Original Research

Journal of Advanced Veterinary Research
(2024) Volume 14, Issue 1, 117-121

Effects of storage period on nutritive value of broilers feeds and their remedy through some dietary treatments

Mohamed N. Makled*, Khaled Abouelezz*, Ale E. Galal, Marwa A. Massoud, Hossam S. Elhabaty

Department of Poultry Production, Faculty of Agriculture, Assiut University, Assiut 71526, Egypt.

ARTICLE INFO

Recieved: 20 October 2023

Accepted: 10 December 2023

***Correspondence:**

Corresponding author: Mohamed N. Makled
E-mail address: makled@yahoo.com

Corresponding author: Khaled Abouelezz
E-mail address: abollez@aun.edu.eg

Keywords:

Broilers performance
Feed storage
Feed antioxidants
Amino acids
Blood analyses

ABSTRACT

There is scarce information on the effects of feed storage period on poultry performance, which has not been investigated thus far. The aim of this study was to evaluate the effect of feeding broilers on feeds stored for different periods, with or without supplemental amino acids and vitamins. This study followed a factorial design (3 x 3), i.e. three dietary treatments and three feed storage periods. The three dietary treatments consisted of 1) a basal diet, 2) a basal diet including additional 5g methionine and 13 g lysine/kg, and 3) a basal diet including 3 g vitamin premix/kg. The 3 dietary treatments were tested with feeds stored for three different periods (Fresh, 4-months, and 6-months). A total of 450 one-day old broiler chicks were randomly assigned to the 9 treatments, each treatment contained 5 replicates of 10 chicks each (n= 50/group). The experiment lasted for 42 days. The results indicated that the final BWs of the birds received 4- or 6-months stored feed were greater than that of those fed fresh diets. The best total BWG was seen in the 4-month-stored feed group. There was no significant effect of storage period on total FC and FCR during period from 0 to 6 weeks of age, but the FCR tended to be better with the feed stored for 4 months. Haemoglobin was significantly higher in the birds fed fresh or 4-month stored feed. The percent of liver and spleen of the birds received 6-month-stored feed were the highest ($P < 0.01$). Plasma content of albumin was higher in the birds received 4-or 6-month stored feed than the fresh diet. The use of additional amino acids increased the final BW and total BWG than those of the control and vitamin-supplemented treatments. The main effect of dietary treatments on percentages of immune organ weight was insignificant. The control (basal diet) recorded higher lymphocytes percentage, while T2 (BD + double level of amino acids) recorded higher eosinophil, heterophil and H/L ratio, whereas T3 (BD + double level of vitamins) recorded higher levels of haemoglobin, eosinophil, and monocytes. The main effect of dietary treatments on blood plasma proteins was insignificant. The tested treatments did not show a significant effect on broilers mortality. The detected values of AF1, AF2, AG1, and AG2 were very low in all 9 diets. In conclusion, enrichment of broiler feeds with higher levels of lysine and methionine (double level of NRC, 1994) together with antioxidants supplement could prolong their shelf-life to 6 months without any deleterious impact on productive performance of broiler chickens.

Introduction

Few research evaluated the effects of storage period on the nutritive value of some individual ingredients used in animal diets; furthermore, no information on the storage effects of mixed/manufactured diets on poultry performance. Storage of poultry feed may reduce its nutritive value and increase fungal and mold infection, which would present high economic losses in poultry enterprises due to increased mortality and reduced performance. Additionally, the legislation made by the Egyptian Ministry of Agriculture estimated the maximal storage period of manufactured/mixed poultry feed as 6 months. However, there are doubts that diets may expire in shorter periods and lose part of their nutrient contents or be contaminated with mycotoxins resulting from growth of mold. This study evaluated the feeding value of diets stored for 4 and 6 months based on broilers' performance.

Long-term storage may result in a decrease in the fat content of the grains and an increase in their free fatty acids; factors that may reduce their energy content. This indicates that some fatty acids oxidize during storage (Pomeranz, 1982). Moreover, storing corn may increase lipid oxidation and free fatty acid content (Galliard, 1986). Reed *et al.* (2007) evaluated the effect of storage of corn for two months on its nutrient composition; the results showed that the percentage of protein in maize was significantly decreased by the duration of storage, also, the fat content of the grain decreased by more than 10%. In this regard, storage may affect the protein content in corn as indicated by changes in the lysine content (Bartov, 1996). Similarly, Yin *et al.* (2017) reported that the content of amino acids and crude protein in corn generally decreased with increasing the storage period. García-Rosas *et al.* (2009) reported that corn storage might cause starch retrogradation and lead to formation of resistant starch. Chen (1990) reported a 20% to 70% loss of vitamin A activity when three commercial cross-linked vitamin A were stored for three months at high temperature and relative humidity. Higher losses were reported in activity in vitamin premixes blended with inorganic trace minerals as compared to vitamin premixes without inorganic trace minerals stored for 120 days (Shurson *et al.*, 2011). The latter authors also observed 9% and 8.6% loss of activity per month for vitamins A and B6, respectively.

