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Effect of probiotic preparations (EM) and sex on morphometric characteristics of the digestive system and leg bones, and caecal microflora in broiler chickens

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ABSTRACT

The objective of the study was to determine the effect of probiotic preparations Pro-Biotyk EM-15 and EMFarma™ and sex on morphometric characteristics of the digestive system and leg bones, and caecal microflora in Ross 308 broiler chickens. One-day chicks were distributed into two groups in two rooms (control, experimental). Each facility contained 9000 Ross 308 chicks and was divided into four compartments. A total of 48 Ross 308 broiler chickens aged 42 days were evaluated, including 12 males and 12 females which received no probiotics, and 12 males and 12 females supplemented with EM preparations. At the age of 42 days, EM supplemented broilers had significantly greater total intestine length, higher intestine–body length ratio and higher proventriculus percentage in body weight. Regardless of the treatment, males exhibited higher body weight, duodenum, jejunum, ileum lengths and total intestine length, higher intestine–body length ratio and spleen weight, and lower duodenum diameter and liver percentage. The group × sex interaction was significant for smallest breadth of the corpus of femur bone and greatest diagonal of proximal end, smallest breadth of the corpus and smallest breadth of distal end of tibia bone.

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KEYWORDS

Broiler; effective microorganisms; intestine; leg bones; sex

Introduction

The intensification of poultry production increased the preference for highly productive specialized breeds and commercial hybrids, which are demanding in terms of the environmental and feeding conditions, veterinary and preventive measures, and show increased susceptibility to disease, including gastrointestinal diseases. By 2006 in the European Union, antibiotic growth promoters significantly reduced the incidence of gastrointestinal diseases while having a positive effect on growth performance. A ban on the use of in-feed antibiotics in the European Union has created a gap in preventing poultry against pathogenic bacteria. Acidifiers, herbal preparations and bioactive substances, such as probiotics, prebiotics and synbiotics, began to be used in agricultural practice (Yegani and Korver 2008; Yang et al. 2009; Przenioslo-Siwczynska and Kwiatek 2013).

Research (Bengmark 1998; Dalloul et al. 2005; Willis and Reid 2008; Dhama et al. 2011) has shown that probiotics inhibit the growth of bacteria (E. coli, Salmonella enteritidis, Salmonella typhimurium, Campylobacter jejuni) and coccidia oocysts in poultry. The use of probiotics reduced the incidence of diseases in poultry and the risk of food-borne intoxication in humans, caused by Salmonella, Campylobacter and E. coli pathogenic bacteria (Swiatkiewicz and Koreleski 2007). Many experiments also determined the impact of probiotics on histomorphological measurements of the small intestine (Olnood et al. 2015; Al-Baadani et al. 2016; Gesek et al. 2018; Souza de et al. 2018), development of internal organs (Mahajan et al. 1999; Pelicia et al. 2004; Takahashi et al. 2005; Zamanzad et al. 2011; Olnood et al. 2015; Malik et al. 2016) and selected characteristics of leg bones (Mutus et al. 2006; Ziaie et al. 2011; Parvaneh et al. 2014).

Research has also been conducted for several years to determine the efficiency of effective microorganisms (EM) in broiler chicken production (Wondmeneh et al. 2011; Jwher et al. 2013). EM probiotics are a mixture of microorganisms (including bacteria, fermenting fungi, radiolaria) selected and formulated in the 1980s. When added to feed or water, they ameliorate the course of food-borne intoxications, reduce the incidence of diarrhea and small intestinal pH, thus creating less favourable conditions for pathogenic bacteria to develop. Spray or mist application of EM probiotics is used for hygienization and biodisinfection of livestock buildings.

The lack of studies concerning the effect of the probiotic preparations EM Pro-Biotyk EM-15 and EMFarma™ on morphometric characteristics of the digestive system and leg bones encouraged us to perform this study. Showing a positive effect of EM preparations on the analysed traits could provide an incentive for larger scale application of these preparations in commercial broiler production.

The objective of the study was to determine the effect of probiotic preparations Pro-Biotyk EM-15 and EMFarma™ and sex on body weight and length, morphometric characteristics of the intestine and leg bones, proportion (g, %) of main internal organs in the body, and composition of caecal microflora in broiler chickens.
Materials and methods

Birds and housing

The experiment used 48 Ross 308 broiler chickens aged 42 days. Carcasses and eviscerated viscera were purchased from a commercial poultry slaughterhouse. Before slaughter, birds were raised on a commercial breeder farm located in the Opolskie Voivodeship (Poland). Eighteen thousand-day-old unsexed chicks were randomly distributed into two groups. Chickens were kept in a confinement building separated by a technical room into two production houses (each with 9000 birds), each having an area of 500 m². Each production room was divided into four compartments with a similar number of chickens. The two houses provided the same environmental conditions depending on the age of the birds. During the first week of rearing, temperature was maintained at 33°C followed by a gradual decrease to 17°C (heating with heaters), relative humidity ranged from 55% to 70%, and air exchange ranged from 0 to 4.2 m³/h/kg of body weight.

