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EFFICACY OF ZEROFLY® HERMETIC AND POLYPROPYLENE STORAGE BAGS FOR CONSERVATION OF MAIZE IN LAYER POULTRY FARMS IN GHANA

Opoku B[1], Osekre EA[1], Opit GP[2], Bosomtwe A[2,3] and K Adomako[4]*

Kawaku Adomako

*Corresponding author email: adomakokwaku5@gmail.com

[1]Department of Crop and Soil Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

[2]Department of Entomology and Plant Pathology, Oklahoma State University, 127 Noble Research Center, Stillwater, Oklahoma 74078, USA

[3]CSIR–Plant Genetic Resources Research Institute, Bunso, Ghana

[4]Department of Animal Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

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ABSTRACT

Maize forms a key component in poultry feed formulation in Ghana. Nonetheless, there is an existing challenge of low-quality maize due to insect infestation and mycotoxin contamination resulting in poor chicken growth and performance including diminished egg laying. This study compared the effectiveness of ZeroFly® Hermetic (ZFH) storage bags to polypropylene (PP) bags to mitigate insect pest and mycotoxin levels over a 6-month storage period. The study was conducted in three-layer poultry farms in Dormaa Ahenkro, Bono Region, Ghana. One storehouse was used in each poultry farm. In each storehouse, there were thirty-two 100-kg-capacity ZFH and same number of 100-kg PP bags, each containing 100 kg of maize, that is, altogether sixty-four 100-kg-capacity bags in each storehouse. The experiment was set up in a Randomized Complete Block Design with two treatments (ZFH and PP bags), three replications (storehouses), and two sub-replications. In each storehouse, two bags in each treatment were destructively sampled monthly. After 6 months of storage, the predominant insects found were *Sitophilus* spp. and *Sitotroga cerealella*, and a larger mean number of insects (live and dead) per 500-g sample was found in the PP bags (149.67 ± 8.67), compared to ZFH bags; 4.67 ± 0.73. The percentage of insect damaged kernels after 6 months was significantly higher in PP bags (67.67 ± 2.62) than ZFH bags (5.00 ± 0.50). Weight loss in the PP bags was > 30%, compared to < 0.3% in ZFH bags. Aflatoxin and fumonisin levels of maize in both types of bags were below the recommended safe threshold of 15 ppb and 4 ppm, respectively. The moisture content of maize in ZFH bags was maintained over the 6-month period whereas that of maize in PP bags increased. Also, crude protein, crude fat, and carbohydrate levels in maize were higher in ZFH bags than PP bags. Data showed ZFH bags conserved maize quality better than the PP bags thereby making maize wholesome for food and feed. Further research to compare the growth and performance of layer poultry in terms of egg laying related to diets of feed from maize stored in ZHF and PP bags is recommended.

Key words: Post-harvest loss, stored product insect pest, mycotoxin, poultry, white maize

INTRODUCTION

Ghana is among the top three producers of maize in West Africa [1]. The crop is a staple food and makes a substantial contribution to the diets of both urban and rural populations [2]. Maize is also used as feed for poultry and about 30% of all maize produced in Ghana is used by the poultry industry [3]. Poultry production contributes substantially to Ghana's agricultural sector and adds approximately 14% to the Gross Domestic Product [4]. Additionally, the poultry industry in Ghana contributes approximately 34% of domestic meat output [4]. The value chain of the industry is a source of employment, income, nutrition, and food security for many individuals [5]. The main products of poultry (chicken meat and eggs) are considered as less expensive and relatively available sources of animal protein, with eggs as the most consumed and readily available [3]. Egg consumption per capita in Ghana reached 1.07 kg in 2020, a 1.90% increase over the previous year. The total consumption of chicken meat in Ghana in 2021 was 400,000 MT and was predicted to increase by 15% to 460,000 MT in 2022 [3].

In spite of increasing demand for maize in Ghana as a result of its use in poultry feed in the burgeoning poultry industry, there is a challenge with the supply of quality affordable maize and proper storage in poultry farms. Although maize production in Ghana has increased in recent times in terms of annual area planted, lack of effective storage technologies and handling practices along the postharvest value chain led to high insect pest infestation and aflatoxin contamination, resulting in substantial quantitative and qualitative losses [6, 7, 8].