Additionally, contamination of poultry feeds with mycotoxins due to infection and growth of mold during storage, especially under humid and hot climates, represents a global challenge to poultry producers and feed manufacturers (Moretti *et al.*, 2017). Aflatoxins, which are the predominant mycotoxins, do not habitually occur at the pre-harvest stage like other mycotoxins; since the associated fungi (Aspergillus flavus and Aspergillus parasiticus) are commonly seen as storage mold (Afolabi *et al.*, 2019; Leggieri *et al.*, 2020). The aflatoxins infection and non-regulation of their metabolites in stored feed ingredients are associated with economic losses in livestock enterprises including elevated mortality, decline in productivity, and augmented veterinary costs (Zain, 2011). Knowledge of aflatoxin concentrations in stored poultry feeds remains scarce (Nakavuma *et al.*, 2020)

This study, therefore, aimed to evaluate the effect of different storage periods (fresh, 4 months and 6 months), and assess to what extent supplementing the stored feed with additional levels of amino acids and vitamins may alleviate the negative impact (if any) of storage period on productive performance of broilers.

ISSN: 2090-6277/2090-6269/

Materials and methods

This study was carried out at the Poultry Research Farm, Faculty of Agriculture, Assiut University, Assiut, Egypt. This study has been approved by the Council of Poultry Production Department, Faculty of Agriculture, Assiut University, with the approval number of (312022-120-3).

Birds and experiment design

A total number of 450 one-day-old, unsexed Hubbard broiler chicks, having a similar average body weight (BW) (42.50±0.33 g), were used. All chicks were wing-banded, individually weighed, and randomly allotted into 45 equal replicates of 10 chicks each. Each replicate was housed in floor pen (2 m length × 0.75 m width). This experiment was carried out in a factorial design (32). It consisted of three dietary treatments (T1: a basal diet, T2: a basal diet supplemented with additional 5g methionine and 13 g lysine/kg diet, and T3: a basal diet supplemented with 3 g vitamin premix/kg diet). The 3 dietary treatments were tested using feeds stored for three different periods (Fresh, 4-months, and 6-months). Therefore, the 45 replicates were equally divided among nine treatment groups (5 replicates per group, N = 50 birds/group) as follows: G1 (Fresh feed T1), G2 (Fresh feed T2), G3 (Fresh feed T3), G4 (4 months stored feed T1), G5 (4 months stored feed T2), G6 (4 months stored feed T3), G7 (6 months stored feed T1). G8 (6 months stored feed T2), G9 (6 months stored feed T3).

The feed ingredients were purchased from Alsalam Feed Factory (Assiut), and the diets were formulated based on yellow corn, soybean meal, gluten, methionine, lysine, table salt, and premix. To ensure that the dietary ingredients are fresh, upon reception of them, they were carefully inspected regarding texture, hardness, intactness, appearance, colour, appearance, and infections with insects, or moulds. All signs indicated that they are fresh.

Before starting the experiment, the total amounts of feed ingredients required for 450 broiler chickens (from 0 to 42 days of age) were calculated. Then, the first third of this amount was mixed and stored for 6 months before starting the experiment, the second third was mixed and stored for 4 months before starting the experiment, and the last third of this amount was mixed just before starting the experiment (fresh).

Feed storage conditions

Feed was stored indoors (in a hall) and away from vermin. Feed was kept in wholesome plastic bags which were closed tightly and stood away from the hall walls and from the concrete floor by placing them on wooden pallets that allow air between the feed and the floor. The bags were also kept away from any source of moisture or direct light (sunlight or electric). Good ventilation was provided all the time to prevent condensation. The place was also under control to prevent rats, mice, and insects. During the feed storage period, the temperatures average was 35ºC, ranging between 28.5°C and 41.5°C, and humidity average was 53.5%, ranging between 26.8% and 80.5%.

