Effect of diet form and enzyme supplementation on stress indicators and bone mineralisation in heat-challenged broilers fed wheat-soybean diet

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ABSTRACT
A study was carried out to evaluate the effect of dietary xylanase supplementation and feed form on the bone mineralisation and tibia breaking strength, serum enzyme activities, plasma minerals concentration, and heat stress biomarkers of broilers fed wheat-based diet under high ambient temperature (33°C for 10 h, from 07.00 to 17.00 h, and 22°C from 17.00 to 07.00 h). Two hundred and forty 1-d-old Ross 308 male broilers were allocated in six experimental treatments, each of which was replicated five times with eight broilers per replicate. A 2 × 3 factorial design was used in the study, and the main factors were composed of xylanase (with or without 300 mg/kg) and feed form (mash, crumbles, and pellets). Broilers fed crumbles or pellets had higher tibia breaking strength, plasma P concentration, and serum ALP activity than broilers fed mash. The tibia ash, Ca, and P contents in ash were greater (p < .001) for broilers fed pellets than for broilers fed mash or crumbles. The circulating heterophil-to-lymphocyte ratio, creatine kinase level, and heat shock protein 70 mRNA of breast muscle were also decreased (p < .05) by the crumbles and pellets diets. Xylanase significantly increased tibia ash, tibia Ca and P contents, ALP activity, plasma Ca and P concentrations (p < .05). The results indicate that crumbling or pelleting of the diets improved minerals retention and down-regulated heat stress biomarkers of broilers subjected to heat challenge. The results of the current study suggested that dietary addition xylanase has the potential to improve bone mineralisation. It could be concluded that feeding crumbled and pelleted diets may effective in partially ameliorating the resistance to heat stress in the birds under high ambient temperature.

ARTICLE HISTORY
Received 12 February 2017
Revised 17 April 2017
Accepted 19 April 2017

KEYWORDS
Broilers; heat stress biomarkers; feed form; wheat; xylanase

Introduction
Heat stress (HS) occurring in temperate countries as well as in the tropical world, exerts deleterious effects on productive performance and mortality rate of broilers (Attia & Hassan 2017). In east of Iran, the environmental temperature during the greater part of the year remains well beyond the upper limit of the thermoneutral zone. It has been well demonstrated that exposing broilers to continuously high temperature, especially during the finisher period, leads to chronic HS (Sahin et al. 2003; Attia et al. 2006). High ambient temperature has been shown to influence immunity status and induce multiple physiological disturbances, such as systemic immune dysregulation, endocrine disorders, and electrolyte imbalance (Teeter et al. 1985; Sohail et al. 2010, 2012). Most of the reduction in feed consumption is due to reduced maintenance requirement. In broilers, growth rates, feed efficiency, and carcase quality are negatively affected (Attia et al. 2011; Sohail et al. 2012). In this respect, feed processing is among the most preferred and practical way to alleviate the effect of high ambient temperature on broilers performance (Hosseini & Afshar 2017). Pelleted feed also has the benefits of decreased feed ingredient separation, decreased feed wastage, starch gelatinisation, and improved palatability (Abdollahi et al. 2011; Attia et al. 2012, 2014a, 2014b). The application of xylanase-based enzymes in wheat diets for broilers has been established as a commercial routine in countries where wheat is the most cost-effective cereal (Bedford 2000). Marked improvements in the nutritive value of wheat, when supplemented with xylanase, have been reported (Schutte et al. 1995).

However, less is known about the effect of feed processing on ameliorating impairment of
performance and minerals retention of broilers subjected to HS. We hypothesised that high ambient temperature might induce impairments of bone mineralisation, liver function, and immune system in broilers, and crumbling or pelleting of the feed and dietary addition of a xylanase enzyme might have beneficial effects to ameliorate these impairments in broilers fed wheat-based diets. There are no studies reported which simultaneously compare mash, crumbles, pellets, heat-conditioned, wheat, and xylanase enzyme. Therefore, the aim of the current study was to evaluate the effects of feed form and xylanase supplementation plus possible interactions on serum enzyme activity, minerals retention, and heat stress biomarkers of broilers under high ambient temperature.

