was not reduced. In conclusion, dietary inclusion of YML did not affect the growth, meat yield or organ weights of the birds. The YML reared on DON-contaminated wheat (up to 30,730 ug/kg) and included in broiler diets at 5% could be an effective means of converting salvage wheat into a safe and sustainable source of protein.

Keywords: broiler, insect, mycotoxin, growth performance

1. Introduction

Fusarium contamination of crops is a global problem for food and feed production. *Fusarium* is a genus of fungi of which more than 16 species cause a disease in cereal crops called *Fusarium* head blight (FHB; Dweba *et al.*, 2017). *Fusarium* species produce a variety of mycotoxins, such as deoxynivalenol (DON) that can have toxic effects when consumed by livestock (Ferrigo *et al.*, 2016). With current global warming effects related to increasing temperatures and unstable weather, major outbreaks of FHB will likely be more frequent in the future, especially during conditions with high humidity (Dweba *et al.*, 2017).

Fusarium graminearum is the most prevalent species in Western Canada and is harmful due to its production of mycotoxins such as DON, 3-acetyl-DON (3ADON), and 15-acetyl-DON (15ADON) (Tittlemier *et al.*, 2019). Due to the effects DON can have in animals, the Canadian Food Inspection Agency (CFIA) have set limits on the concentrations allowed in food and feed. The CFIA have further proposed limits on mycotoxins in single feed ingredients which would limit DON concentrations to a maximum of 10,000 µg/kg (CFIA, 2018). Any crops measuring above these concentrations would be condemned as salvage which have no economic value, representing a loss to producers, or would be blended into crops with low contamination. If large quantities of salvage crops are produced due to outbreaks of fungal disease, blending will not be an option, and crops would be burnt or buried which can have negative environmental effects.

One method to utilise DON-contaminated crops could be to rear insects such as the yellow mealworm larvae (YML; *Tenebrio molitor*) on the grain. Studies by Van Broekhoven *et al.* (2017) and Ochoa-Sanabria *et al.* (2019) fed YML DON-contaminated wheat, at concentrations up to 12,000 µg/kg, and reported no impacts on larval survival, fecundity, or growth. Van Broekhoven *et al.* (2017) and Ochoa-Sanabria *et al.* (2019) reported excretion of DON in frass at 14 to 41% and 6-15% of ingested concentrations respectively. Van Broekhoven *et al.* (2017) did not detect the presence of any mycotoxins in the YML reared on DON-contaminated wheat, while Ochoa-Sanabria detected approximately 130 µg/kg DON, which was still much less than the ingested concentrations indicating that they may have some means of metabolising DON.

Yellow mealworms in their larval stage that were reared on DON-contaminated wheat are rich in crude fat (CF) and crude protein (CP) at 34.4% and 50.2%, respectively (Ochoa-Sanabria *et al.*, 2019). YML also have an amino acid profile similar to soybean meal, with the exception of lower concentrations of methionine in YML, which could make them excellent as both a protein and energy source when included in animal diets (Bovera *et al.*, 2016). These observations indicate that YML may have potential when reared on DON-contaminated crops to be a cost effective, highly nutritious, and safe ingredient for use in poultry diets. The aim of this study was to determine if YML raised on low or high DON wheat could be used as a feed ingredient for broiler diets, and investigate any effects that may occur in growth, survival, and efficiency.

2. Materials and methods

Wheat samples

Two sources of Canadian Western Red Spring wheat were purchased from producers in Saskatchewan, Canada that had low or high infection with *Fusarium*. Mycotoxin panel testing was conducted at Prairie Diagnostic Services (Saskatoon, Canada) using HPLC-tandem MS. The mycotoxin panel included mycotoxins DON, Nivalenol (NIV), 3ADON, and 15ADON. The low and high DON wheat (LDW and HDW) had 630 and 30,730 µg/kg DON, respectively.