Smallholder farmers in most developing countries typically sell or use their produce immediately after harvest due to inadequate storage and/or lack of effective storage technologies [9]. The lack of appropriate storage structures and/or technologies results in grains being stored under humid and warm conditions which facilitate grain spoilage [10]. One of the most common means of storing grains in many developing countries is the use of jute and polypropylene bags (hereafter referred to as PP bags) [6]. Polypropylene is a thermoplastic polymer; PP storage bags do not have insecticide incorporated into the bag fabric's fibers. PP bags are affordable, easy to use, reusable, and convenient for transportation. Nevertheless, jute and PP bags do not adequately preserve stored commodities [2, 11, 12].

The effectiveness of hermetic storage bags for protection of maize against stored product insect pests and mycotoxin contamination in Ghana has been reported [11, 12]. To ensure conservation of maize for higher feed quality, poultry farmers in Dormaa Ahenkro in Ghana who hitherto store maize in PP bags are now willing to adopt hermetic storage bags, in particular ZeroFly® Hermetic storage bags (hereafter referred to as ZFH bags). The ZFH bag comprises a single inner liner made with polyamide as barrier resins that ensures hermeticity, and an outer layer made of a polypropylene polymer that has deltamethrin insecticide incorporated into the yarns which is slowly released onto the surface of the material in a controlled and sustained manner to prevent infestation from outside [11, 13]. Hermeticity of the single inner liner kills insects infesting the commodity inside the bag whereas the outer deltamethrin insecticide incorporated layer serves as a barrier to infestation from outside the ZFH bag. ZeroFly Hermetic bags effectively protect maize against insect pests and mycotoxin contamination, are durable, affordable, and re-usable [11, 12].

The adoption of ZFH bags will become more attractive to poultry farmers and widespread if perceived benefits are demonstrable. However, there is limited field data on the performance of ZFH bags for preserving maize on layer poultry farms. Considering this, the current study was conducted to evaluate the effectiveness of the ZFH bags compared to PP bags to protect maize against insect infestation and mycotoxin contamination in layer poultry farms in Ghana.

MATERIALS AND METHODS

Study site

The study was conducted in storehouses of three poultry farms; Evans Joes Farms, M.M Unity Farms, and T.K. Takyi Farms, all in Dormaa Ahenkro in the Bono Region of Ghana (7°17'N 2°53'W). This area is the main center for commercial poultry farming in Ghana. It lies in the Forest Savannah agro-ecological zone, with mean annual rainfall between 125 and 175 mm, relative humidity ranging from 70 to 80%, temperatures ranging from 26 to 30 ˚C, and average wind speed of 9.5 kmh^{1}.

Set up of treatments

Experimental treatments were the two types of storage bags; ZFH bags and PP bags. The ZFH bags were purchased from a distributor in Nigeria (Turner Wright Nigeria Limited 15, Adenekan Salako Close, Ogba, Lagos, Nigeria). The PP bags were purchased in Kumasi (Bentronic Productions, Kumasi, Ghana). The study design was a Randomized Complete Block Design (RCBD) with two treatments. Each treatment was replicated three times with each poultry farm storehouse being a replicate or block, and there was a sub-replication of two, where two bags in each treatment in each farm, were destructively sampled monthly. One hundred and ninety-two 100-kg bags of clean untreated (insecticide-free) white maize of ~13% moisture content were purchased from a single producer and transported to a single storehouse location in PP bags provided by the producer. The maize was emptied onto a tarpaulin and thoroughly mixed, and 100 kg was transferred to each of the 100-kg-capacity ZFH and PP bags used for the study. In each storehouse, there were thirty-two 100-kg-capacity ZFH and the same number of 100-kg PP bags, each

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containing 100 kg of maize, that is, altogether sixty-four 100-kg-capacity bags in each storehouse. Thirty-two bags for each treatment were arranged on two wooden pallets, that is; sixteen bags on each of the pallets. Arrangement of pallets in each storehouse and checking of the integrity of the ZFH bags were conducted as described in [12]. Wooden pallets were used to prevent maize in the bags from absorbing moisture from the floor. The pallets for each treatment were separated by 2 meters of free space. The integrity of the inner liners of the hermetic bags was checked to ensure there were no punctures before use and were then checked again to ensure they were tightly sealed after maize was put in to maintain hermeticity. The outer polypropylene layers were equally checked to ensure they were not torn before putting maize into the bags.