The chicks were fed their diets in a mash form that m*et al*l nutrient requirements recommended by NRC (1994). During the experimental period (0 – 6 wks.), they were fed a starter diet from 0 - 3 weeks (23% CP, 2950 kcal ME/kg, 1.35% Lysine, 0.536% Methionine) and a grower diet (21% CP, 3100 kcal ME /kg, 1.05% Lysine, 0.41% Methionine) from 4 - 6 weeks of age. Supplementations of double level of amino acids (lysine and methionine) or vitamins premix were provided in the diets from day 1 to day 42 of age. All birds involved in the study were kept under similar managerial and hygienic conditions and were offered feed and water ad libitum. The chicks were maintained on a 24-h constant light regimen throughout the experimental period. All chicks were vaccinated against the most popular diseases. It is noteworthy to mention that we tried in this experiment to simulate the managerial conditions common in most small broiler raising farms that do not have enough advanced facilities to control temperature or humidity specially at summertime.

The studied criteria

Growth performance

The initial and final body weights (BW) were recorded on an individual basis. The feed consumption (FC) was weekly recorded on a per replicate basis for the whole experimental period according to Attia *et al*. (1995). The total body weight gain (BWG)= [(Final BW − initial BW), and feed conversion ratio (FCR)= (FC / BWG) were calculated. The number of dead birds was recorded, and mortality rate (%) was calculated for each treatment.

Carcass traits and internal organs

At the end of the experiment (42 days of age), a slaughter test was performed using 5 birds from each group (1 bird from each replicate around the average LBW of each group). The birds were fasted for 6 hours then individually weighed to the nearest gram and slaughtered using a sharp, sterile knife. After bleeding, the birds were individually weighed to determine the blood weight. Then, the birds were scaled and defeathered manually and weighed to calculate feather weight by difference. The measurements recorded from the carcass were: giblets weight (liver, heart and empty gizzard), abdominal fat weight, carcass parts weights (breast, back, head, thigh, and drumstick), and dressed carcass weight. Moreover, the immunity organs (liver, bursa, and spleen) were separated and weighed. All weights of such organs and parts were also expressed as a percent of pre-slaughter body weight.

Blood analyses

Blood samples were collected at the time of slaughter at 42 days of age from 45 previously mentioned birds in heparinized tubes (10.0 ml). Haemoglobin, total protein, albumin, and globulin were measured.

Haemoglobin concentration

The haemoglobin (Hb) concentration was determined in the collected blood samples, using Drabkin reagent (a chemical kit purchased from Hem. React Co.) and the absorbance was measured by a UV- visible spectrophotometer (Optizen Pop, Mecasys – Korea) using 3 ml sealed quartz-glass cuvettes with a path length of 1 cm).

Plasma analyses

Blood samples were collected from each of the 45 slaughtered birds in heparinised tubes (10.0 ml) to obtain plasma. Total protein, and albumin, were measured in blood plasma by an auto-analyser (SHIMADZU CL-8000 automatic autoanalyzer, Cairo, Egypt) and using commercial kits purchased from Biodiagnostic CO. 29 El-Tahrer St. - Dokki- Giza - Egypt. Globulin was calculated as the difference between total protein and albumin of the same sample.

Detection of Aflatoxins

Detection of Aflatoxins was done at the Central Laboratory, Health Building, Faculty of Veterinary Medicine, Assiut University. Feed samples were taken from fresh, 4-months and 6-months stored feeds for determining AF1, AF2, AG1, and AG2. Aflatoxins were detected according to the procedure of Benvenuti and Burgess (2010) as follows: 1. Standard Preparation: Two stock solutions were purchased from Supelco; 46319-U and 46304-U. From these, intermediate stocks were prepared in meth-

anol and diluted with 1% acetic acid (aq.) to produce six standard concentrations. These concentrations bracket the concentration of the final extract following the sample preparation procedure. From each standard, 20 µL was injected in triplicate. 2. Sample Preparation: Individual samples of broiler diets were divided into two 25 g portions. One portion was kept as a blank. The other was spiked with aflatoxin standards at the EU levels. Both portions were then carried through the VICAM Afla Test cleanup procedure. 3. Spiking Procedure: Each sample was spiked at 4 µg/kg total B and G (1.54 µg/kg B1). This was achieved by adding 384.6 µL of a 0.26 mg/L solution to the blender for the sample preparation. Afla test sample was Prepared using a blender, blend 25 g sample, 5 g sodium chloride, and 100 mL of 80:20 methanol: water (HPLC grade), mixed at high speed for 1 min. This solution was filtered through fluted Whatman filter paper (filtrate 1), 10 mL of filtrate was mixed with 40 mL water, filtered through glass microfiber filter paper (filtrate 2). Then, 10 mL were loaded of filtrate 2 onto a VICAM Afla Test Affinity Column, part no. G1024, and washed with two 10 mL portions of HPLC-grade water. Finally, they were eluted with 1 mL HPLC grade methanol.