Yellow mealworm larval production

Tenebrio molitor beetles sourced from Bug Order Inc. (Morinville, Canada) were placed into 50×32×15 cm bins containing either LDW or HDW which was fed whole kernel. Approximately 500 beetles were placed in each bin. No supplementary or wet feeds were provided. Beetles were left to breed and lay eggs which then hatched into larvae that consumed the wheat. All bins had paper towels laid on top of the wheat which were misted with water 3 times per week to maintain humidity. All larvae weighing a minimum of 110 mg were harvested, fasted for 24 hours, rinsed with water to remove dust, then euthanised by freezing stored at -20 °C. Bins were harvested weekly until all larvae were removed. The rearing room was maintained with an 8-hour photoperiod, a temperature between 22-26 °C and a minimum relative humidity of 50%. The frozen larvae were oven-dried at 110 °C for 40 minutes. The dried larvae were then ground using a Cuisinart Model CH-4DCC food processor (Stamford, CT, USA) to produce mealworm meal and stored at -20 °C.

Mycotoxin and nutritional analysis of yellow mealworm larvae meal

CP of the YML meals was determined using the Dumas-Combustion method by placing duplicate 0.11 g samples in gel capsules and combusting at 800 °C, (AOAC, 1997; method 990.03). CF was analysed using a Goldfisch extraction apparatus model 3500 (Kansas City, MO, USA) by processing and extracting 1.3 g of samples in duplicate for 5 hours using the ethyl ether extraction gravimetric method (AOAC, 2000; method 920.39). Moisture content was analysed in 2.0 g samples in aluminium dishes that were in an oven at 135 °C for 2 hours. The samples were then cooled, sealed, and weighed again to determine the loss from drying (AOAC, 1990; method 930.15). Acid detergent fibre (ADF) was determined by running duplicate 0.5 g samples in an ANKOM^{200} fiber analyser (New York, NY, USA; AOAC, 1990; method 973.18). The remaining portions of the samples were used to estimate CP and determine acid detergent insoluble nitrogen (ADIN). Chitin was calculated from ash-free ADF and ADIN as described by Marono *et al.* (2015). Proximate analyses are summarised in Table 1. The amino acid profile was determined by the nutrition lab in the Faculty of Agricultural and Food Sciences at University of Manitoba, (Winnipeg, Canada; AOAC, 1995; method 994.12) utilising the S2100 Sykam amino acid analyser (Eresing, Germany; Table 2). Amino acid digestibility values by Matin (2019) were used to estimate digestible amino acid profiles of the YML meals. The YML meal was analysed for mycotoxins as described by Ochoa-Sanabria *et al.* (2019) at Prairie Diagnostic Services. DON was the only mycotoxin detected in the YML meal produced on HDW at concentrations of 17.5 µg/kg while no mycotoxins were detected in YML meal produced on LDW. The panel included: 3ADON, 15ADON, aflatoxin B1, zearalenol, DON, fumonisins B1 and B2, NIV, ochratoxin A, T-2 toxin, HT-2 toxin, and zealalenone.

Diets

A research exemption was obtained from the Animal Feed Division of the Canadian Food Inspection Agency to include the YML meals in the diets. Approval was also obtained from the Animal Research Ethics Board at the University

Table 1. Proximate analyses of yellow mealworm larvae grown on low or high deoxynivalenol (DON) wheat (% fresh matter).

	Treatments[1]	
Parameter	**LML**	**HML**
Dry matter	94.65	93.60
Crude protein[2]	45.28	47.71
Crude fat	38.41	35.66
Acid detergent fibre	6.94	7.71
Acid detergent insoluble nitrogen	3.69	4.18
Chitin[3]	3.25	3.53

[1] LML = yellow mealworm larvae meal produced on low DON wheat (630 µg/kg DON); HML = yellow mealworm larvae meal produced on high DON wheat (30,730 µg/kg DON).
[2] Crude protein was analysed using the Dumas combustion method.
[3] Chitin calculated based on Marono *et al.* (2015).

Table 2. Amino acid profiles of yellow mealworm meal produced on low or high deoxynivalenol (DON) wheat (% fresh matter).[1]

	Amino acid profile	
Parameter	**LML**	**HML**
Aspartate	3.638	4.009
Threonine	1.682	1.852
Serine	2.110	2.351
Glutamine	5.293	5.764
Proline	2.783	2.920
Glycine	2.315	2.412
Alanine	3.770	4.039
Cysteine	0.371	0.399
Valine	2.947	3.004
Methionine	0.573	0.617
Isoleucine	2.041	2.069
Leucine	3.316	3.534
Tyrosine	3.294	3.560
Phenylalanine	1.635	1.794
Histidine	2.857	3.661
Lysine	2.408	2.668
Arginine	2.469	2.651
Tryptophan	0.440	0.491

[1] LML = yellow mealworm larvae meal produced on low DON wheat (630 µg/kg DON); HML = yellow mealworm larvae meal produced on high DON wheat (30,730 µg/kg DON).

of Saskatchewan to conduct this experiment according to the recommended guidelines of the Canadian Council of Animal Care (2009).