Sampling and data collection
Initial sampling was conducted at the start of the study; samples were subsequently taken monthly for six months. Randomization of the bags in each storehouse to determine when each bag would get destructively sampled was conducted as described in [12], except the randomization involved 16 bags of each treatment, that were on each pallet, in each storehouse. The randomization was in accordance with a sub-replication of two. To reiterate, the bags in the various treatments were destructively sampled each month; this means that bags of maize in each treatment, in each storehouse, that were sampled each month were discontinued from the study.

Determination of moisture content (MC)
Moisture content (MC) of maize in each bag was determined as described in [12]. Three different readings were taken for each bag, and the mean MC for each bag was computed.

Grain sampling
Sampling of maize in the selected bags was conducted as described in Opoku *et al.* [12]. The samples were used to determine insect infestation and weight loss per 500-g sample. Samples for mycotoxin analysis were collected and handled as described in Opoku *et al.* [12]. Estimation of the percentage of insect damaged kernels on a number basis (IDKnb), weight loss, extraction of insects from samples, mycotoxin analysis, and proximate analysis were all conducted as described in Opoku *et al.* [12].

Data analyses
The experimental design was a randomized complete block design (RCBD) with sub-replication. Statistical analyses were performed with SAS Version 9.4 (SAS Institute, Cary, NC). Effects of sampling month (Month) and type of storage bag (Bag) were assessed using a 2-way analysis of variance (ANOVA) with poultry farms as the

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blocking factor (PROC MIXED). Analyses of the numbers of live insects were conducted with the use of a square root transformation, but untransformed values are reported. The simple effects of the type of storage bag in a given month were assessed with protected planned contrasts (SLICE option in an LSMEANS statement). Additionally, the SLICE option was used to assess simple effects of month in a given type of storage bag. Data analyses for response variables expressed as percentages were conducted with the use of an arcsine square root transformation to stabilize variances, but untransformed percentages are reported.

RESULTS AND DISCUSSION

Insect infestation level in maize

The dominant insect pest found in the maize samples was *Sitophilus* spp. (adults) (Coleoptera: Curculionidae), whereas *Sitotroga cerealella* (Lepidoptera: Gelechiidae) comprised only a small percentage of insects found in bags. However, the two species were counted together in each sampling month (Tables 1 and 2). For the number of insects found in maize samples, there was a significant interaction ($P < 0.05$) between the sampling month and type of storage bag (Table 1). The main effects sampling month and type of storage bag were also significant ($P < 0.05$) (Table 1). The initial numbers of insects in PP (1.00 ± 0.29) and ZFH (1.5 ± 0.29) storage bags were not significantly different ($P > 0.05$). However, after two months of storage, the number of insects in PP bags was consistently larger throughout the sampling period compared to ZFH bags. There were also differences ($P < 0.05$) in the number of insects in the PP bags for the different sampling months, with month 5 recording the largest number of 245.17 ± 11.35 in PP (Table 2). PP bags are one of the most popular methods of storing grains in sub-Saharan Africa, however, they are not durable and offer little or no protection against insect pests, rodents and water. According to Baoua *et al*. [2] PP bags are relatively easy for adult insects to penetrate by pushing through the interstices of the mesh. High insect infestation of maize in PP bags has been reported in studies in Ghana [11, 12, 14]**.** For the first three months, the number of live insects in the ZFH bags increased marginally, and after this period all the insects found were dead. This presupposes that initial live adult insects were likely able to lay eggs and hatch during early exposure to hypoxia conditions, but as exposure time increased, insects died out of desiccation. A study by Yan *et al*. [15] showed that low oxygen did not significantly affect the number of eggs laid by cowpea bruchids during short exposure times to limiting oxygen conditions, but increasing maternal and egg exposure time depressed reproductive output and resulted in lower adult emergence. The results of this study demonstrate that ZFH storage bags offer superior protection to stored maize against insect pests than PP bags by killing insects through limiting oxygen conditions. According to Murdock *et al*. [16] the major source of water for stored product insects is through their own metabolic processes, however, when oxygen is limited, insects cannot use it to produce enough water leading them to stop growing, developing and reproducing, and they eventually die of desiccation instead of suffocation. Additional protection offered by ZFH bags is the deltamethrin incorporated in the yarns of the outer layer serving as a barrier to infestation from outside [11, 12].