Statistical analysis

The variables presented as percentages were arcsine-transformed before analysis. Each replicate was considered as an experimental unit. The influence of the experimental factors (storage period and dietary treatments) on the measured variables was analyzed by ANOVA following a completely randomized design, and the statistical analysis was conducted using the General Linear Model (GLM) procedure, SAS version 9.4. Significant differences among treatment means were determined using Duncan multiple range tests (Duncan, 1955).

The following model was used: $Y_{ijk} = M + S_i + T_j + ST_{ij} + E_{ijk}$

Where, Y_{ijk} = observed value, M = overall mean, S_i = Storage period effect, T_i = Dietary treatment effect, ST_{ij} = Interaction effect, and E_{ijk} = random error.

Results

Growth performance measurements

The results of the effect of storage periods and dietary treatments on broilers growth performance measurements are shown in Table 1. The final BWs of the birds that received feed stored for 4 or 6 months were greater than that of those fed fresh diets, and the best total BWG was seen in the 4-months-stored feed group. There was no significant effect of storage period on total FC and FCR during period from 0 to 6 weeks of age, but the FCR tended to be better with the feed stored for 4 months.

Moreover, the birds of T2 (BD + double level of amino acids) showed significantly higher final BW and total BWG than those of T1 and T3. These results revealed that feeding diets supplemented with higher levels of lysine and methionine could alleviate the effect of storage for 6 months. Moreover, these results indicated that raising the lysine and methionine level to the double of its level in the basal diet had a significant positive effect on final BW, i.e. at 6 weeks of age. Besides, raising the lysine and methionine level to the double of its level in the basal diet (T2) showed a significant positive effect on FC and FCR from 0 to 6 weeks of age. Regarding the mortality rate, there were only 4 cases during the experimental periods which were from different four groups (one per group). The whole number of birds under study was 450 birds. Therefore, the mortality percentage was less than 1%. It is obvious that the effects of storage period and dietary supplements were insignificant.

Carcass, meat yield, and immune organs

The results presented in Table 2 illustrate the effect of storage periods and dietary treatments on percentages of carcass parts, immune organs, and meat yield of broiler chickens. The relative weights of legs (thigh and drumstick), and breast were not affected by feed storage periods, but the abdominal fat percentages tended to be higher in the birds fed fresh feed. The effect of storage period on dressed carcass percentage was inconsistent since it was lower at using 4-month stored feed than the fresh feed, then they did not differ at using 6-month stored feed than the fresh feed. The liver percent and the total giblets percent were significantly higher for the birds received the feed stored for 6 months, and the gizzard percent was higher for the birds of T2 (BD + vitamins). The percent of the liver, spleen and total immune organs for the birds received 6-month stored feed were significantly higher ($P < 0.01$).

The relative weights of legs (thigh and drumstick), and breast were not affected by dietary treatments. Regarding the effect of dietary treatments on dressed carcass percentage, the results revealed significantly higher percentages for the birds of T2 (BD + double level of amino acids). The main effect of dietary treatments on percentages of immune organs weight was insignificant, however; there was a trend to increase in the percent of spleen and bursa of T3 birds (those received BD + double level of vitamins).

Plasma proteins

The effect of storage periods and dietary treatments on plasma total protein, albumin, and globulin are presented in Table 3. The main effect of storage period on blood plasma proteins was significant for albumin ($P<0.01$). Albumin percent was significantly higher in blood plasma of

Table 1. Effect of storage period and dietary treatments on growth performance of broiler chickens aged 0-6 weeks.