Diets were formulated (Table 3) to meet or exceed Ross 708 (2019) performance standards (Aviagen, 2019) using the results of the analyses and were produced as a mash. Corn that contained undetectable concentrations of DON and soybean meal made up the majority of the diets. The treatments consisted of the control containing no insect meal (CD), containing 5% YML grown on LDW (LMD), and the last diet containing 5% YML grown on HDW (HMD). Porcine meat meal was included in the control diets to meet the valine requirements. The meat meal and the insect meals had similar compositions with the exception of glycine, which was present in higher concentrations in the meat meal. Diets were fed in two phases with a starter/grower fed for the first three weeks (days 1-21) and a finisher fed during the last two weeks (days 21-35). The finisher included titanium dioxide (TiO_2) as a marker to determine CP retention and DM digestibility. Nutrient composition (DM, moisture, CP, calcium, phosphorus, and sodium) of the diets were determined by Central Testing Laboratory Ltd. (Winnipeg, Canada; AOAC, 1951; method 935.13 AOAC, 1990; method 968.08; AOAC, 1990; method 930.15; AOAC, 1996; method 985.01; AOAC, 1997; method 990.03).

Broiler performance

Seventy-five male Ross day-of-hatch 708 broilers were obtained from Lilydale Hatchery (Edmonton, Canada) and were randomly split into groups of five birds and placed into one of 15 cages (five replications/treatment, 46 cm high × 51 cm wide × 51 cm long) in a temperature-controlled room at the University of Saskatchewan Poultry Research Center (Saskatoon, Canada). Bird numbers were reduced to four birds/treatment on day 14 to comply with space requirements for housing, the removed birds were selected randomly.

The initial temperature of the room housing the broilers was 34 °C. The temperature was gradually reduced to 22.3 °C by day 28. Light intensity was initially 40 lux for a 22-hour photoperiod which was reduced to 20 hours on day 2, 20 lux on day eight, then to 10 lux on day nine. Birds had free access to feed (tray feeders) and water (nipple drinkers) for the duration of the trial. All mortalities and culled birds were necropsied for cause of death or morbidity by an independent veterinary service (Prairie Diagnostic Services). Broiler body weight (BW, g) and feed intake (FI, g) was measured at 1, 7, 14, 21, 28, and 35 days. Feed to gain ratios (g feed/g weight gain; F:G) were calculated per cage.

Excreta

Excreta was collected over a period of 48 hours on days 33 and 34 of the trial. Samples were pooled, dried at 50 °C for 72 hours and ground using a Retsch ZM 100 Ultra Centrifugal Mill (Haan, Germany) using a 1000-micron

Table 3. Ingredients (% fresh matter) and nutrient composition (% fresh matter) of experimental diets.[1]