Maize kernels damaged by insects

For percent insect damaged kernels (%IDK), there was a significant interaction between sampling month and type of storage bag ($P < 0.05$). The main effects sampling month and type of storage bag were also significant ($P < 0.05$) (Table 1). At the start of the study, %IDK was similar for the two types of bags (Table 2). However, thereafter, %IDK in PP bags were higher compared to ZFH bags throughout the 6-month storage period. From an initial mean of 1.17 ± 0.17%, %IDK in the PP bags increased about 65 times to 76.17 ± 0.44 after five months of storage (Table 2). Stored product insect pests damage grains by consuming the germ and partial or complete consumption (hollowing) of the kernels [17]. The increase in % IDK in the PP bags was directly proportional to the storage period, as higher percentages were recorded with increasing time of storage. This higher damage was attributed to feeding by the internal feeding insects, that is, *Sitophilus* spp. and *S. cerealella* found in the PP bags. Primary feeders are internal feeders and are more destructive because their larvae feed inside infested grains, and when they exit grains as adults, they leave highly visible exit holes which ultimately increases the number of insect damaged kernels [18]. In the case of the ZFH bags, the highest %IDK was recorded after three months of storage (12.33 ± 2.40), nonetheless, it was significantly lower ($P < 0.05$) in months 4, 5, and 6 of storage (Table 2). Damage was generally kept in check in the ZFH bags. The low %IDK found in the ZFH bags corroborates the findings of other studies on the storage of maize in hermetic bags [11, 12]. The Ghana Standards Authority (GSA) considers percent insect damaged kernels as one of the critical parameters in grading maize, and 5% is the acceptable threshold by wholesalers, retailers, consumers and Ghana storage industry [19]. The percent damaged kernels in ZFH bags at the end of the 6 months was 5% compared to approximately 68% in the PP bags, making maize in PP bags unwholesome for consumption.

Weight loss of maize

There was a significant ($P < 0.05$) interaction between the sampling month and storage bag regarding the percent weight loss (%WL) of maize. The main effects of sampling month and type of storage bag were also significant ($P < 0.05$) (Table 1). Significant differences ($P < 0.05$) were also recorded between PP and ZFH bags, and within the same bag type for the different months' sampling was conducted (Table 1). Mean %WL of 2.76 ± 1.56 and 1.24 ± 0.27 in the PP and ZFH bags,

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respectively, after the first two months of storage were not different (P > 0.05). Thereafter, consistent and significant increases in %WL were recorded in PP bags compared to ZFH bags. The highest recorded %WL in the PP bags of 32.02 ± 7.53 in month 6 of storage was approximately 267 times the initial %WL (Table 2), compared to the highest (3.66 ± 0.54) in the ZFH bags recorded after five months of storage. This weight loss in the PP bags is attributed to the high numbers of live *Sitophilus* spp. in the PP bags. Some of the leading causes of grain weight loss worldwide are grain storage pests including rodents, insects, and mites [20]. It is estimated that approximately 64% of grain weight losses occur because of grain insects within the first three to six months of storage [20]. Studies in Africa have reported above 21% grain weight losses caused by *S. zeamais* after 6 months of grain storage in traditional materials [2, 12].

Moisture content of maize

In the case of percent moisture content (MC), the interaction between the sampling month and storage bag was significant (P < 0.05). The main effects of sampling month and type of storage bag were also significant (P < 0.05) (Table 1). The differences in MC of maize in the two types of storage bags were significant (P < 0.05), as well as differences in sampling month for PP bags (Table 1). The initial percent moisture content of the maize was approximately 12.5%, which is within the acceptable moisture content threshold (< 13%) recommended for safe storage of maize [21]. Moisture content levels in maize from PP bags increased consistently and significantly to 15.55 ± 0.16%, compared to maize in the ZFH bag (12.61 ± 0.04%) after six months of storage (Table 2). Maize stored in the ZFH bags generally maintained moisture during the 6 months storage period. The ZFH bags appear to retain the initial grain moisture and preserve it against changes influenced by seasonal variations in humidity. Similar observations have been reported in other parts of West Africa in comparative studies on maize storage using hermetic bags [2, 11, 12]. The hermetic layers of ZFH bags are water tight, thus retain existing water in the bag when it is tied, and also prevent moisture ingress from the ambient environment [2]. Another reason for the fairly consistent moisture is the minimal insect activities in the hermetic bags which would increase grain temperature and moisture as a result of respiration [22]. By contrast, the increase in grain moisture in PP bags could be linked to the porous nature of the PP bags which are essentially open to the ambient air [2]. Additionally, the high numbers of live insects may have resulted in high respiration rate and moisture production [22].