Treatments	Variables (0-6 weeks of age)				
	Final BW (g/bird)	Total BWG (g/bird)	Total FI (g/bird)	FCR (g feed: g gain)	Mortality*
Storage Period					
Fresh	2172^{b}	2116.8^{b}	3620.4	1.71	1
4 Month	2288^{a}	2217.6^{a}	3662.4	1.65	2
6 Month	2246^{a}	2171.4^{ab}	3683.4	1.7	1
SEM	22.17	0.27	1.09	0.02	-
P-Value	0.00	0.09	0.62	0.3	-
Dietary Treatment					
T1	2152^{b}	2079^{b}	3574.2^{b}	1.72^{a}	1
T2	2357^{a}	2284.8^{a}	3750.6^{a}	1.64^{b}	2
T3	2199^{b}	2146.2^{b}	3637.2^{ab}	1.69^{ab}	1
SEM	22.17	0.27	1.09	0.02	-
P-Value	<.001	0.00	0.03	0.09	-

$^{a, b, c,d}$ Means within the same column without mutual superscripts are significantly different ($P \leq 0.05$). T1: control; T2: amino acids; T3: vitamins; *: number of birds.

the birds received 4- or 6-month stored feed. The main effect of dietary treatments on blood plasma proteins was insignificant, however; the percentages of globulins and total proteins tended to be higher in T2 (BD + amino acids) and T3 (BD + vitamins).

Detection of Aflatoxins

The results presented in Table 4 revealed the values of different types of aflatoxins detected in the experimental diets at different storage periods (fresh, 4-months, and 6-months). The detected values of AF1, AF2, AG1, and AG2 were very low. They - even in some samples- were lower than the limit of detection (LOD). It is globally (USDA, FDA, and EFSA) and locally (Egyptian Ministry of Agriculture) accepted that all animal feeds containing less than 20 parts per billion aflatoxins are considered safe. These results indicate that diets supplemented with antioxidants could be safely used after 4 to 6 months storage period without any risk of unaccepted level of aflatoxins.

Discussion

This study aimed at evaluating the effects of storage period of broiler's feed, mainly based on bird's performance. Broilers feed here was stored for up to 6 months, which is the expiry date set by the Egyptian Ministry of Agriculture. As mentioned in the introduction, there are few studies evaluated the effects of storage period of poultry feed on performance. Some studies evaluated the effect of storage period of individual ingredients including corn and barley, which indicated a severe decline in their nutritive value in one or more nutrients including energy, fat, starch, total protein, amino acids, Lysine, vitamins, or increased aflatoxin content (Bartov, 1996; Reed *et al*., 2007; García-Rosas *et al*., 2009; Shurson *et al*., 2011; Yin *et al*., 2017). Any reduction in the dietary nutrient levels, feed spoilage, or aflatoxin contamination, would show large negative impacts on broilers performance and liveability. However, the obtained results here did not indicate any negative effect of feed storage periods on growth performance measurements or liveability, which implies that

Table 2. Effects of storage period and dietary treatments on carcass traits and immune organs of broiler chickens at 6 weeks of age.

Treatment	Variables (% of live body weight)								
	Legs (thigh +drumstick)	Breast	Dressing	Abdominal Fat	spleen	Bursa	Liver	Heart	Gizzard
Storage Period									
Fresh	22.67	22.49	79.20^{a}	1.61^{b}	0.21^{b}	0.16	1.86^{b}	0.49	1.71
4 Month	22.64	23.52	78.59^{b}	1.26^{a}	0.22^{b}	0.16	2.00^{ab}	0.49	1.6
6 Month	22.89	23.59	79.15^{a}	1.36^{b}	0.25^{a}	0.17	2.18^{a}	0.52	1.69
SEM	0.22	0.47	0.15	0.08	0.01	0.01	0.07	0.01	0.04
P-Value	0.69	0.21	0.01	0.01	0.03	0.33	0.01	0.27	0.24
Dietary Treatment									
T1	23.01	22.72	78.60^{b}	1.37	0.23^{ab}	0.15	2	0.49	1.76^{a}
T2	22.57	23.77	79.51^{a}	1.35	0.21^{b}	0.16	2.01	0.51	1.59^{b}
T3	22.63	23.12	78.83^{b}	1.5	0.25^{a}	0.18	2.04	0.5	1.65^{ab}
SEM	0.22	0.47	0.15	0.08	0.01	0.01	0.07	0.01	0.04
P-Value	0.33	0.33	0.01	0.38	0.08	0.13	0.89	0.75	0.06

$^{a, b, c,d}$ Means within the same column without mutual superscripts are significantly different ($P \leq 0.05$).T1: control; T2: amino acids; T3: vitamins

Table 3. Effects of storage period and dietary treatments on plasma proteins and haemoglobin concentration of broiler chickens at 6 weeks of age.