Materials and methods

Birds and diets

Two hundred and forty 1-d-old Ross 308 male broiler chicks were randomly allocated to six groups, each of which was replicated five times with eight broilers per replicate. The experiment was designed according to a 2 × 3 factorial arrangement of treatments, and the main factors consisted of xylanase enzyme (0 and 300 mg/kg) and feed form (mash, crumbles, and pellets). The diets were subjected to three different forms: (1) no pelleting (mash), (2) pelleted with a 3.0-mm die and crumbled, or (3) pelleted with a 3.0-mm die. Pellet quality of the diets was measured in quadruplicate on samples taken at different intervals during the pelleting process by placing a weighed amount of feed (approximately 500 g) in a Tyler Sieve Shaker (Tyler Co., Mentor, OH) for 30 s at a rate of 278 oscillations/min. The percentage of pellets retained on a 2-mm screen was used to determine pellet quality. The percentages of fines were 97% for mash, 58% for the crumble, and 17% for the pellet diets. The xylanase supplementation (Econase XT 25; AB Vista Feed Ingredients, Chesterfield, MO) was used in powder form that was intrinsically thermostable up to 95°C. There was no difference in initial BW (43.2 ± 0.2 g) among the treatment groups. The feeding programme consisted of a starter phase from 1 to 21 d of age and a finisher phase from 22 to 42 d of age. Wheat/soybean-meal-based basal diets were formulated according to the NRC (1994) recommendations (Table 1). In the experiment period, birds were subjected to cyclic HS by exposing them to 33°C for 10 h, from 0700 to 1700 h, and 22°C from 1700 to 0700 h. The lighting programme was 23L:1D during the entire period.

Air humidity was kept at 70% throughout the experimental period. The birds were reared in pens (90 × 120 × 70 cm, length × width × height) and given ad libitum access to feed and water. The animal care protocol in this experiment was approved by the Animal Ethics Committee of the University of Birjand.

Size of different organs

At 42 d of age, two randomly chosen birds per replicate (10 birds per treatment) were randomly selected and slaughtered through cutting of jugular veins and carotid arteries, and processed manually and collections were made following a 4-h fast. The weights of the pancreas, liver, spleen and bursa were recorded. The organs were cleaned with physiological saline solution, dried with filter paper and weighed. The weight of each organ was expressed relative to the pre-slaughter body weight (g/100 g BW).

Bone mineralisation

The right tibia of the same killed birds was removed and stored at −18°C to assess their total ash and mineral content at day 42 of age. Tibia ash was determined by removing the adhering tissue, drying the bone at 110°C for 12 h and extraction of fat with ether. The dry fat-free bones were ashed in a muffle

| Table 1. Ingredients and calculated and analysed compositions of the basal diets. |
|------------------|-----|-----|
| Item             | Starter (0–21 d) | Grower (22–42 d) |
| Ingredients, %   |     |     |
| Wheat (12.5% CP) | 50.46 | 51.00 |
| Corn             | 14.00 | 19.32 |
| Soybean meal (48% CP) | 29.00 | 22.00 |
| Soybean oil      | 2.50  | 4.00  |
| Limestone        | 1.10  | 1.00  |
| Dicalcium phosphate | 1.70  | 1.50  |
| NaHCO3           | 0.20  | 0.20  |
| Salt             | 0.15  | 0.10  |
| L-Lysine         | 0.20  | 0.22  |
| a-Methionine     | 0.14  | 0.13  |
| L-Threonine      | 0.05  | 0.03  |
| Vitamin–mineral premixa | 0.50 | 0.50 |
| Calculated composition |     |     |
| Metabolizable energy, MJ/kg | 12.18 | 12.81 |
| Available phosphorus, g/kg | 4.50 | 4.00 |
| Lysine, %        | 1.25  | 1.07  |
| Methionine, %    | 0.45  | 0.41  |
| Methionine + cysteine, % | 0.90 | 0.80 |
| Analyzed composition, g/kg |     |     |
| Crude protein (N × 6.25) | 21.15 | 18.53 |
| Crude fibre      | 2.65  | 2.51  |
| Calcium          | 0.90  | 0.80  |

*Vitamin and mineral premix supplied the following per kilogram of diet: vitamin A (from vitamin A acetate), 10,000 U; vitamin D3, 9790 U; vitamin E (dl-a-tocopheryl acetate), 30 U; vitamin B12, 20 μg; riboflavin, 4.4 mg; calcium pantothenate, 40 mg; niacin, 22 mg; choline, 840 mg; biotin, 30 μg; thiamine, 4 mg; zinc sulphate, 60 mg; copper sulphate, 100 μg; selenium (sodium selenate), 0.2 mg; iodine, 1 mg; manganese oxide, 60 mg.
furnace at 550°C for 3 h, according to AOAC International (2000, method 932.16). Ash weight was calculated as a percentage of dry-fat-free bone weight. The tibia contents of Ca, P, and Mg were measured using dry-ashed bone samples. The meat was removed before bone-breaking strength analyses of the right tibia.

