*Animal Science Papers and Reports* vol. 36 (2018) no. 3, 297-310
Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

# The activity of glycosidases in turkey muscles as influenced by the form and level of Cu, Zn, Mn dietary supplementation*

**Artur Jóźwik[1]**, Nina Strzałkowska[1], Joanna Marchewka[1], Olaf Horbańczuk[3], Małgorzata Szumacher-Srabel[2], Adam Cieślak[2], Maciej Gogulski[2], Agnieszka Kamińska[4], Monika Michalczuk[3], Małgorzata Łysek-Gładysińska[5], Atanas G. Atanasov[1,6]**

[1] Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzębiec, Postępu 36A,05-552 Magdalenka, Poland

[2] Department of Animal Nutrition and Feed Management, Poznań University of Life Sciences, Wołyńska 33, 60-637 Poznań, Poland

[3] Warsaw University of Life Sciences-SGGW, Nowoursynowska 159c, 32, 02-776 Warsaw, Poland

[4] Department of Family Studies, Cardinal Stefan Wyszynski University in Warsaw, Dewajtis 5, 01-815 Warszawa, Poland

[5] Department of Cell Biology and Electron Microscopy, Institute of Biology, University of Jan Kochanowski, Swietokrzyska 15, 25-406 Kielce, Poland

[6] Department of Pharmacognosy, University of Vienna, 1090 Vienna, Austria

*(Accepted June 19, 2018)*

**The aim of the study was to estimate the influence of the different levels of Cu, Zn, and Mn nanoparticles provided in the diet on the activity of glycosidases in turkey meat. An experiment was carried out on 144 Hybrid Converter turkey hens. The birds were divided into groups fed with the standard- and nanoparticle-supplementation of different levels of copper (Cu 20, 10, 2 mg/kg), zinc (Zn 100, 50, 10 ppm), and manganese (Mn 100, 50, 10 ppm), covering respectively 100, 50, and 10% of the physiological demands for those minerals in the diet. The largest changes in glycosidases**

*The financial support of the GUTFEED-Innovative nutrition in sustainable poultry production 267659/7/NCBR/2015, Poland.
**Corresponding author: aa.jozwik@ighz.pl

**activity in both the breast and thigh muscles were found after the use of 10% Zn and Cu additive in the form of nanoparticles.**
**The use of a 10% dose of Mn nanoparticles in turkey feeding caused the inhibition of glycosidase activity in the breast muscle. Changes in glycosidase activity in the turkey hens' thigh and breast muscle after supplementation of Zn, Cu and Mn as nanoparticles can be an indicator of muscle metabolism levels.**

**KEY WORDS: glycosidases / nanoparticles / nutrition / supplementation / turkey meat**

Intensive poultry production requires modern, balanced and sustainable feeding technology [Czech *et al.* 2017] increasing product quality and animal health [Horbańczuk *et al*. 1998, Cooper and Horbańczuk 2004, Poławska *et al*. 2011, 2013 Marchewka *et al*. 2013, 2015]. One of the innovative technologies is implementation of nanoparticles in poultry diet that can be an alternative solution as compared to conventional form of trace elements supplementation. The application of nano-molecules in birds' diet leads to improved minerals absorption in the digestive tracts, contributing to better bird's performance [Liu *et al*. 2015, Uniyal *et al.* 2017, Jóźwik *et al.* 2018]. In turn, over supplementation of trace minerals including zinc or copper can affect negatively the bird's health status and product quality. It may even lead to disturbances of homeostasis and metabolic diseases of affected birds. It can be manifested with reduced growth rates and increased lameness issues, which remain a major health and welfare problems in poultry production [Richard 2005]. The metabolic processes in bird's cells depend on synthesis and degradation rates of the basic energetic compounds, i.e. proteins, carbohydrates, and lipids [Longato *et al*. 2017]. These processes are determined by activity of inter alia glycosidases enzymes playing an important role in physiological homeostasis, which can be affected by different stress factors, including diet [Bechet *et al*. 2005, Jóźwik *et al.* 2013]. Glycosidases are generally glycoproteins of lysosomal origin that catalyse the hydrolysis of glycoproteins, glycolipids, and glycosaminoglycans, as well as synthetic substrates [Hahn *et al.* 2001, Jóźwik *et al*. 2003]. Biological regulation of enzyme functions including glycosidases depends on the sufficient levels of zinc, copper and manganese in the diet, while their bioavailability depends on the supplementation form affecting changes in biochemical processes in many tissues of the birds' organism. Therefore, the aim of the study was to assess the effect of different levels of copper, zinc and mangnese supplementation either in nanoparticles or in traditional form in bird's diet on the activity of glycosidases in turkey muscles.

## Material and methods

### Animals and diet

The experiment was conducted on a commercial turkey farm located in the north-eastern part of Poland, using144 turkey hen Hybrid Converter (n=8 birds).

The birds were kept in pens, each with an area of 3.7 $m^2$ and with a wood chip bedding. The stocking density was at the level of 4.8 birds/ $m^2$ for the first 6 weeks and

3.2 birds/ $m^2$ from 7 weeks onward until the end of production cycle (98 days). The experimental building was equipped with automatically controlled lighting, heating and ventilation system. Light programs and temperature were in accordance with the recommendations of Hybrid Turkeys (Hendrix Genetics, The Netherlands). Birds had ad libitum access to drinking water and feed mixtures. All birds were fed according to the diet shown in the Table 1.