Aflatoxin and fumonisin in maize

The interaction between sampling month and type of storage bag was significant (P < 0.05) for the levels of aflatoxin and fumonisin in maize. The main effects sampling month and type of storage bag were also significant (P < 0.05) (Table 3) for both aflatoxin and fumonisin. There were also statistical differences (P < 0.05) in the two storage bags as well as between different sampling months in the PP bags (Table 3) for both aflatoxin and fumonisin. After one month of storage, aflatoxin levels were consistently higher (P < 0.05) in PP bags than in the ZFH bags throughout the five sampling months, reaching the highest levels of 14.78 ± 0.12 ppb in month six (Table 4). This highest aflatoxin level in the PP bags was 5.1 ppb more than the initial 9.68 ± 0.46 ppb. However, aflatoxin levels found in ZFH bags during all the sampling months were similar to the initial 9.82 ± 0.54 (Table 4). Fumonisin levels increased in PP bags from 0.27 ± 0.009 ppm at the start of the study to 0.73 ± 0.067 ppm, after 6 months of storage, at the end of the study. Fumonisin levels were always higher in PP bags than ZFH bags after 1 month of storage; the only exception was after 3 months of storage. Fumonisin levels in ZFH bags remained similar throughout the 6 months of storage.

The presence of mycotoxins in food has long been a matter of health concern, particularly in developing countries where exposure to mycotoxins is widespread [23]. The economic and health implications of mycotoxins including aflatoxins and fumonisins contamination in human food and animal feeds require greater attention and control [24]. Levels of aflatoxin and fumonisin in both storage bag types were below the safe threshold of 15 ppb and 4 ppm, respectively [19]. Nonetheless, significant increases in levels in the PP bags could be related to high insect infestation, greater percent insect damage kernel, and the likely fungal infection associated with insect feeding and respiration. A number of other studies reported comparatively higher aflatoxin contamination in extensively damaged maize than less damaged or insect free maize [11, 12]. When maize is not adequately dried and safely stored, it is prone to aflatoxin contamination [25]. Aflatoxins in poultry feed adversely affect poultry performance including decreasing egg production, lowering growth rate and causing illness in birds [26]. Poultry feed needs to be safe for proper growth and performance of poultry birds because the ingestion of any unsafe food material could not only affect the health of the birds, but would also find its way through the food chain to humans [27].

Proximate composition of maize

The interaction between sampling month and type of storage bag was significant (P < 0.05; Table 5) for proximate analyses for all the variables except for percent ash content (%AC) (P > 0.05) (Table 5). Initial and final levels of all proximate composition response variables tested were not different (P > 0.05) at the start and end of the study in the case of ZFH bags (Table 6). Maize in ZFH bags maintained nutrient levels during the 6-month storage period whereas %PRO, %CHO, and %FAT levels in the PP bags decreased significantly (P < 0.05) (Table 6). Slower or no depletion of nutrients in maize stored under hermetic condition compared to traditional PP bags has been reported [12, 28]. Stored grain insect pests bore into kernels and feed on the endosperm and the germ portion, reducing the quality of grains [24]. The decrease in nutritional content of maize stored in the PP bags could be attributed to the high insect infestation and its associated damage. Insects that are internal feeders including *Sitophilus* spp. and *S. cerealella* consume parts of seeds which contain substantial amounts of nutrients especially crude protein, carbohydrate, protein, and fat content [29]. Additionally, insect infestation decreased the carbohydrate content of the maize stored in PP bags, causing a relative increase in the proportion of fiber (%CF) [29]. It has been reported that high unrefined fiber in poultry feed has adverse effects on nutrient utilization and feed conversion, and causes decrease in body weight gain [30].

CONCLUSION AND RECOMMENDATIONS FOR DEVELOPMENT

ZeroFly® Hermetic storage bags conserved stored maize by killing insects already in the maize at the start of the study and those that attempted to enter the bags from outside. The ZFH bags maintained the nutrient content of stored maize, and also kept the aflatoxin and fumonisin within safe levels for human and animal use. Therefore, poultry farmers are encouraged to patronize hermetic storage bags to preserve quality and quantity of maize for food and feed for improved food security and profitability.