Ingredients	Starter diets (days 1-21)			Finisher diets (days 21-35)		
	CD	LMD	HMD	CD	LMD	HMD
Corn	60.081	56.753	57.045	65.965	67.805	68.100
Soybean meal	29.655	32.624	32.334	22.861	21.178	20.566
Meat meal	5.973	0.0	0.0	5.942	1.968	2.238
LML	0.0	5.000	0.0	0.0	5.000	0.0
HML	0.0	0.0	5.000	0.0	0.0	5.000
Dicalcium phosphate	1.543	1.744	1.755	0.291	1.008	0.969
Calcium carbonate	0.0	1.331	1.128	0.409	0.807	0.779
Canola oil	1.000	1.000	1.000	2.864	0.422	0.546
Poultry vit/min premix[2]	0.500	0.500	0.500	0.500	0.500	0.500
Titanium dioxide	0.0	0.0	0.0	0.300	0.300	0.300
Dl-methionine	0.368	0.356	0.355	0.274	0.279	0.280
Lysine HCl	0.315	0.345	0.258	0.197	0.228	0.224
Salt	0.177	0.265	0.349	0.180	0.288	0.284
Choline chloride	0.165	0.165	0.165	0.150	0.150	0.150
L-threonine	0.149	0.115	0.111	0.068	0.067	0.064
L-isoleucine	0.038	0.0	0.0	0.0	0.0	0.0
Valine	0.035	0.0	0.0	0.0	0.0	0.0
Nutrient concentrations (% as fed)						
Metabolisable energy (MJ/kg)	13.0	13.0	13.0	13.4	13.4	13.4
Dry matter	87.60	87.32	87.56	87.26	87.00	87.54
Crude protein	21.66	20.92	21.25	18.53	18.74	17.52
Crude fat	4.05	5.42	5.28	5.92	5.24	5.24
Digestible lysine	1.28	1.28	1.28	1.03	1.03	1.03
Digestible methionine	0.67	0.66	0.66	0.55	0.55	0.55
Calcium	1.12	1.02	1.01	1.04	0.87	1.00
Phosphorus	1.02	0.85	0.81	0.69	0.68	0.75
Sodium	0.14	0.18	0.16	0.13	0.15	0.17

[1] LML = yellow mealworm larvae meal produced on low deoxynivalenol (DON) wheat (630 µg/kg DON); HML = yellow mealworm larvae meal produced on high DON wheat (30,730 µg/kg DON). CD = control diet; LMD = 5% inclusion yellow mealworm reared on low DON wheat; HMD = 5% inclusion yellow mealworm reared on high DON wheat.

[2] One kg premix contains 2,200,000 IU vitamin A, 440,000 IU vitamin D, 6,000 IU vitamin E, 400 mg menadione, 300 mg thiamine, 1,200 mg riboflavin, 800 mg pyridoxine, 4 mg vitamin B12, 12,000 mg niacin, 2,000 mg pantothenic acid, 120 mg folic acid, 30 mg biotin. 2,000 mg copper, 16,000 mg manganese, 160 mg iodine, 16,000 mg zinc, 60 mg selenium, 100,000 mg calcium carbonate, 125 mg antioxidant, 807,879 mg wheat middlings (DSM Nutritional Products Canada Inc. ON, Canada).

screen. This was used for TiO_2, CP, and DM analyses to determine DM and CP retention.

Carcass traits

Meat yield and organ weights were collected from all birds on day 35 of the trial. Birds were euthanised by T-61 euthanasia solution injected into the left brachial vein. The carcasses were then scalded in 66±2 °C water for 25 seconds and feathers plucked by hand. Breasts, skin-on-bone-in wings, skin-on-bone-in drums, bone-in-skinless thighs, abdominal fat pads, livers, spleens, and bursa were removed from the carcasses and weighed.

Crude protein and dry matter retention

Excreta and feed samples were measured for CP and DM retention in duplicate for TiO_2 using a protocol adapted from Myers *et al.* (2004). Titanium dioxide was measured by placing samples weighing 0.5 and 1.0 g for excreta and feed respectively into 250 ml macro-Kjeldahl digestion tubes. A catalyst tablet containing 3.5 g of K_2SO_4 and 0.4 g of $CuSO_4$ was added to each tube. Thirteen millilitres of concentrated sulfuric acid were added, and samples were digested at 420 °C for 2 hours. Samples were allowed to cool for 30 minutes, 10 ml 30% H_2O_2 (v/v) was added and left to cool for 30 minutes. Samples were transferred into

125 ml Erlenmeyer flasks and distilled water was added to bring the liquid weight up to 100 g. Samples were filtered using 541 Whatman paper then transferred into cuvettes and placed into a spectrophotometer set to 410 nm to measure absorbance. A standard was made using 0.2 g TiO_2 with the same procedure and was serial diluted using 1:1 standard solution to distilled water and measured in the spectrophotometer to determine the standard curve to which the samples were compared to determine TiO_2 concentrations.