**Table 1.** Feed and nutrient composition of turkey hens at different ages

| Item | Diet and age (days) | | |
|---|---|---|---|
| | Starter | Grover | Finisher |
| Protein (%) | 26.50 | 23.00 | 18.50 |
| *Fiber* (%) | 3.40 | 3.98 | 3.57 |
| Fat (%) | 4.23 | 7.16 | 7.37 |
| Amino acids | | | |
| arginine (%) | 1.76 | 1.52 | 1.18 |
| lysine (%) | 1.74 | 1.50 | 1.17 |
| methionine (%) | 0.71 | 0.57 | 0.45 |
| methionine.+ cysteine (%) | 1.13 | 0.95 | 0.78 |
| threonine (%) | 1.05 | 0.93 | 0.68 |
| tryptophan (%) | 0.32 | 0.29 | 0.22 |
| Minerals | | | |
| Ca (%) | 1.15 | 1.05 | 0.65 |
| P (%) | 0.55 | 0.45 | 0.30 |
| Na (%) | 0.15 | 0.13 | 0.13 |
| Energy (kcal/kg) | 2750 | 2950 | 3100 |

In order to test the effect of supplementation form in turkey feeding, the birds were divided into two groups. Control groups received standard form of the studied minerals, while the test groups received the mineral in the nanoparticles form. The birds in both groups were supplemented with different levels of copper (Cu 20, 10, 2 mg/kg), manganese (Mn 100, 50, 10 ppm), and zinc (Zn 100, 50, 10ppm) covering respectively 100, 50 and 10% of the physiological demands for those minerals in the diet.

**Sampling**

The turkey broiler breast and thigh muscle meat samples were collected immediately after slaughter (max 40 min) and frozen in liquid nitrogen (-80ºC). Then, the samples were homogenised in 0.1Mol phosphate buffer (pH 7.0) with 0.1% Triton X100 according to the modified method Marzella and Glaumann [1980].

**Glycosidase assay procedure**

The activities of acid phosphatase (AcP – EC 3.1.3.2); β-glucuronidase (BGRD – EC 3.2.1.31); β-galactosidase (BGAL – EC 3.2.1.23); β-glucosidase (BGLU – EC 3.2.1.21); N-acetyl-β- hexosaminidase (HEX – EC 3.2.1.52), α-glucosidase (AGLU – EC 3.2.1.20), and mannosidase (MAN – EC 3.2.1.25) were determined in the supernatant. The activity of AcP, BGRD, BGAL, BGLU, AGLU, MAN, and HEX were measured as 4-nitrophenyl derivatives at 420 nm (spectrophotometer UV-VIS CarryBio 50) according to Barrett and Heath's method [1972].

The chemical assays, as well as substrates for determination of enzymes and proteins were purchased from Sigma-Aldrich Co.

**Statistical analysis**

A generalised linear mixed model analysis was performed on all measured parameters: AcP, BGRD, BGAL, BGLU, AGLU, MAN, and HEX including "supplementation form", "dose" and their interaction as fixed factors. Separate model was run for each of the minerals: Cu, Mn and Zn in each of two types of muscle: breast and thigh, The validity of the models was tested by using Akaike's information criterion. PROC GLIMMIX of SAS v 9.3 (SAS Institute Inc., Cary, NC, USA) including the Tukey adjustment option was used to conduct the analysis. The least square means for all significant effects in the models ($P \leq 0.05$) were computed using the LSMEANS option.

## Results and discussion

The effects of the minerals supplementation in three doses (100, 50 and 10% of the requirement) and two forms (nanonaparticles and standard) into turkey diet on the AcP, BGRD, BGAL, BGLU, HEX, AGLU and MAN activity levels in turkey muscles are presented in Tables 2-7.

**Copper**. There was a significant effect of the interaction between Cu supplementation form and dose on most of the indicators on all parameters in breast and thigh muscle, except MAN in breast muscle (Tab. 2 and 3). When Cu was supplemented as nanoparticle, the lowest dose (10% of the requirement) increased significantly HEX and AcP activity (539.8±37.6 and 1363.8±29.9, respectively) in breast muscle, when compared to the same dose provided in the standard form (138.9±25.8 and 176.4±16.8, respectively) – Table 2. The same interaction of the lowest dose with the nano-form of Cu decreased significantly AGLU activity (122.5±5.1), compared to standard form (150.0±7.5) in breast (Tab. 2). Activity of BGAL in breast muscle did not differ for the lowest dose (10%) between supplementation forms, however activity of this enzyme was significantly higher when 50 and 100% doses were provided in standard (163.1±5.2 and 163.9±4.1; respectively) form compared to nano-form (87.3±7.8 and 89.0±4.3, respectively).

In thigh muscle the nano-form of Cu supplementation on the lowest level (10%) decreased significantly activity of AcP and BGAL (657.3±26.8 and 41.0±2.2), when compared to standard form (787.5±17.7 and 123.5±2.6) – Table 3. When providing Cu in the middle nano form dose (50%) activity of the HEX (342.5±21.0) increased in thigh muscle compared to the standard form (209.0 ± 5.9) – Table 2. No significant differences in the activity of any enzymes were found in thigh muscle between nano and standard Cu supplementation form when full dose was provided (100%).

**Manganese.** There was a significant effect of the interaction between Mn supplementation form and dose on all parameters in breast and in thigh muscle, except BGRD in thigh muscle (Tab. 4 and 5).

**Table 2.** The effect of Cu supplementation in three doses (100%, 50% and 10% of the requirement) and two forms (nanonaparticles and standard) into turkey diet on the AcP, BGRD, BGAL, BGLU, HEX, aglu, MAN activity levels (mean±SE) in breast muscle