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Conflict of interest

The authors declare no conflict of interest.

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Table 1: ANOVA for main effects sampling month (Month) and type of storage bag (Bag) and interactions (*) for number of insects (# insects), percent insect damaged kernels (%IDK), percent weight loss (%WL), and percent moisture content (%MC). Maize samples were taken monthly during the months of March–August 2022 (a 6-month period)

Variable	Source	df	*F*	*P*
# insects	Month	6,26	89.36	<.0001
	Bag	1,26	643.01	<.0001
	*	6,26	89.10	<.0001
%IDK	Month	6,28	175.97	<.0001
	Bag	1,28	1141.61	<.0001
	*	6,28	94.93	<.0001
%WL	Month	6,26	17.78	<.0001
	Bag	1,26	83.61	<.0001
	*	6,26	15.31	<.0001
%MC	Month	6,26	48.38	<.0001
	Bag	1,26	372.69	<.0001
	*	6,26	46.09	<.0001

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Table 2: Mean (± SE) number of insects, percent insect damaged kernels (%IDK), percent weight loss (%WL), and percent moisture content (%MC) from Polypropylene (PP) and ZeroFly® Hermetic (ZFH) bags (treatments) sampled monthly during the months of March–August 2022 (a 6-month period)

Variable	Month	PP	ZFH
# insects	0	1.00 ± 0.29eA	1.5 ± 0.29aA
	1	6.83 ± 1.86eA	1.83 ± 0.44aA
	2	44.83 ± 7.49dA	9.50 ± 2.29aB
	3	87.83 ± 23.09cA	13.67 ± 4.11aB
	4	242.33 ± 9.88aA	4.50 ± 1.26aB
	5	245.17 ± 11.35aA	4.33 ± 1.86aB
	6	149.67 ± 8.67bA	4.67 ± 0.73aB
%IDK	0	1.17 ± 0.17eA	1.17 ± 0.44dA
	1	6.67 ± 0.67dA	1.00 ± 0.50dB
	2	24.67 ± 2.77cA	9.67 ± 1.74abB
	3	42.00 ± 1.50bA	12.33 ± 2.40aB
	4	72.17 ± 1.17aA	7.33 ± 2.32bB
	5	76.17 ± 0.44aA	6.33 ± 0.33bB
	6	67.67 ± 2.62aA	5.00 ± 0.50bcB
%WL	0	0.12 ± 0.12cA	0.13 ± 0.13cdA
	1	1.20 ± 0.33cA	0.00 ± 0.00dB
	2	2.76 ± 1.56cA	1.24 ± 0.27bcA
	3	8.05 ± 2.43bA	2.26 ± 0.36bB
	4	10.51 ± 2.79bA	0.84 ± 0.66bcdB
	5	7.67 ± 0.91bA	3.66 ± 0.54abA
	6	32.02 ± 7.53aA	0.25 ± 0.25cdB
% MC	0	12.62 ± 0.09eA	12.45 ± 0.04bA
	1	12.65 ± 0.05eA	12.49 ± 0.05bA
	2	13.06 ± 0.14dA	12.55 ± 0.03bB
	3	13.44 ± 0.19cA	12.80 ± 0.02aB
	4	13.58 ± 0.19cA	12.21 ± 0.14bB
	5	14.23 ± 0.18bA	12.34 ± 0.02bB
	6	15.55 ± 0.16aA	12.61 ± 0.04abB

For each response variable, significant differences within the same treatment for the 6 months are denoted with different lower-case letters and differences between treatments within each month are denoted by different upper-case letters. ($P < 0.05$, SAS). For #insects, %IDK, and %WL values are for 500-g samples

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Table 3: ANOVA for main effects sampling month (Month) and type of storage bag (Bag) and interactions (*) for levels of aflatoxin (ppb) and fumonisin (ppm). Maize samples were taken monthly during the months of March–August 2022 (a 6-month period)

Variable	Source	df	*F*	*P*
Aflatoxin	Month	6,26	24.19	<.0001
	Bag	1,26	130.75	<.0001
	*	6,26	16.57	<.0001
Fumonisin	Month	6,28	11.46	<.0001
	Bag	1,28	65.52	<.0001
	*	6,28	7.98	<.0001