CP of excreta samples was determined using the Dumas-Combustion method by placing duplicate 0.11 g samples of YML meal in gel capsules and combusting at 800 °C, (AOAC, 1997; method 990.03). Moisture in excreta was analysed by placing 2.0 g samples into aluminium dishes and placing into a 135 °C oven for 2 hours. The samples were then cooled, sealed, and weighed again to determine loss from drying from which dry matter was calculated (AOAC, 1990; method 930.15). The equation used to determine retention was: % retention = 100 – (100 × [% marker in diet/% marker in excreta] × [% nutrient in excreta/% nutrient in diet])

Statistical analyses

All data were analysed as a one-way ANOVA in a complete randomised design using MIXED procedure in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). There were 5 replicates for the BW, FI, F:G, CP and DM retention (unit was the cage). For meat yield and organ weights there were 19, 19 and 16 reps for CD, LMD, and HMD, respectively. Results were tested for normality using the Shapiro-Wilk test. Treatment means were compared using the Tukey-Kramer HSD test at $P<0.05$ determining significance. All tests conducted on the YML meal used a pooled sample, thus were not statistically analysed but the values are reported.

3. Results

Broiler performance

There were zero mortalities in the first 14 days of the experiment after which the bird number was reduced from five to four birds per cage from five to meet animal care requirements for space. One broiler from each on the LMD and HMD diets was culled for leg issues at 21 days and 30 days, respectively bringing mortality/morbidity to 0, 5, and 5% for CD, LMD, and HMD respectively. Growth performance of the broilers are summarised in Table 4. Feed intake was reduced in birds fed HMD (261.8 g) between days 8-14 ($P=0.002$) compared to 292.0 and 293.6 in CD and LMD respectively. Feed intake for days 1-35 ($P=0.003$) was 2,469.0 g in birds fed HMD compared to 2,709.1 and 2,762.4 g in CD and LMD (Table 4). Feed intake tended to be reduced in birds fed HMD during days 15-21 ($P=0.094$) and 29-35 ($P=0.074$). Live weights of birds (Table 4) were reduced in birds fed HMD on day 14 ($P=0.029$) averaging 344.2 while broilers fed CD and LMD averaged 370.2 and 373.4 g. There was a tendency for broilers fed HMD to be lighter on day 21 ($P=0.082$) although final body weights were not different ($P=0.204$). The diets did not have an impact on F:G ratio in the broiler chickens ($P>0.10$; Table 4).

Meat and organs

Diets did not influence meat yield nor organ weights in the male broilers (Table 5; $P>0.10$), although fats pads (g) of birds fed the CD tended to be heavier than those on LMD and HMD, respectively ($P=0.055$). Gizzard weights (% BW) had a tendency to be heavier relative to body weight in broilers fed HMD (Table 5). Four mis-sexed females were removed from this analysis in total: one from CD and three from HMD.

Crude protein and dry matter retention

Dry matter and CP retention results are displayed in Table 6. CP retention was increased in broilers fed LMD and HMD relative to those fed CD ($P=0.0091$). Retention in broilers fed LMD and HMD was 68.17 and 68.61% respectively while CD was 66.17%. Dry matter retention was increased in HMD at 76.80% compared to LMD and CD at 74.93 and 74.88% respectively ($P=0.0046$).

4. Discussion

DON was the only mycotoxin detected in the wheat (30,730 ug/kg) that was also identified in the YML meal (17.5 ug/kg) produced on HDW. This was lower than the 130 µg/kg Ochoa-Sanabria *et al.* (2019) detected when feeding up to 12,000 µg/kg DON to YML. This could be due to genetic differences in the breeding colonies, as the YML were obtained from two different sources. Van Broekhoven *et al.* (2017) did not detect any presence of mycotoxins in YML grown on up to 8,000 µg/kg DON. The exact mechanisms used by the YML to metabolise DON are currently unknown, it is most likely a mixture of microbial and gut enzymatic activity. Genta *et al.* (2006) noted an adaptation of the gut microbiota in YML and that certain digestive enzymes disappeared in larvae treated with antibiotics indicating that they might have been microbial in origin. These microbes may also play a role in the adaptation process to exposure to mycotoxins.