| Group* | | | AcP | | BGRD | | BGAL | | BGLU | | HEX | | AGLU | | MAN | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | mean | SE | mean | SE | mean | SE | mean | SE | mean | SE | mean | SE | mean | SE |
| Suppl. form effect | | | | | | | | | | | | | | | | |
| | N | | 853.1$^{a}$ | 76.3 | 19.0 | 0.9 | 113.4$^{b}$ | 8.0 | 27.7$^{b}$ | 2.7 | 337.1$^{a}$ | 33.0 | 114.3 | 3.3 | 120.9 | 5.0 |
| | S | | 450.0$^{b}$ | 42.8 | 18.0 | 1.0 | 167.5$^{a}$ | 2.6 | 34.2$^{a}$ | 3.4 | 224.7$^{b}$ | 26.7 | 120.9 | 6.5 | 133.5 | 4.5 |
| Dose effect | | | | | | | | | | | | | | | | |
| | | 10 | 770.1$^{a}$ | 154.2 | 17.9$^{b}$ | 1.1 | 169.8$^{a}$ | 2.7 | 33.3$^{a}$ | 1.6 | 339.3$^{a}$ | 56.2 | 136.3$^{a}$ | 5.6 | 129.7 | 6.5 |
| | | 50 | 620.2$^{b}$ | 16.1 | 21.4$^{a}$ | 1.2 | 125.2$^{b}$ | 10.8 | 45.9$^{b}$ | 2.8 | 326.4$^{b}$ | 21.3 | 122.7$^{b}$ | 4.5 | 134.1 | 5.8 |
| | | 100 | 564.3$^{c}$ | 13.0 | 16.2$^{b}$ | 0.8 | 126.4$^{b}$ | 10.1 | 13.7$^{c}$ | 0.8 | 177.1$^{b}$ | 8.3 | 93.8$^{c}$ | 3.1 | 117.7 | 5.1 |
| Suppl. form x dose | | | | | | | | | | | | | | | | |
| | N | 10 | 1363.8$^{a}$ | 29.9 | 16.0$^{bc}$ | 1.3 | 163.9$^{a}$ | 3.4 | 33.7$^{b}$ | 2.2 | 539.8$^{a}$ | 37.6 | 122.5$^{bc}$ | 5.1 | 118.3 | 7.8 |
| | N | 50 | 597.6$^{bc}$ | 21.0 | 22.3$^{a}$ | 1.7 | 87.3$^{b}$ | 7.8 | 38.5$^{b}$ | 2.3 | 265.2$^{c}$ | 15.5 | 116.8$^{bc}$ | 6.7 | 134.8 | 7.9 |
| | N | 100 | 597.8$^{bc}$ | 10.9 | 18.6$^{abc}$ | 0.7 | 89.0$^{b}$ | 4.3 | 11.1$^{c}$ | 0.6 | 206.4$^{cd}$ | 4.4 | 103.5$^{cd}$ | 2.8 | 109.5 | 8.6 |
| | S | 10 | 176.4$^{d}$ | 16.8 | 19.8$^{ab}$ | 1.6 | 175.7$^{a}$ | 3.2 | 32.9$^{b}$ | 2.3 | 138.9$^{d}$ | 25.8 | 150.0$^{a}$ | 7.5 | 141.1 | 9.1 |
| | S | 50 | 642.8$^{b}$ | 23.0 | 20.5$^{ab}$ | 1.7 | 163.1$^{a}$ | 5.2 | 53.4$^{a}$ | 3.5 | 387.6$^{b}$ | 25.3 | 128.5$^{ab}$ | 5.7 | 133.5 | 8.9 |
| | S | 100 | 530.9$^{c}$ | 16.9 | 13.7$^{c}$ | 0.5 | 163.9$^{a}$ | 4.1 | 16.2$^{c}$ | 0.6 | 147.7$^{d}$ | 5.7 | 84.1$^{d}$ | 2.6 | 125.9 | 4.4 |
| Source of variation | | | | | | | | | P value | | | | | | | |
| Suppl. form effect | | | <.0001 | | 0.3977 | | <.0001 | | 0.0008 | | <.0001 | | 0.1420 | | 0.0579 | |
| Dose effect | | | <.0001 | | 0.0011 | | <.0001 | | <.0001 | | <.0001 | | <.0001 | | 0.1122 | |
| Suppl. form x dose | | | <.0001 | | 0.0078 | | <.0001 | | 0.0034 | | <.0001 | | 0.0003 | | 0.3007 | |

*100S – 100% of the dose in a standard form; 100N – 100% of the dose in a nanoparticle form; 50S – 50% of the dose in a standard form; 50N – 50% of the dose in a nanoparticle form; 10S – 10% of the dose in a standard form; 10N – 10% of the dose in a nanoparticle form.

$^{ab\cdots}$Means in the same column with different letters differ significantly at $P<0.05$ separately between the group, between the species and between the additives.

**Table 3.** The effect of Cu supplementation in three doses (100%, 50% and 10% of the requirement) and two forms (nanonaparticles and standard) into turkey diet on the AcP, BGRD, BGAL, BGLU, HEX, aglu, MAN activity levels (mean±SE) in thigh muscle