Table 4: Mean (± SE) levels of aflatoxin (ppb) and fumonisin (ppm) found during the months of March–August 2022 (a 6-month period)

Variable	Month	PP	ZFH
Aflatoxin (ppb)	0	9.68 ± 0.46dA	9.82 ± 0.54aA
	1	9.84 ± 0.30dA	9.93 ± 0.38aA
	2	11.55 ± 0.21cA	10.54 ± 0.30aB
	3	11.89 ± 0.67cA	10.17 ± 0.18aB
	4	13.21 ± 0.19bA	10.53 ± 0.14aB
	5	14.12 ± 0.02abA	9.91 ± 0.16aB
	6	14.78 ± 0.12aA	10.64 ± 0.47aB
Fumonisin (ppm)	0	0.27 ± 0.009cA	0.28 ± 0.017aA
	1	0.26 ± 0.005cA	0.28 ± 0.018aA
	2	0.43 ± 0.016bA	0.27 ± 0.010aB
	3	0.48 ± 0.030bA	0.36 ± 0.032aA
	4	0.68 ± 0.006aA	0.30 ± 0.020aB
	5	0.65 ± 0.120aA	0.35 ± 0.057aB
	6	0.73 ± 0.067aA	0.32 ± 0.039aB

For each response variable, significant differences within the same treatment for the 6 months are denoted with different lower-case letters and differences between treatments within each month are denoted by different upper-case letters. ($P < 0.05$, SAS)

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Table 5: ANOVA for main effects sampling month (Month) and type of storage bag (Bag) and interactions (*) for percent crude protein (%PRO), percent carbohydrate (%CHO), percent fat (%FAT), percent crude fiber (%CF), percent ash content (%AC), and percent moisture content (%MC). Maize samples analyzed were taken at the start (March 2022) and end of the study (August 2022)

Variable	Source	df	*F*	*P*
%PRO	Month	1,6	176.04	<.0001
	Bag	1,6	176.04	<.0001
	*	1,6	187.04	<.0001
%CHO	Month	1,6	492.92	<.0001
	Bag	1,6	451.86	<.0001
	*	1,6	521.28	<.0001
%FAT	Month	1,8	9.49	0.0151
	Bag	1,8	8.97	0.0172
	*	1,8	9.49	0.0151
%CF	Month	1,6	7.29	0.0355
	Bag	1,6	7.29	0.0355
	*	1,6	7.56	0.0333
%AC	Month	1,6	6.0	0.0498
	Bag	1,6	6.0	0.0498
	*	1,6	0.0	1.0000
%MC	Month	1,6	90.81	<.0001
	Bag	1,6	103.54	<.0001
	*	1,6	113.63	<.0001

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Table 6: Mean (± SE) percent crude protein (%PRO), percent carbohydrate (%CHO), percent fat (%FAT), percent crude fiber (%CF), percent ash content (%AC), and percent moisture content (%MC) from Polypropylene (PP) and ZeroFly® Hermetic (ZFH) bags (treatments) sampled at the start (March 2022) and at the end of the layer poultry study, 6 months later (August 2022)

Variable	Month	PP	ZFH
%PRO	0	8.72 ± 0.04aA	8.68 ± 0.03aA
	6	6.40 ± 0.16bB	8.73 ± 0.02aA
%CHO	0	77.56 ± 0.08aA	77.42 ± 0.01aA
	6	73.48 ± 0.18bB	77.48 ± 0.02aA
%FAT	0	2.37 ± 0.003aA	2.37 ± 0.008aA
	6	1.73 ± 0.200bB	2.38 ± 0.021aA
%CF	0	2.37 ± 0.007bA	2.37 ± 0.002aA
	6	3.66 ± 0.535aA	2.37 ± 0.010aB
%AC	0	2.17 ± 0.012aA	2.18 ± 0.009aA
	6	2.15 ± 0.023aA	2.16 ± 0.006aA
%MC	0	12.29 ± 0.089bA	12.36 ± 0.040aA
	6	15.17 ± 0.325aA	12.22 ± 0.094aB

For each response variable, significant differences within the same treatment at the start and end of the study are denoted with different lower-case letters and differences between treatments at the start and then also at end of the study are denoted by different upper-case letters. ($P < 0.05$, SAS)

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