The primary objective of this study was to determine if YML reared on high DON wheat (30,730 µg/kg) were safe for consumption when included in poultry diets and if any effects on growth performance, organ weights, and organ size could be observed. Final body weight and FI in the broilers were lower by 14.5 to 19.9% and 13.4 to 22.6% respectively when compared to the performance objective

Table 4. Effect of the dietary inclusion of yellow mealworm larval meal produced on low or high deoxynivalenol (DON) wheat on body weight gain, feed intake and feed-to-gain ratios of broilers.[1]

Period	Treatment[2]			SEM	*P*-value
	CD	LMD	HMD		
Body weight (g)					
Day 1	40.0	39.4	38.4	0.86	0.775
Day 7	136.0	131.4	130.3	2.37	0.236
Day 14	370.2[a]	373.4[a]	344.2[b]	7.31	0.003
Day 21	723.8	788.9	698.6	26.47	0.082
Day 28	1,232.9	1,252.1	1,173.5	31.69	0.229
Day 35	1,908.7	1,877.0	1,786.1	47.19	0.204
Feed intake (g)					
Day 1-7	136.3	137.2	135.1	2.26	0.752
Day 8-14	292.0[a]	293.6[a]	261.8[b]	5.41	0.002
Day 15-21	485.2	510.6	454.1	16.62	0.094
Day 22-28	742.1	768.3	710.3	22.34	0.223
Day 29-35	1,053.6	993.8	951.8	28.48	0.074
Day 1-35	2,709.1[a]	2,762.4[a]	2,469.0[b]	45.43	0.003
Feed-to-gain ratios					
Day 1-7	1.422	1.497	1.485	0.0288	0.190
Day 8-14	1.247	1.215	1.225	0.0175	0.438
Day 15-21	1.375	1.252	1.286	0.0481	0.213
Day 22-28	1.480	1.609	1.500	0.0874	0.593
Day 29-35	1.562	1.594	1.552	0.0301	0.606
Day 1-35	1.448	1.443	1.440	0.0134	0.413

[1] Mean of replicates (n=5) per treatment presented on a per bird basis. Different letters indicate statistical difference: $P<0.05$.

[2] CD = control diet; LMD = 5% inclusion yellow mealworm reared on low DON wheat; HMD = 5% inclusion yellow mealworm reared on high DON wheat.

set out by Aviagen (2019). This was likely at least partially due to the physical form of the feed which was fed as a mash instead of a crumble or pellet which have been shown to improve BW, FI, and F:G ratios in broilers (Abdollahi *et al.*, 2018). Feed intake was reduced in broilers fed HMD during days 8-14 which likely led to the reduction in BW measured on day 14. Feed intake also had a tendency to be lower during days 15-21 and days 29-35. This may be indicative of the presence of DON-like metabolites or a modified mycotoxin present in the YML which could be accumulating through the mechanisms used in detoxification. Due to the difference in structure of the molecules, the mycotoxin would not be detected when using traditional means (Freire and Sant'Ana, 2018). The broilers fed CD and LMD had similar growth performance throughout the trial which agrees with the results found by Biasato *et al.* (2018). Elahi *et al.* (2020) also found no differences in broiler performance with inclusion of 0, 2, 4, and 8% YML meal in broiler diets. Bovera *et al.* (2016) however, found improved growth performance with reductions in feed intake and F:G ratios observed when completely replacing soybean meal with YML meal. The numerical differences between treatments, such as BW, are large with broilers fed CD weighing 1,908.7 g compared to broilers fed HMD weighing 1,786.1 g. Increasing replicates may have resulted in more statistical differences being observed. Constraints in space and time resulted in limitations in the number of YML that were able to be produced for the experiment. Most studies evaluating YML meal as a feed ingredient have formulated diets based on total amino acids which is not ideal when formulating for broiler chickens (Elahi *et al.*, 2020). Matin (2019) recently published results pertaining to amino acid digestibility of various insect species including YML, therefore more research formulating based on digestible amino acid profiles of insects will likely take place in the future.