| Group* | | | AcP | | BGRD | | BGAL | | BGLU | | HEX | | AGLU | | MAN | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | mean | SE | mean | SE | mean | SE | mean | SE | mean | SE | mean | SE | mean | SE |
| Suppl. form effect | | | | | | | | | | | | | | | | |
| | N | | 722.1$^{b}$ | 24.6 | 23.2$^{a}$ | 0.7 | 95.1$^{b}$ | 8.5 | 25.5 | 1.0 | 307.2$^{a}$ | 11.0 | 120.4 | 4.6 | 130.7 | 5.6 |
| | S | | 799.3$^{a}$ | 17.6 | 20.8$^{b}$ | 0.7 | 124.8$^{a}$ | 3.5 | 26.5 | 1.0 | 262.2$^{b}$ | 14.9 | 123.3 | 7.3 | 120.0 | 5.3 |
| Dose effect | | | | | | | | | | | | | | | | |
| | | 10 | 722.4$^{b}$ | 22.9 | 22.6$^{ab}$ | 1.0 | 82.2$^{b}$ | 10.8 | 28.9$^{a}$ | 1.1 | 244.9$^{b}$ | 8.5 | 143.8$^{a}$ | 6.6 | 113.0$^{b}$ | 5.6 |
| | | 50 | 702.8$^{b}$ | 25.1 | 23.2$^{a}$ | 0.7 | 124.0$^{a}$ | 5.0 | 27.5$^{a}$ | 1.1 | 275.8$^{b}$ | 20.2 | 123.7$^{b}$ | 6.3 | 137.6$^{a}$ | 7.8 |
| | | 100 | 856.8$^{a}$ | 17.6 | 20.1$^{b}$ | 1.0 | 123.7$^{a}$ | 4.6 | 21.5$^{b}$ | 0.6 | 333.5$^{a}$ | 11.7 | 98.0$^{c}$ | 4.2 | 125.5$^{ab}$ | 5.6 |
| Suppl. form x dose | | | | | | | | | | | | | | | | |
| | N | 10 | 657.3$^{b}$ | 26.8 | 24.7$^{a}$ | 1.5 | 41.0$^{b}$ | 2.2 | 26.5$^{ab}$ | 1.2 | 262.6$^{bc}$ | 13.1 | 129.9$^{ab}$ | 7.5 | 123.9$^{ab}$ | 7.8 |
| | N | 50 | 647.4$^{b}$ | 18.3 | 22.4$^{ab}$ | 1.2 | 116.2$^{a}$ | 5.7 | 28.9$^{a}$ | 1.8 | 342.5$^{a}$ | 21.0 | 122.4$^{b}$ | 10.4 | 151.4$^{a}$ | 10.4 |
| | N | 100 | 861.5$^{a}$ | 27.4 | 22.5$^{ab}$ | 1.0 | 128.3$^{a}$ | 6.2 | 21.0$^{c}$ | 0.9 | 316.6$^{ab}$ | 10.0 | 108.9$^{bc}$ | 2.9 | 116.7$^{ab}$ | 6.9 |
| | S | 10 | 787.5$^{a}$ | 17.7 | 20.6$^{ab}$ | 1.0 | 123.5$^{a}$ | 2.6 | 31.3$^{a}$ | 1.5 | 227.2$^{c}$ | 7.0 | 157.7$^{a}$ | 8.5 | 102.0$^{b}$ | 6.1 |
| | S | 50 | 758.2$^{ab}$ | 38.7 | 24.0$^{a}$ | 0.7 | 131.8$^{a}$ | 7.5 | 26.1$^{abc}$ | 1.1 | 209.0$^{c}$ | 5.9 | 125.1$^{b}$ | 8.0 | 123.7$^{ab}$ | 9.8 |
| | S | 100 | 852.2$^{a}$ | 23.9 | 17.8$^{b}$ | 1.2 | 119.2$^{a}$ | 6.7 | 22.0$^{bc}$ | 0.8 | 350.5$^{a}$ | 20.2 | 87.1$^{c}$ | 5.7 | 134.3$^{ab}$ | 8.0 |
| Source of variation | | | | | | | | | P value | | | | | | | |
| Suppl. form effect | | | 0.0009 | | 0.0118 | | <.0001 | | 0.3429 | | 0.0004 | | 0.6384 | | 0.1239 | |
| Dose effect | | | <.0001 | | 0.0222 | | <.0001 | | <.0001 | | <.0001 | | <.0001 | | 0.0186 | |
| Suppl. form x dose | | | 0.0236 | | 0.0144 | | <.0001 | | 0.0157 | | <.0001 | | 0.0082 | | 0.0186 | |

*100S – 100% of the dose in a standard form; 100N – 100% of the dose in a nanoparticle form; 50S – 50% of the dose in a standard form; 50N – 50% of the dose in a nanoparticle form; 10S – 10% of the dose in a standard form; 10N – 10% of the dose in a nanoparticle form.

$^{ab\cdots}$Means in the same column with different letters differ significantly at $P<0.05$ separately between the group, between the species and between the additives.

**Table 4.** The effect of Mn supplementation in three doses (100%, 50% and 10% of the requirement) and two forms (nanonaparticles and standard) into turkey diet on the AcP, BGRD, BGAL, BGLU, HEX, aglu, MAN activity levels (mean±SE) in breast muscle

| Group* | | | AcP | | BGRD | | BGAL | | BGLU | | HEX | | AGLU | | MAN | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | mean | SE | mean | SE | mean | SE | mean | SE | mean | SE | mean | SE | mean | SE |
| Suppl. form effect | | | | | | | | | | | | | | | | |
| | N | | 1245.1$^{a}$ | 24.6 | 52.3 | 0.7 | 808.5 | 8.5 | 76.0 | 1.0 | 785.2 | 11.0 | 86.7 | 4.6 | 174.5 | 5.6 |
| | S | | 1209.0$^{b}$ | 17.6 | 51.7 | 0.7 | 806.5 | 3.5 | 75.6 | 1.0 | 796.1 | 14.9 | 86.2 | 7.3 | 168.0 | 5.3 |
| Dose effect | | | | | | | | | | | | | | | | |
| | | 10 | 1198.9$^{b}$ | 8.3 | 47.9$^{b}$ | 1.1 | 772.6$^{b}$ | 7.4 | 72.9 | 1.8 | 735.1$^{b}$ | 16.9 | 83.6$^{b}$ | 2.0 | 163.8$^{b}$ | 2.4 |
| | | 50 | 1267.8$^{a}$ | 20.3 | 54.0$^{a}$ | 1.1 | 816.8$^{a}$ | 15.6 | 78.2 | 1.6 | 839.9$^{a}$ | 21.2 | 88.8$^{a}$ | 1.7 | 179.0$^{a}$ | 5.0 |
| | | 100 | 1214.5$^{b}$ | 13.6 | 54.0$^{a}$ | 1.8 | 833.2$^{a}$ | 4.9 | 76.2 | 1.8 | 796.9$^{a}$ | 11.1 | 87.0$^{ab}$ | 1.4 | 171.0$^{ab}$ | 2.5 |
| Suppl. form x dose | | | | | | | | | | | | | | | | |
| | N | 10 | 1181.5$^{b}$ | 9.1 | 45.0$^{b}$ | 1.0 | 747.0$^{b}$ | 5.2 | 73.4$^{ab}$ | 2.1 | 690.6$^{b}$ | 16.5 | 78.3$^{b}$ | 2.3 | 159.4$^{b}$ | 3.6 |
| | N | 50 | 1308.4$^{a}$ | 33.8 | 55.5$^{a}$ | 1.9 | 843.0$^{a}$ | 24.9 | 74.5$^{ab}$ | 2.1 | 862.8$^{a}$ | 33.7 | 92.3$^{a}$ | 1.8 | 190.1$^{a}$ | 7.7 |
| | N | 100 | 1245.5$^{ab}$ | 19.2 | 56.4$^{a}$ | 3.4 | 835.6$^{a}$ | 6.1 | 80.1$^{ab}$ | 2.6 | 802.2$^{a}$ | 15.4 | 89.5$^{a}$ | 2.4 | 174.1$^{ab}$ | 4.4 |
| | S | 10 | 1216.3$^{b}$ | 11.1 | 50.8$^{ab}$ | 1.5 | 798.2$^{ab}$ | 4.7 | 72.4$^{b}$ | 3.1 | 779.6$^{ab}$ | 19.8 | 88.8$^{a}$ | 2.1 | 168.2$^{b}$ | 2.6 |
| | S | 50 | 1227.2$^{b}$ | 12.7 | 52.5$^{ab}$ | 0.9 | 790.6$^{ab}$ | 14.9 | 81.9$^{a}$ | 1.4 | 817.0$^{a}$ | 25.4 | 85.4$^{ab}$ | 2.3 | 168.0$^{b}$ | 3.9 |
| | S | 100 | 1183.6$^{b}$ | 12.1 | 51.7$^{ab}$ | 1.2 | 830.8$^{a}$ | 7.9 | 72.3$^{b}$ | 1.6 | 791.7$^{a}$ | 16.8 | 84.5$^{ab}$ | 1.1 | 167.9$^{b}$ | 2.4 |
| Source of variation | | | | | | | | | P value | | | | | | | |
| Suppl. form effect | | | 0.0207 | | 0.6815 | | 0.8519 | | 0.8216 | | 0.5511 | | 0.7788 | | 0.0820 | |
| Dose effect | | | 0.0014 | | 0.0020 | | <.0001 | | 0.0665 | | 0.0001 | | 0.0426 | | 0.0056 | |
| Suppl. form x dose | | | 0.0063 | | 0.0148 | | 0.0010 | | 0.0056 | | 0.0118 | | 0.0002 | | 0.0047 | |