**Bone strength**

Bone-breaking strength was determined by breaking the right tibia on an Instron 4301 tensiometer (model 5500, Instron Corp., Canton, MA), using a three-point bend with supports 60 mm apart and a load applied at 50 mm/min to the midpoint of the long axis of the bone (Knowles & Broom 1990). The parts of the tensiometer in contact with the bone were covered in soft rubber tubing to avoid point stresses. The breaking strength was recorded as the peak load before the bone breakage.

**Blood sampling**

Two chicks from each pen (10 per treatment) were randomly selected and weighed individually after a 4-h fasting period at the end of the experiment (42 days of age, sampling starting after the 6 h of high ambient temperature). Two blood samples were obtained for subsequent determination of serum and plasma constituents. The first sample was collected for plasma measurements and placed into heparinised tubes. The tubes were centrifuged at 839 g for 15 min to obtain plasma, which was stored at −20°C pending analysis. A second coagulated blood sample was centrifuged (839g for 10 min) within 30 min after sampling to obtain serum. Serum samples were divided in two aliquots and kept at −20°C until analysed.

**Chemical analysis of blood components**

Plasma triiodothyronine (T₃) and thyroxine (T₄) were determined by double-antibody RIA using commercially available RIA kits (China Institute of Atomic Energy, Beijing, China) as described by Darras et al. (1992). The reported procedures were used to measure Ca, P, Mg, Fe, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), (Burtis & Ashwood 1998), triglycerides, and cholesterol (Nauck et al. 2002). Haematological analysis was conducted to measure the levels of lymphocytes and heterophils by using an automatic haematological analyser (XE-2100, Automated Hematology Analyzer, Sysmex America, Inc., Kobe, Japan). Values of lymphocytes and heterophils counts were also used to calculate the heterophil-to-lymphocyte (H:L) ratio index.

The breast (pectoralis major) muscles without skin and adipose tissues were also collected and quickly snap-frozen in liquid nitrogen that was further used to analyse creatine kinase (CK) and heat shock protein 70 mRNA (HSP70) levels. The CK activity was measured using a CK kit (Roche Diagnostics, Indianapolis, IN), while HSP70 level was measured using LD-L10 (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The chicken HSP70 gene sequence deposited in GenBank under the accession number J02579 was used as nucleotide sequence in the current research.

**Statistical analysis**

In this experiment, pen considered as experimental unit and data were analysed as a completely randomised design with 3 × 2 factorial treatment arrangements using the GLM procedures of SAS (SAS 2008). The model included the main effects of feed form, xylanase, and associated 2-way interactions. Level of significance was set at 5% and when a significant effect was indicated, treatment means were separated using Tukey–Kramer’s test. Percentage data were submitted to arc sin square root percentage transformations prior to statistical analysis.

**Results**

No interactions between feed form and xylanase enzyme were detected for any of the traits studied, and therefore, only main effects are presented. Data on the effect of feed form and xylanase enzyme on relative visceral weight are presented in Table 2. No significant variation in the relative values for liver, pancreas, spleen, and bursa has been found at 42 d of age, among main effects.

Table 2. Effects of feed form and xylanase enzyme on relative visceral weight of heat-stressed broilers fed wheat-based diet (n = 5).