Treatment	Plasma Proteins (g/dl)			Haemoglobin (g/dl)
	Total protein	Albumin	Globulin	
Storage Period				
Fresh	1.93	0.65^{b}	1.27	13.94^{a}
4 Month	1.95	0.90^{a}	1.05	13.07^{a}
6 Month	1.92	0.87^{a}	1.05	10.22^{b}
SEM	0.08	0.06	0.08	0.41
P-Value	0.97	0.01	0.13	<.0001
Dietary Treatment				
T1	1.77^{b}	0.81	0.96	12.05^{b}
T2	2.01^{ab}	0.8	1.2	11.91^{b}
T3	2.02^{a}	0.8	1.21	13.27^{a}
SEM	0.08	0.06	0.08	0.41
P-Value	0.06	0.99	0.08	0.05

$^{a, b, c,d}$ Means within the same column without mutual superscripts are significantly different ($P \leq 0.05$). T1: control; T2: amino acids; T3: vitamins

Table 4. Aflatoxins detected in fresh and stored feed.

	AFB1 (ppb)	AFB2	AFG1 (ppb)	AFG2 (ppb)	Total Afs (ppb)
Fresh	9.3	>LOD	>LOD	0.4	8.9
4 Month	2.24	0.27	1.27	0.2	0.42
6 Month	0.5	>LOD	0.08	>LOD	0.44

the tested storage periods up-to 6 months have no negative effects on dietary nutrient levels or diet safety.

These results may partially agree with those of Chrastil (1990) who evaluated the effect of storage duration for 110 months on the nutritional value of corn kernels for broiler and his results showed that dry matter, protein, amino acids, and fat contents were not changed in the corn stored for periods from 0 to 110 month, and therefore their growth performance was not negatively affected. On the other hand, our findings contradicted with those of Abera and Rakshit (2004) who evaluated the effect of storage of corn for 2 months on nutrient composition. Their results showed that the percentage of protein in the maize was significantly decreased by the duration of storage, also, the fat content of the grain decreased by more than 10% (Reed *et al*., 2007). Similarly, Yin *et al*. (2017) reported that the content of fatty acids, amino acids, and crude protein in corn generally decreased with increasing the storage period. Moreover, García-Rosas *et al*. (2009) found that corn storage caused starch retro-gradation and increase resistant starch formation.

Different researchers have previously shown significant increases in BWG from broilers receiving diets supplemented with methionine and lysine. Kidd and Kerr (1996) reported that body weight gain of broiler chicks was improved significantly by 110 and 130% of the methionine level recommended by NRC (1994). Ahmed and Abass (2011) found that there were positive effects on meat yield and growth performance in response to supplemental lysine and methionine in male broiler diets from 21 to 41 days of age. Nasr and Kheiri (2011) reported that lysine and methionine may result in enhanced performance, especially with regard to breast meat yield, body weight gain, and feed conversion ratio related to an increase in lean muscle tissue. Wen *et al*. (2017) reported that lysine was found to improve carcass quality and growth performance of broilers. Belloir *et al*. (2019) indicated that healthy broilers responded positively to the high dietary inclusion of amino acids and had a positive effect on the performance. Fagundes *et al*. (2020) illustrated that lysine is one of the limiting amino acids in poultry diets. Additional lysine at 120% of the level of NRC in avian broiler diets optimized BWG, whereas a low level of lysine decreased growth and live weight.

Dale and Fuller (1980) reported that heat-stressed broiler chickens respond positively to increase amino acid consumption. They suggested increasing the amino acid levels in warm environments to compensate for the expected reduction in feed intake. These results are similar to those of Schutte and Pack (1995) who reported that a significant increase in amino acids feed intake (120 and 130% of NRC methionine) improved feed conversion ratio in broiler chicks. Rezaei *et al*. (2004) found that diets supplemented with additional lysine of 0.2% with the standard methionine level of 0.43% in a reduced CP diet gave the best results in terms of FCR. Corzo *et al*. (2005) reported that lysine and methionine may result in enhanced performance, especially regarding breast meat yield, body weight gain, and feed conversion ratio. Saki *et al*. (2007) cleared that amino acid supplementation favours feed conversion efficiency in broilers. Chrystal *et al*. (2020) stated that high amino acids diets have been shown to maximize growth performance, meat yields, and BW and to decrease FCR. Neto *et al*. (1998) reported that the addition of Methionine to high (24%)- or low (17%)-protein diets improved the growth and FCR. Kidd *et al*. (2004) mentioned that dietary synthetic amino acid supplementation to broilers diets improved feed efficiency and reduced nitrogen excretion.