*100S – 100% of the dose in a standard form; 100N – 100% of the dose in a nanoparticle form; 50S – 50% of the dose in a standard form; 50N – 50% of the dose in a nanoparticle form; 10S – 10% of the dose in a standard form; 10N – 10% of the dose in a nanoparticle form.

$^{ab...}$Means in the same column with different letters differ significantly at P<0.05 separately between the group, between the species and between the additives.

**Table 5.** The effect of Mn supplementation in three doses (100, 50 and 10% of the requirement) and two forms (nanonaparticles and standard) into turkey diet on the AcP, BGRD, BGAL, BGLU, HEX, aglu, MAN activity levels (mean±SE) in thigh muscle

| Group* | | | AcP | | BGRD | | BGAL | | BGLU | | HEX | | AGLU | | MAN | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | mean | SE | mean | SE | mean | SE | mean | SE | mean | SE | mean | SE | mean | SE |
| Suppl. form effect | | | | | | | | | | | | | | | | |
| | N | | 1092.9 | 33.3 | 75.3$^{b}$ | 2.4 | 388.9$^{a}$ | 13.1 | 49.1 | 2.3 | 679.0$^{b}$ | 23.0 | 101.2 | 3.6 | 174.2 | 6.9 |
| | S | | 1081.3 | 30.3 | 83.2$^{a}$ | 2.0 | 362.0$^{b}$ | 5.7 | 52.3 | 2.3 | 723.3$^{a}$ | 15.2 | 105.6 | 4.5 | 169.8 | 6.7 |
| Dose effect | | | | | | | | | | | | | | | | |
| | | 10 | 1181.5$^{a}$ | 18.9 | 87.8$^{a}$ | 1.4 | 405.4$^{a}$ | 11.7 | 54.7$^{a}$ | 1.4 | 767.1$^{a}$ | 15.0 | 104.2$^{b}$ | 2.8 | 177.7$^{a}$ | 4.8 |
| | | 50 | 885.5$^{b}$ | 11.9 | 67.1$^{b}$ | 1.6 | 337.1$^{b}$ | 7.5 | 38.5$^{b}$ | 1.2 | 625.2$^{b}$ | 21.8 | 85.1$^{c}$ | 1.9 | 143.2$^{b}$ | 4.3 |
| | | 100 | 1194.1$^{a}$ | 10.6 | 82.8$^{a}$ | 2.3 | 383.7$^{a}$ | 12.1 | 58.9$^{a}$ | 2.5 | 711.2$^{a}$ | 21.5 | 121.0$^{a}$ | 4.7 | 195.1$^{a}$ | 8.8 |
| Suppl. form x dose | | | | | | | | | | | | | | | | |
| | N | 10 | 1222.0$^{a}$ | 31.4 | 86.6 | 2.8 | 436.2$^{a}$ | 14.8 | 58.0$^{ab}$ | 2.3 | 799.8$^{a}$ | 21.8 | 113.4$^{b}$ | 2.8 | 193.9$^{a}$ | 3.8 |
| | N | 50 | 881.0$^{c}$ | 11.1 | 62.0 | 1.5 | 320.2$^{d}$ | 9.7 | 35.6$^{c}$ | 0.6 | 566.5$^{c}$ | 23.1 | 78.6$^{d}$ | 1.2 | 133.7$^{b}$ | 4.1 |
| | N | 100 | 1175.6$^{ab}$ | 9.1 | 77.3 | 1.8 | 410.2$^{ab}$ | 17.8 | 53.7$^{b}$ | 2.7 | 670.8$^{b}$ | 17.2 | 111.6$^{b}$ | 3.0 | 194.9$^{a}$ | 9.0 |
| | S | 10 | 1141.1$^{b}$ | 9.1 | 89.1 | 0.7 | 374.6$^{bc}$ | 10.1 | 51.3$^{b}$ | 0.6 | 734.4$^{ab}$ | 13.5 | 95.1$^{c}$ | 1.5 | 161.4$^{ab}$ | 2.7 |
| | S | 50 | 890.0$^{c}$ | 21.9 | 72.2 | 1.3 | 354.1$^{cd}$ | 8.2 | 41.5$^{c}$ | 1.9 | 683.9$^{b}$ | 23.0 | 91.5$^{cd}$ | 1.3 | 152.7$^{b}$ | 6.0 |
| | S | 100 | 1212.6$^{ab}$ | 17.3 | 88.3 | 3.3 | 357.3$^{cd}$ | 10.7 | 64.1$^{a}$ | 3.5 | 751.5$^{ab}$ | 34.8 | 130.3$^{a}$ | 7.9 | 195.3$^{a}$ | 15.8 |
| Source of variation | | | | | | | | | P-values | | | | | | | |
| Suppl. form effect | | | 0.4473 | | <.0001 | | 0.0107 | | 0.0820 | | 0.0244 | | 0.1536 | | 0.5185 | |
| Dose effect | | | <.0001 | | <.0001 | | <.0001 | | <.0001 | | <.0001 | | <.0001 | | <.0001 | |
| Suppl. form x dose | | | 0.0073 | | 0.0925 | | 0.0005 | | 0.0011 | | 0.0007 | | <.0001 | | 0.0109 | |

*100S – 100% of the dose in a standard form; 100N – 100% of the dose in a nanoparticle form; 50S – 50% of the dose in a standard form; 50N – 50% of the dose in a nanoparticle form; 10S – 10% of the dose in a standard form; 10N – 10% of the dose in a nanoparticle form.