| Main effect | Liver, g/100 g BW | Pancreas, g/100 g BW | Spleen, g/100 g BW | Bursa, g/100 g BW |
|-------------|------------------|---------------------|--------------------|-------------------|
| Feed form   |                  |                     |                    |                   |
| Mash        | 22.09            | 1.95                | 1.53               | 1.90              |
| Crumble     | 21.89            | 1.92                | 1.60               | 1.84              |
| Pellets     | 21.95            | 1.90                | 1.61               | 1.78              |
| Xylanase    |                  |                     |                    |                   |
| +           | 21.96            | 1.95                | 1.58               | 1.79              |
|              | 22.00            | 1.90                | 1.57               | 1.90              |
| SEM         | 1.41             | 0.08                | 0.07               | 0.06              |
| P-value     |                  |                     |                    |                   |
| Feed form   | 0.225            | 0.417               | 0.965              | 0.265             |
| Xylanase    | 0.215            | 0.326               | 0.887              | 0.564             |
| Feed form × xylanase | 0.198 | 0.265 | 0.659 | 0.265 |
Feed form and xylanase supplementation significantly affected the bone ash and tibia Ca and P contents (Table 3). Tibia ash (p = .002), tibia Ca (p = .033), and P (p = .013) contents were greater for chicks fed pellets than for broilers fed mash, with broilers fed crumbles being intermediate. Breaking strength was higher (Figure 1; p < .05) for broilers fed crumbles or pellets than for broilers fed mash. Addition of xylanase supplementation significantly increased tibia ash (p = .036), breaking strength (p < .05), and Ca (p = .024) and P (p = .028) contents in ash.

Chicks fed pellets or crumbles have shown no statistical changes for serum ALT, AST, and LDH at d 42, when compared with the birds fed mash diet (Table 4). Only ALP values have significantly (p = .002) increased in both the crumbles and pellets diets used in this study; whereas, in the broilers fed diets supplemented with xylanase, this value was not significant.

As Table 5 shows, the plasma P concentration was higher for broilers fed pellets than for broilers fed crumbles and both were higher than broilers fed mash (p = .008). Xylanase supplementation significantly increased plasma Ca (p = .014) and P concentrations (p = .025).

Data on the effect of feed form and xylanase enzyme on selected blood metabolites are presented

### Table 3. Effects of feed form and xylanase enzyme on bone mineralisation of heat-stressed broilers fed wheat-based diet (n = 5).

| Main effect          | Ash, % | Ca, % | P, % | Mg, % |
|----------------------|--------|-------|------|-------|
| Feed form            |        |       |      |       |
| Mash                 | 46.02b | 39.29b| 19.22b| 0.86  |
| Crumble              | 47.05ab| 39.52ab| 19.19ab| 0.88  |
| Pellets              | 48.54a | 40.14a| 19.29a| 0.87  |
| Xylanase             |        |       |      |       |
| –                    | 46.62b | 39.05b| 19.20b| 0.87  |
| +                    | 47.80a | 40.26a| 19.27a| 0.88  |
| SEM                  | 0.57   | 0.84  | 0.23 | 0.07  |
| p-value              |        |       |      |       |
| Feed form            | .002   | .033  | .013 | .453  |
| Xylanase             | .036   | .024  | .028 | .276  |
| Feed form x xylanase| .065   | .098  | .327 | .089  |

*Mean values within a column with different letters differ significantly (p < .05).

### Table 4. Effects of feed form and xylanase enzyme on the serum enzymes activity of heat-stressed broilers fed wheat-based diet (n = 5).

| Main effect          | ALP, U L⁻¹ | AST, U L⁻¹ | ALT, U L⁻¹ | LDH, U L⁻¹ |
|----------------------|------------|------------|------------|------------|
| Feed form            |            |            |            |            |
| Mash                 | 14,321b    | 240        | 1.7        | 720        |
| Crumble              | 14,334a    | 240        | 1.6        | 720        |
| Pellets              | 14,333a    | 241        | 1.7        | 719        |
| Xylanase             |            |            |            |            |
| –                    | 14,320     | 240        | 1.7        | 720        |
| +                    | 14,324     | 241        | 1.7        | 719        |
| SEM                  | 166        | 3.3        | 0.62       | 6.2        |
| p-value              |            |            |            |            |
| Feed form            | .002       | .257       | .302       | .659       |
| Xylanase             | .211       | .183       | .125       | .752       |
| Feed form x xylanase| .131       | .235       | .329       | .466       |

*Mean values within a column with different letters differ significantly (p < .05).

ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LDH: lactate dehydrogenase.

![Figure 1. Tibia-breaking strength (breaking force divided by bone weight expressed as Newton per gram) by xylanase enzyme and feed form in heat-stressed broilers fed wheat-based diet. Values are mean ± SE (n = 5). Within the graph, bars with different letters (a–c) are significantly different (p < .05).](image-url)
in Table 6. Feed form and xylanase supplementation did not significant effect on cholesterol and triglycerides concentrations, and thyroid hormones.