The obtained results in this study partially agree with those of Cave *et al*. (2008) who reported that the addition of amino acids and metabolic intermediates to diets may lower abdominal fat deposition in broilers. Schutte and Pack (1995) reported that broiler chicks consuming diets with methionine higher than NRC level showed a significant increase in absolute and relative breast weight and a significant decrease in abdominal fat. Ahmed and Abbas (2011) showed that the broiler chicks fed with a diet containing higher methionine level than National Research Council requirements (NRC, 1994) exhibited a significant increase in relative and absolute weight of the breast and a significant reduction in abdominal fat.

The absence of any negative effect of storage period on most percentages of carcass parts may be considered as a favour for feed stored for up to 6 months. These results are partially in agreement with those of Nutautaitė *et al*. (2020) who reported that modified amino acid inclusion can reduce mortality and improve health status of broiler chickens.

Conclusion

Based on the results of the present study it could be concluded that, under Egyptian circumstances, enrichment of broiler feeds with higher levels of lysine and methionine (double level of NRC recommendations) together with supplemental antioxidants could prolong their shelf-life to 6 months without any deleterious impact on productive performance of broiler chickens.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Abera, S., Rakshit, S.K., 2004. Effect of dry cassava chip storage on yield and functional properties of extracted starch. Starch-Stärke 56, 232-240.

Afolabi, C.G., Ezekiel, C.N., Ogunbiyi, A.E., Oluwadairo, O.J., Sulyok, M., Krska, R., 2019. Fungi and mycotoxins in cowpea (Vigna unguiculata L) on Nigerian markets. Food Additives and Contaminants 13, 52–58.

Ahmed, M.E., Abbas, T.E., 2011. Effects of dietary levels of methionine on broiler performance and carcass characteristics. International Journal of Poultry Science 10, 147-151.

Attia, Y. A., Burke, W.H., Yamani, K.A., Jensen, L.S., 1995. Daily energy allotments and performance of broiler breeders. 2. Females. Poultry Science 74, 261-270.

Bartov, I., 1996. Effect of storage duration on the nutritional value of corn kernels for broiler chicks. Poultry Science 75, 1524-1527.

Belloir, P., Lessire, M., Lambert, W., Corrent, E., Berri, C., Tesseraud, S., 2019. Changes in body-composition and meat quality in response to dietary amino acid provision in finishing broilers. Animal 13, 1094-1102.

Benvenuti, M.E., Burgess, J.A., 2010. Rapid analysis of aflatoxins in corn, cereals, and almonds using ACQUITY UPLC H-Class System with fluorescence detection. Corporation W, editor. Milford, MA2010.

Cave, M.C., Hurt, R.T., Frazier, T.H., Matheson, P.J., Garrison, R.N., McClain, C J., McClave, S.A., 2008. Obesity, inflammation, and the potential application of pharmaconutrition. Nutrition in Clinical Practice 23, 16-34.

Chrastil, J., 1990. Protein-starch interactions in rice grains. Influence of storage on oryzenin and starch. Journal of Agricultural and Food Chemistry 38, 1804-1809.

Chrystal, P.V., Moss, A.F., Khoddami, A., Naranjo, V.D., Selle, P.H., Liu, S.Y., 2020. Effects of reduced crude protein levels, dietary electrolyte balance, and energy density on the performance of broiler chickens offered maize-based diets with evaluations of starch, protein, and amino acid metabolism. Poultry Science 99, 1421-1431.

Corzo, A., Kidd, M.T., Burnham, D.J., Miller, E.R., Branton, S.L., Gonzalez-Esquerra, R., 2005. Dietary amino acid density effects on growth and carcass of broilers differing in strain cross and sex. Journal of Applied Poultry Research 14, 1-9.

Dale, N.M., Fuller, H.L., 1980. Effect of diet composition on feed intake and growth of chicks under heat stress: II. Constant vs. cycling temperatures. Poultry Science 59, 1434-1441.

Duncan, D.B., 1955. Multiple range and multipletests. Biometrics 11, 1-42.

Fagundes, N.S., Milfort, M.C., Williams, S.M., Da Costa, M.J., Fuller, A.L., Menten, J.F., Rekaya, R., Aggrey, S.E., 2020. Dietary methionine level alters growth, digestibility, and gene expression of amino acid transporters in meat-type chickens. Poultry Science 99, 67-75.

Galliard, T., 1986. Hydrolytic and oxidative degradation of lipids during storage of wholemeal flour: Effects of bran and germ components. Journal of Cereal Science 4, 179-192.