$^{ab\cdots}$Means in the same column with different letters differ significantly at $p<0.05$ separately between the group, between the species and between the additives.

When Mn was supplemented in the lowest dose (10% of the requirement) as nanoparticle it decreased the activity of AGLU in breast muscle (78.3±2.3) compared to when standard form was provided (88.8±2.1). Opposite effect was observed in thigh muscle where: AGLU activity increased from 95.1±1.5 when standard form was given to 113.4±2.8 when nanoform was applied; BGAL activity increased from 374.6±10.1 when standard form was given to 436,2±14,8 when nanoform was applied and for AcP which activity increased from 1141.1±9.1 when standard form was given to 1222.0±31.4 when nanoform was applied.

When providing Mn in the middle nano form dose (50%) activity of the AcP and MAN (1308.4±33.8 and 190.1±7.7, respectively) increased in breast muscle compared to the same dose provided in the standard form (1227.2±12.7 and 168.0±3.9, respectively) – Table 4. No significant differences in the activity of any enzymes were found in breast muscle between nano and standard Mn supplementation form when full dose was provided (100%), while in thigh muscle BGAL activity increased from 357.3±10.7 for standard form up to 410.2±17.8 when nano form of Mn covering 100% of the requirement was delivered. BGLU decreased activity from 64.1±3.5 when standard form of Mn covering 100% of the requirement was delivered down to nano form (53.7±2.7) – Table 5.

**Zinc**. There was a significant effect of the interaction between Zn supplementation form and dose on all parameters in breast and in thigh muscle, except AGLU in breast muscle (Tab. 6 and 7).

When Zn was supplemented as nanoparticle the lowest dose (10% of the requirement) increased the activity of MAN in breast muscle (70.0±1.6) compared to standard form supplementation (56.8±2.9) and of AcP, BGRD, AGLU and MAN (1692.4±42.8, 148.1±8.3, 351.3±25.7 and 349.7±26.5) in thigh muscle, when compared to supplementation on the same level using standard form of Zn (1340.0±80.6, 127.6±2.6, 277.3±21.1 and 274.9±9.6).

When providing Zn in the middle nano form dose (50%) activity of the AcP (1424.2±42.0) and BGRD (65.0±5.8) in the breast compared to standard form (1710.2±52.9 and 80.4±3.4, respectively) and of AcP (1288.1±21.5) in thigh muscle decreased compared to when standard form was provided (1542.9±72.1).

Significant differences in the activity of BGRD, BGAL and BGLU were found in breast muscle between nano (48.5±2.1, 450.7±12.0 and 14.9±0.7, respectively) and standard Zn supplementation form (72.0±1.3, 579.7±20.6 and 22.5±0.9) when full dose was provided (100%) and of AcP, BGLU and MAN between nano (1070.9±12.8, 125.0±2.4 and 238.9±3.0, respectively) and standard Zn supplementation form (1364.3±40.9, 198.7±6.5 and 333.1±16.5 respectively) in thigh muscle when full dose was provided (100%).

Lysosomes, including glycosidases are metabolic signaling hubs maintaining cellular and organismal energy homeostasis, through regulation of the metabolism of metals in the cells. They provide an important source of energy metabolites and ions [Xiong and Zhu 2016]. Glycosidase activity has also implication for the postmortem

**Table 6.** The effect of Zn supplementation in three doses (100, 50 and 10% of the requirement) and two forms (nanonaparticles and standard) into turkey diet on the AcP, BGRD, BGAL, BGLU, HEX, aglu, MAN activity levels (mean±SE) in breast muscle