The effects of diet form and xylanase on heterophil (H) and lymphocyte (L) concentrations and H:L ratio of heat-stressed broilers are shown in Table 7. Feed form and xylanase supplementation had little effect on the biomarkers of heat stress. In fact, the only effect observed was for the H:L ratio that was lower ($p = .023$) in chicks fed crumbles or pellets diets than in chicks fed mash diet. It was noted that heat stressed-broilers fed with crumbled and pelleted diets had lower the CK activity in breast muscle (Figure 2) in comparison with the chicks fed mash diet. The relative value for HSP70 mRNA expression in breast muscle is presented in Figure 3. Real-time PCR analysis showed that crumblles and pellets diets down-regulated the HSP70 gene expression in breast muscle of heat-challenged broilers.

### Discussion

Francesch and Geraert (2009) found that reducing dietary nutrient levels resulted in reduced bone ash, calcium, and phosphorus contents in comparison with a standard diet. Moreover, those authors revealed that supplementing the diet with a xylanase improved the bone mineralisation of broilers, promoting similar results as those obtained with a standard diet. The tibial ash content obtained in 42-d-old broilers in the present study are similar to the response as reported by Shaw et al. (2011), who found that the highest xylanase addition level promoted similar ash content as those found in the broilers fed the highest dietary phosphorus level. The tibial ash, P, and Ca contents results of the present study are in agreement with those obtained by Oliveira et al. (2008), who found effect of the addition of xylanase on the tibial ash and minerals content of broilers. Onyango et al. (2005) reported that xylanase supplementation of a low-phosphorus diet restored broiler growth rate, but ash content remained low. In addition, Walk et al. (2011), evaluating xylanase in broiler diets with reduced available phosphorus and calcium levels, found negative effect of enzyme supplementations on the ash content. Possibly, the reduced dietary Ca and P contents, as well as the reduced energy levels, limited not only muscle growth, but also skeletal growth. Birds may have presented smaller bones, but ash, calcium, and phosphorus contents were similar. It is important to assure that any improvement observed in bone mineralisation with extra xylanase is related to a reduction in anti-nutritional effects of NSPs (Karimi et al. 2013). By breaking down arabinoxylan polymers from wheat grain, xylanase has been shown to reduce gut viscosity and nutrient entrapment, and lead to better digestion in broilers. Hosseini and Afshar (2017) reported that xylanase supplementation reduced the competition for nutrient utilisation from intestinal microflora and more nutrients were available for the birds (Hosseini & Afshar 2017).

### Table 5. Effects of feed form and xylanase enzyme on plasma minerals concentration of heat-stressed broilers fed wheat-based diet ($n = 5$).

| Main effect | Ca, mg dL$^{-1}$ | P, mg dL$^{-1}$ | Mg, mg dL$^{-1}$ | Fe, μg dL$^{-1}$ |
|-------------|-----------------|----------------|-----------------|-----------------|
| Feed form   |                 |                |                 |                 |
| Mash        | 11.1            | 6.3            | 2.2             | 216             |
| Crumble     | 11.0            | 6.8            | 2.0             | 217             |
| Pellets     | 11.1            | 7.1            | 2.2             | 213             |
| Xylanase    |                 |                |                 |                 |
| -           | 10.8            | 6.6            | 2.2             | 215             |
| +           | 11.4            | 7.0            | 2.2             | 216             |
| SEM         | 1.7             | 1.2            | 0.06            | 7.5             |
| p-value     |                 |                |                 |                 |
| Feed form   | 0.25            | 0.08           | 0.235           | 0.328           |
| Xylanase    | 0.014           | 0.025          | 0.175           | 0.216           |
| Feed form x xylanase | .098 | .089 | .118 | .166 |

*Mean values within a column with different letters differ significantly ($p < 0.05$).

### Table 6. Effects of feed form and xylanase enzyme on selected blood metabolites of heat-stressed broilers fed wheat-based diet ($n = 5$).

| Main effect | Cholesterol, mg dL$^{-1}$ | Triglycerides, mg dL$^{-1}$ | $T_4$, ng dL$^{-1}$ | $T_3$, ng dL$^{-1}$ |
|-------------|---------------------------|-----------------------------|--------------------|--------------------|
| Feed form   |                           |                             |                    |                    |
| Mash        | 123.5                     | 62.04                       | 1.690              | 16.141             |
| Crumble     | 122.5                     | 61.03                       | 1.694              | 16.139             |
| Pellets     | 124.6                     | 61.12                       | 1.691              | 16.138             |
| Xylanase    |                           |                             |                    |                    |
| –           | 123.6                     | 61.94                       | 1.693              | 16.136             |
| +           | 123.4                     | 60.85                       | 1.691              | 16.134             |
| SEM         | 0.33                      | 0.06                        | 0.002              | 0.069              |
| p-value     |                           |                             |                    |                    |
| Feed form   | 0.25                      | 0.357                       | 0.623              | 0.166              |
| Xylanase    | 0.178                     | 0.336                       | 0.453              | 0.344              |
| Feed form x xylanase | .145 | .257 | .358 | .132 |