CP retention was increased in broilers fed LMD and HMD relative to those fed CD. Improved CP retention has been associated with a reduction in abdominal fat deposition in broilers (Rao *et al.*, 2018). CP retention in CD was 66.17% which is higher than 53.5 to 57.2% with 0 to 9% inclusion of meat and bone meal in diets reported by Bolarinwa *et al.* (2012). Dry matter retention was increased in broilers

Table 5. Effects of dietary inclusion of yellow mealworm larval meal produced on low or high deoxynivalenol (DON) wheat on meat yield and organs weights of male broiler chickens.[1]

Parameter	Treatment[2]			SEM	*P*-value
	CD (n=19)	LMD (n=19)	HMD (n=16)		
Body weight (BW) (g)	1,916.6	1,886.6	1,789.4	59.04	0.329
Breasts (g)	434.7	453.8	414.7	21.37	0.470
Breasts (% BW)	22.6	23.8	23.0	0.54	0.263
Thighs (g)	200.0	192.8	186.5	6.96	0.426
Thighs (% BW)	10.4	10.2	10.4	0.14	0.419
Drums (g)	176.1	175.0	166.4	5.15	0.392
Drums (% BW)	9.2	9.3	9.3	0.14	0.835
Wings (g)	145.6	145.7	141.3	4.37	0.749
Wings (% BW)	7.6	7.7	7.9	0.11	0.158
Fat pad (g)	21.9	17.3	17.5	1.48	0.055
Fat pad (% BW)	1.15	0.92	0.97	0.077	0.103
Bursa (g)	3.8	3.6	3.6	0.22	0.734
Liver (g)	46.7	45.4	42.2	1.52	0.131
Liver (% BW)	2.45	2.42	2.37	0.067	0.683
Spleen (g)	1.9	1.7	1.9	0.10	0.270
Proventriculus (g)	6.8	6.6	6.7	0.20	0.655
Gizzard (g)	22.5	23.6	23.5	0.56	0.355
Gizzard (% BW)	1.18	1.28	1.33	0.044	0.092

[1] Mean of replicates. Different letters indicate significance: $P<0.05$. n=number of birds.

[2] CD = control diet; LMD = 5% inclusion yellow mealworm reared on low DON wheat; HMD = 5% inclusion yellow mealworm reared on high DON wheat.

Table 6. Effect of dietary inclusion of yellow mealworm larval meal produced on low or high deoxynivalenol (DON) wheat on dry matter and crude protein retention in broiler chickens.[1]

Parameter	Treatment[2]			SEM	*P*-value
	CD	LMD	HMD		
Dry matter (%)	74.88^{b}	74.93^{b}	76.80^{a}	0.37	0.0046
Crude protein (%)	66.17^{b}	68.17^{a}	68.61^{a}	0.49	0.0091

[1] Mean of replicates (n=5). Different letters indicate statistical difference: $P<0.05$.

[2] CD = control diet; LML = yellow mealworm larvae meal produced on low DON wheat (630 µg/kg DON); HML = yellow mealworm larvae meal produced on high DON wheat (30,730 µg/kg DON).

fed HMD, although broiler performance was not improved, the exact reasons for this increase are unknown.

Meat yield and organs were not affected by the diets when analysed on an absolute weight or as a percentage of live weight basis, indicating no effects of carcass traits. This agrees with research Biasato *et al.* (2018), and Elahi *et al.* (2020) where YML inclusion in broiler diets ranging from 0 to 15% had no observed effects on carcass traits. Elahi *et al.* (2020) observed no effects of YML meal on meat quality when included in broiler diets. The tendency for the YML meals to reduce abdominal fat pad weights may in part be caused by the hypolipidemic and hypocholesterolemic properties of chitin that can result in reductions in body fat of broilers (Gasco *et al.*, 2018). Marono *et al.* (2017) reported lower serum cholesterol and triglyceride concentrations in layer hens which were attributed to chitin being able to bind bile acids and free fatty acids in the gastrointestinal tract.

Chitin can also act as a prebiotic to increase *Lactobacillus* populations in the gastrointestinal tract (Islam and Yang, 2017) which has been observed to reduce carcass fat of broilers (Kalavathy *et al.*, 2008).

In conclusion, these results suggest that YML grown on wheat contaminated with DON up to 30,730 ug/kg can be used as an effective feed ingredient for use in poultry production. Larger scale experiments with high inclusion levels of YML should be conducted to further assess safety and confirm lack of impact on performance. Further research is required to establish how YML metabolise DON, at the current level of inclusion there was no effect on poultry production.

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