| Group* | | | AcP | | BGRD | | BGAL | | BGLU | | HEX | | AGLU | | MAN | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | mean | SE | mean | SE | mean | SE | mean | SE | mean | SE | mean | SE | mean | SE |
| Suppl. form effect | | | | | | | | | | | | | | | | |
| | N | | 1424.5$^{b}$ | 46.4 | 61.3$^{b}$ | 3.0 | 608.8 | 29.6 | 20.4$^{b}$ | 1.0 | 790.0 | 47.2 | 328.1 | 19.9 | 54.8 | 3.2 |
| | S | | 1602.1$^{a}$ | 30.4 | 74.3$^{a}$ | 1.9 | 630.1 | 17.1 | 23.4$^{a}$ | 0.8 | 779.1 | 26.4 | 361.3 | 19.5 | 52.8 | 2.4 |
| Dose effect | | | | | | | | | | | | | | | | |
| | | 10 | 1613.5$^{a}$ | 30.6 | 70.6$^{a}$ | 2.5 | 751.2$^{a}$ | 13.2 | 24.9$^{a}$ | 0.9 | 971.6$^{a}$ | 27.4 | 441.7$^{a}$ | 19.9 | 63.4$^{a}$ | 2.3 |
| | | 50 | 1567.2$^{a}$ | 49.3 | 72.7$^{a}$ | 3.8 | 592.0$^{b}$ | 13.9 | 22.1$^{b}$ | 0.8 | 769.9$^{b}$ | 30.7 | 317.2$^{b}$ | 11.6 | 58.9$^{a}$ | 2.0 |
| | | 100 | 1359.1$^{b}$ | 53.2 | 60.3$^{b}$ | 3.3 | 515.2$^{c}$ | 20.3 | 18.7$^{c}$ | 1.1 | 612.2$^{c}$ | 26.7 | 275.1$^{b}$ | 17.7 | 39.2$^{b}$ | 2.1 |
| Suppl. form x dose | | | | | | | | | | | | | | | | |
| | N | 10 | 1656.7$^{a}$ | 50.4 | 70.5$^{ab}$ | 3.4 | 780.6$^{a}$ | 17.6 | 23.6$^{a}$ | 1.0 | 1019.1$^{a}$ | 44.7 | 448.5 | 17.0 | 70.0$^{a}$ | 1.6 |
| | N | 50 | 1424.2$^{b}$ | 42.0 | 65.0$^{b}$ | 5.8 | 595.1$^{b}$ | 19.2 | 22.7$^{a}$ | 1.2 | 809.6$^{bc}$ | 51.5 | 301.4 | 13.7 | 58.2$^{b}$ | 3.1 |
| | N | 100 | 1192.7$^{b}$ | 38.8 | 48.5$^{c}$ | 2.1 | 450.7$^{c}$ | 12.0 | 14.9$^{b}$ | 0.7 | 541.4$^{d}$ | 30.8 | 234.5 | 3.6 | 36.3$^{c}$ | 1.7 |
| | S | 10 | 1570.4$^{ab}$ | 30.9 | 70.6$^{ab}$ | 3.9 | 721.7$^{a}$ | 13.8 | 26.1$^{a}$ | 1.6 | 924.2$^{ab}$ | 24.0 | 435.0 | 37.3 | 56.8$^{b}$ | 2.9 |
| | S | 50 | 1710.2$^{a}$ | 52.9 | 80.4$^{a}$ | 3.4 | 588.8$^{b}$ | 21.4 | 21.6$^{a}$ | 1.1 | 730.2$^{c}$ | 30.7 | 333.1 | 17.8 | 59.6$^{ab}$ | 2.5 |
| | S | 100 | 1525.6$^{ab}$ | 52.1 | 72.0$^{ab}$ | 1.3 | 579.7$^{b}$ | 20.6 | 22.5$^{a}$ | 0.9 | 682.9$^{cd}$ | 26.0 | 315.7 | 29.4 | 42.1$^{c}$ | 3.8 |
| Source of variation | | | | | | | | | P value | | | | | | | |
| Suppl. form effect | | | <.0001 | | <.0001 | | 0.1499 | | 0.0020 | | 0.7122 | | 0.0797 | | 0.3624 | |
| Dose effect | | | <.0001 | | 0.0026 | | <.0001 | | <.0001 | | <.0001 | | <.0001 | | <.0001 | |
| Suppl. form x dose | | | <.0001 | | 0.0073 | | <.0001 | | 0.0013 | | 0.0029 | | 0.1238 | | 0.0032 | |

*100S – 100% of the dose in a standard form; 100N – 100% of the dose in a nanoparticle form; 50S – 50% of the dose in a standard form; 50N – 50% of the dose in a nanoparticle form; 10S – 10% of the dose in a standard form; 10N – 10% of the dose in a nanoparticle form.

$^{ab\cdots}$Means in the same column with different letters differ significantly at $p<0.05$ separately between the group, between the species and between the additives.

**Table 7.** The effect of Zn supplementation in three doses (100%. 50% and 10% of the requirement) and two forms (nanonaparticles and standard) into turkey diet on the AcP. BGRD. BGAL. BGLU. HEX. aglu. MAN activity levels (mean±SE) in thigh muscle.

| Group* | | | AcP | | BGRD | | BGAL | | BGLU | | HEX | | AGLU | | MAN | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | mean | SE | mean | SE | mean | SE | mean | SE | mean | SE | mean | SE | mean | SE |
| Suppl. form effect | | | | | | | | | | | | | | | | |
| | N | | 1350.4 | 56.0 | 98.3 | 8.0 | 498.9 | 26.9 | 188.2 | 10.1 | 809.8 | 44.6 | 242.0 | 18.4 | 289.1 | 13.1 |
| | S | | 1415.7 | 41.4 | 98.3 | 4.7 | 528.1 | 8.2 | 198.3 | 4.0 | 842.7 | 27.4 | 225.8 | 11.3 | 296.2 | 10.2 |
| Dose effect | | | | | | | | | | | | | | | | |
| | | 10 | 1516.2$^{a}$ | 63.3 | 137.9$^{a}$ | 4.9 | 595.9$^{a}$ | 28.3 | 211.7$^{a}$ | 5.5 | 913.0$^{a}$ | 59.2 | 314.3$^{a}$ | 18.7 | 312.3 | 16.7 |
| | | 50 | 1415.5$^{a}$ | 49.0 | 77.3$^{b}$ | 2.3 | 486.7$^{b}$ | 12.1 | 206.1$^{a}$ | 6.6 | 787.1$^{b}$ | 26.8 | 193.2$^{b}$ | 4.1 | 279.6 | 10.1 |
| | | 100 | 1217.6$^{b}$ | 43.2 | 79.7$^{b}$ | 3.0 | 458.0$^{b}$ | 14.1 | 161.8$^{b}$ | 10.1 | 778.7$^{b}$ | 37.1 | 194.3$^{b}$ | 8.9 | 286.0 | 14.6 |
| Suppl. form x dose | | | | | | | | | | | | | | | | |
| | N | 10 | 1692.4$^{a}$ | 42.8 | 148.1$^{a}$ | 8.3 | 627.2$^{a}$ | 55.4 | 218.9$^{a}$ | 4.3 | 998.7$^{a}$ | 101.6 | 351.3$^{a}$ | 25.7 | 349.7$^{a}$ | 26.5 |
| | N | 50 | 1288.1$^{c}$ | 21.5 | 74.5$^{c}$ | 1.4 | 463.0$^{bc}$ | 16.4 | 220.7$^{a}$ | 11.0 | 725.1$^{b}$ | 7.2 | 207.0$^{c}$ | 4.0 | 278.6$^{bc}$ | 8.6 |
| | N | 100 | 1070.9$^{d}$ | 12.8 | 72.2$^{c}$ | 4.5 | 406.6$^{c}$ | 5.0 | 125.0$^{b}$ | 2.4 | 705.5$^{b}$ | 39.6 | 167.8$^{c}$ | 3.0 | 238.9$^{c}$ | 3.0 |
| | S | 10 | 1340.0$^{bc}$ | 80.6 | 127.6$^{b}$ | 2.6 | 564.5$^{ab}$ | 9.7 | 204.6$^{a}$ | 9.9 | 827.4$^{ab}$ | 51.0 | 277.3$^{b}$ | 21.1 | 274.9$^{bc}$ | 9.6 |
| | S | 50 | 1542.9$^{ab}$ | 72.1 | 80.1$^{c}$ | 4.2 | 510.5$^{bc}$ | 14.1 | 191.5$^{a}$ | 1.5 | 849.1$^{ab}$ | 43.8 | 179.4$^{c}$ | 0.9 | 280.5$^{bc}$ | 19.1 |
| | S | 100 | 1364.3$^{bc}$ | 40.9 | 87.1$^{c}$ | 1.7 | 509.4$^{bc}$ | 8.8 | 198.7$^{a}$ | 6.5 | 851.8$^{ab}$ | 52.9 | 220.8$^{bc}$ | 11.4 | 333.1$^{ab}$ | 16.5 |
| Source of variation | | | | | | | | | | | | | | | | |
| Suppl. form effect | | | 0.1272 | | 0.9916 | | 0.1588 | | 0.0826 | | 0.4801 | | 0.1780 | | 0.5863 | |
| Dose effect | | | <.0001 | | <.0001 | | <.0001 | | <.0001 | | 0.0384 | | <.0001 | | 0.1050 | |
| Suppl. form x dose | | | <.0001 | | 0.0008 | | 0.0064 | | <.0001 | | 0.0123 | | 0.0003 | | <.0001 | |