*Mean values within a column with different letters differ significantly ($p < 0.05$).
They also found that xylanase increases the villus height or villus height-to-crypt depth ratio in the small intestine, resulting in a larger surface for nutrient digestion and absorption. These factors facilitated the nutrient digestibility, demonstrated by the addition of xylanase, and enhanced minerals retention in birds.

A number of enzymes are used in the clinical biochemistry as tools for differential diagnosis, such as ALT and AST. As the bulk of each is located in different tissues, their abnormal appearance in the blood plasma can give a hint to specific muscle or organ damages (Pech-Waffenschmidt et al. 1995). The increase in the activities of AST and ALT enzymes in plasma is an indication of liver damage and thus causes alterations in liver function (Kim et al. 2008). In this study, feed form could increase serum ALP activity in broilers exposed to high ambient temperature. Total serum ALP measures a composite of several isoenzymes of Zn metalloenzymes.

**Figure 2.** Creatine kinase (expressed as U/mg of protein) level of chicken breast muscle by xylanase enzyme and feed form in heat-stressed broilers fed wheat-based diet. Values are mean ± SE (n = 5). Within the graph, bars with different letters (a,b) are significantly different (p < .05).

**Figure 3.** Heat shock protein 70 mRNA (HSP70) expression of chicken breast muscle by xylanase enzyme and feed form in heat-stressed broilers fed wheat-based diet. Values are mean ± SE (n = 5). Within the graph, bars with different letters (a,b) are significantly different (p < .05).
by cells in a number of organs (liver, bone, muscle, small intestine, and kidney) (Nourmohammadi et al. 2016). Huff et al. (1998) observed that serum ALP levels decreased in chicks that received high levels of phosphorus, most likely the result of a down regulation of ALP activity, suggesting that the synthesis of this protein is dependent on phosphorus levels. The increase in serum ALP activity associated with the crumbled and pelleted diets might reflect the up-regulation of this enzyme caused by decreased availability of phosphorus. These findings are inconsistent with data from bone mineral retention. The inconsistency in results probably is associated with environmental temperature or physical feed form and particle size. Potential effects of diet form in serum enzyme activity and mineral retention under high ambient temperature needs to be further investigated.

That chickens give priorities to their mineral requirements for vital functions in compromise of body growth is indicated by the normal concentrations of the minerals in the plasma of the control birds. Similar findings have been attributed to a diluting effect as a result of rapid growth rate on the adequate diets and poor growth rate on the control diet. It indicates that the assessment of trace mineral status is difficult and remains an important, tricky challenge. The results of the current research demonstrated that xylanase enzyme supplementation and feed form can be an appropriate strategy for improvement of minerals retention in broilers under high ambient temperature fed wheat-based diets.

It is well documented that environmental stressors (such as heat stress) can increase levels of HSP70 in different tissues of poultry with a concomitant increase in circulating H:L ratio and CK (Garriga et al. 2006). The results of the present study indicate that HS biomarkers were modulated by feed form, which probably are in an indication of improving effects of feed processing against heat challenge. In other words, the resistance to high ambient temperature was improved in the broilers fed pelleted and crumbled diets as evidenced by lower HSP70 gene expression and CK level. Based on these results it can be concluded that the diet form were protective effects against the onset of oxidative damage. A strong relationship among synthesis of HSP70 and oxidation in stressed cells has been detected (Akbarian et al. 2013).

Conclusions

It was concluded that dietary use of xylanase supplementation as a feed additive and feed form may be a practical nutritional strategies for broilers to overcome or reduce the disadvantageous effects of high ambient temperature in broiler production. Based on these findings, it also was concluded that the dietary supplementation of xylanase and pelleted can help in decreasing harmful effects of high ambient temperature in broiler chickens fed wheat-based diets.

Disclosure statement

No potential conflict of interest was reported by the authors.

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