García-Rosas, M., Bello-Pérez, A., Yee-Madeira, H., Ramos, G., Flores-Morales, A., Mora-Escobedo, R., 2009. Resistant starch content and structural changes in maize (Zea mays) tortillas during storage. Starch-Stärke 61, 414-421.

Kidd, M.T., Kerr, B.J., 1996. L-threonine for poultry: A review. Journal of Applied Poultry Research 5, 358-367.

Kidd, M.T., McDaniel, C.D., Branton, S.L., Miller, E.R., Boren, B.B., Fancher, B.I., 2004. Increasing amino acid density improves live performance and carcass yields of commercial broilers. Journal of Applied Poultry Research 13, 593-604.

Leggieri, M.C., Lanubile, A., Dall'Asta, C., Pietri, A., Battilani, P., 2020. The impact of seasonal weather variation on mycotoxins: maize crop in 2014 in northern Italy as a case study. World Mycotoxin Journal 13, 25–36.

Moretti, A., Logrieco, A.F., Susca, A., 2017. Mycotoxins: an underhand food problem. In: Mycotoxigenic Fungi. Humana press, New York, pp 3–12.

Nakavuma, J.L., Kirabo, A., Bogere, P., Nabulime, M.M., Kaaya, A.N., Gnonlonfin, B., 2020. Awareness of mycotoxins and occurrence of aflatoxins in poultry feeds and feed ingredients in selected regions of Uganda. International Journal of Food Contamination 7, 1-10.

Nasr, J., Kheiri, F., 2011. Effect of different lysine levels on Arian broiler performances. Italian Journal of Animal Science 10, e32.

Neto, M.G., Pesti, G.M., Bakilli, R.I., 1998. Effects of betaine and methionine levels in broilers chickens fed different levels of energy and protein. Poultry Science 77, 84.

NRC., 1944. Recommended Nutrient Allowances for Poultry. National Academy, Washington.

Nutautaitė, M., Alijošius, S., Bliznikas, S., Šašytė, V., Vilienė, V., Pockevičius, A., Racevičiūtė-Stupelienė, A., 2020. Effect of betaine, a methyl group donor, on broiler chicken growth performance, breast muscle quality characteristics, oxidative status and amino acid content. Italian Journal of Animal Science 19, 621-629.

Pomeranz, Y., 1982. Biochemical, functional and nutritive changes during storage. Storage of cereal grains and their products. U.S. Grain Marketing Res. Lab., Manhattan, KS 66506, USA

Reed, C., Doyungan, S., Ioerger, B., Getchell, A., 2007. Response of storage molds to different initial moisture contents of maize (corn) stored at 25°C, and effect on respiration rate and nutrient composition. Journal of Stored Products Research. 43, 443-458.

Rezaei, M., Moghaddam, H.N., Reza, J.P., Kermanshahi, H., 2004. The effects of dietary protein and lysine levels on broiler performance, carcass characteristics and N excretion. International Journal of Poultry Science 3, 148-152.

Saki, A.A., Pour, H.A., Ahmdi, A., Akhzar, M.T., Tabatabie, M.M., 2007. Decreasing broiler crude protein requirement by methionine supplementation. Pakistan Journal of Biological Sciences 10, 757-762.

SAS institute, 2009. User's Guide Version 9.2, SAS Institute Inc., Cary, NS, USA.

Schutte, J.B., Pack, M., 1995a. Effects of dietary sulphur-containing amino acids on performance and breast meat deposition of broiler chicks during the growing and finishing phases. British Poultry Science 36, 747-762.

Shurson, G.C., Salzer, T.M., Koehler, D.D., Whitney, M.H., 2011. Effect of metal specific amino acid complexes and inorganic trace minerals on vitamin stability in premixes. Animal Feed Science and Technology 163, 200-206.

Wen, C., Jiang, X.Y., Ding, L.R., Wang, T., Zhou, Y.M., 2017. Effects of dietary methionine on growth performance, meat quality and oxidative status of breast muscle in fast-and slow-growing broilers. Poultry Science 96, 1707-1714.

Yin, D., Yuan, J., Guo, Y., Chiba, L.I., 2017. Effect of storage time on the characteristics of corn and efficiency of its utilization in broiler chickens. Animal Nutrition 3, 252-257.

Zain, M.E., 2011. Impact of mycotoxins on humans and animals. Journal of Saudi Chemical Society 15, 129–144.