*100S – 100% of the dose in a standard form; 100N – 100% of the dose in a nanoparticle form; 50S – 50% of the dose in a standard form; 50N – 50% of the dose in a nanoparticle form; 10S – 10% of the dose in a standard form; 10N – 10% of the dose in a nanoparticle form.

$^{ab\cdots}$Means in the same column with different letters differ significantly at $p<0.05$ separately between the group. between the species and between the additives.

proteolysis and weakening of the muscle fibers leading to meat tenderization. With the reduced proteolytic potential in faster growing poultry lines, the gylocisdased are characterized by the lower activity therefore causing decreased quality of meat including tenderization [Kuttappan *et al*. 2013].

Cu, Zn or Mn are the minerals with significant roles in the animal organism, while their requirements need to be covered by the optimally balanced nutrition [Sirri *et al.* 2016]. The diets containing the trace minerals including Cu, Zn or Mn affect growth performance, immune responses, and meat quality of production animals [Sahoo *et al.* 2014, Sirri *et al*. 2016, Uniyal *et al*. 2017]. Changes in levels of minerals like Cu, Zn, and Mn in the diet affect digestion, absorption and functionality of enzymes in the cells.

Copper belongs to a group of metals that are essential for the activity of vitally important enzymes, although it is toxic when in excess. Thus, copper uptake and supply have to be strictly regulated [Gonzales-Eguia *et al*. 2009]. Zinc serves many essential functions in the body under normal conditions and may be an activator of lysosomal functions including glycosidase and other enzymes. However increased free zinc levels in a cell can be highly toxic. Liu [2015] showed that dietary Zn supplementation in the traditional form increased the Zn contents in the breast and thigh muscle of broilers. It is therefore important to control the potential oversupplementation of this mineral in the commercial poultry diet, while not disturbing the lysosomal enzymes functions. Mn is essential to animal health, acting as a co-factor in the active centers of various enzymes, and is required for normal development, maintenance of nerve and immune cell functions, among other functions. Overexposure to this metal, however, can be toxic to many organs [Keen and Zidenberg-Cherr 1994, Smith *et al*. 2017].

In present study, two forms of supplementation and three different levels covering 100, 50 and 10% of the requirement for Zn, Cu and Mn demand were used in turkey feeding. There was an effect of the form of application and the amount of Zn on the activity of the glycolytic enzymes studied in the breast muscles and thighs of turkeys.

The largest changes in enzymes activity in both the breast and thigh muscles were reported after the use of 10% zinc additive in the form of nanoparticles. Similar results were obtained for other groups of lysosomal enzymes (aminopetidases) in previous study by Jóźwik *et al*. [2018].

In current study the use of copper nanoparticles in birds feeding led to an increase in the activity of enzymes in the lysosomal degradation pathway. The lowest dose (10%) of Cu provided in the nanoform resulted in an increase in the activity of the majority of the studied enzymes in the breast muscle and decreased their activity in the thigh muscle. Differentiation in the reaction of glycosidases in the examined muscles may be related to their physiological character. Differences between slow and fast growing birds in the proteolytic and glycolitic capacity of the muscles, indicated by the higher enzyme to inhibitor ratio in the slower growing birds were observed [Dransfield and Sosnicki 1999]. It suggested that the increased growth and muscle mass in modern poultry lines could be largely governed by reduced protein catabolism. Our previous study showed an increase in activity of other lysosomal enzymes, like

aminopeptidases, in the thigh muscle after using the lowest doses of Cu nanoparticles in turkey diet [Jóźwik *et al*. 2018].

In turn, the use of a 10% dose of Mn nanoparticles in turkey feeding caused the inhibition of glycosidase activity in the breast muscle, but increasing the dose up to 50% of the Mn in the form of nanoparticles caused opposite response in the examined muscles. In breast muscle, this dose caused an increase in enzymatic activity, but in the leg muscle we observed decrease of the activity of all estimated glycosidases.

The use of Zn, Cu and Mn in turkey diet modulates the activity of the studied enzymes already when small doses are applied into the diet (10 and 50% of the requirement for those minerals), and especially in case of the nanoparticle form. This may indicate that Zn, Cu and Mn in the nano form are more bioavailable when compared to traditionally used standard for of those minerals. Moreover changes in glycosidase activity in the turkey hens' breast and thigh muscle after supplementation of Zn, Cu and Mn as nanoparticles can be an indicator of muscle metabolism levels.

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