Feeding programme

Throughout rearing, birds were fed ad libitum complete diets and had 24 h access to water. For the first 8 days of rearing, birds received a commercial complete starter diet for broilers in crumble form. From 9 to 42 days of age, birds were fed complete grower 1 (9–24 d), grower 2 (25–32 d) and finisher diets (33–42 d), which were finely ground and produced from purchased feed components (protein concentrate, feed wheat, soybean meal, soybean oil, ground limestone, premix) on farm premises. The ingredient composition of the diets produced on the farm is listed in Table 1, and chemical composition of all the diets fed to birds, determined at the laboratory, is presented in Table 2.

Microbial analysis and effective microorganisms Programme

The microbial profile (Table 3) of the Pro-Biotyk (Em-15) and EmFarma™ probiotics was determined using quantitative culture technique (Petri dish method). Lactobacillus spp. bacteria were enumerated in MRS Agar medium (OXOID), Bifidobacterium spp. in TOS-MUP (MERCK), Lactococcus and Streptococcus thermophilus in M17 (OXOID), Bacillus subtilis in TSB (OXOID), Rhodopseudomonas spp. in Van Niel’s medium (ATCC medium 1676) and Saccharomyces cerevisiae yeast with chloramphenicol medium (YGC, BIOCORP). The determinations were made in accordance with Polish standards.

In the house with experimental birds from day 1 of age, ProBiotyk Em-15 was added to water three times per week in the amount of 2 ml/l of water. From 2 weeks of age, a ProBiotyk Em-15 and EMFarma™ mixture solution was sprayed twice per week (1.25 l of each preparation plus 2.5 l of water). The solution was sprayed onto feed and litter.

Birds were reared under supervision of a veterinarian. When preparing the building for the placement of chicks, the control house was disinfected with chemical disinfectants (sodium hypochlorite, Virocid F), and the experimental house, apart from the above chemical disinfectants, was treated with EMFarma™ (30 l of the preparation plus 170 l of water per 500 m² of the house) by spraying the ceiling, poultry equipment, and litter. All chickens (both groups) were vaccinated against Marek’s disease (1 d of age), Gumboro disease (7 and 21 d of age), infectious bronchitis – IB (1 d of age), coccidiosis (1 d of age) and swollen head syndrome – SHS (1 d of age).

Analysis of the digestive system and leg bone traits

On day 42 of rearing, 48 chickens, i.e. 24 birds from each group (12 males and 12 females per group, 3 males and 3 females from each compartment every production room, based on comb growth) were selected for slaughter. The selected birds were banded with padlock tags, individually weighed on an electronic balance (Axis BD 15S, Axis, Gdansk, Poland) with an accuracy level of 5 g, and measured for body length (length of trunk with neck) by measuring the distance from the first cervical vertebra (atlas) and the posterior superior tuberosity of the ischium.

Following live assessment, the birds were slaughtered in a small commercial poultry slaughterhouse (water bath electrical stunning with a current of 125 mA/pcs, stunning time 4 s, mechanical cutting of neck blood vessels, bleeding out). Slaughter date was linked to liquidation of the rest of the flock. After slaughter, birds were mechanically defeathered and manually eviscerated into intestines and other internal

Table 2. Composition of diets for broiler chickens.

| Ingredient                        | Starter 0–8 d | Grower 1 9–24 d | Grower 2 25–32 d | Finisher 33–42 d |
|-----------------------------------|--------------|----------------|-----------------|-----------------|
| Truw Nutrition DKAG Plus Concentrate | 5.0          | 5.0            | –               | –               |
| Lidermix DKA-F E Fito Trouw Nutrition | –            | –              | 2.0             | 2.0             |
| Soybean meal                      | 25.0         | 22.0           | 22.0            | 22.0            |
| Raw soybean oil                   | 3.5          | 4.2            | 4.7             | 4.7             |
| Ground limestone                  | –            | –              | 0.75            | –               |
| Feed wheat                        | 66.5         | 68.8           | 70.55           | –               |
| **ME, MJ/kg**                     | **12.60**    | **13.20**      | **13.33**       | **13.09**       |
| **DM, %**                         | **89.58**    | **87.93**      | **89.79**       | **89.96**       |
| **CP, %**                         | **23.08**    | **22.45**      | **22.10**       | **21.63**       |
| **Crude fat, %**                  | **5.41**     | **5.45**       | **5.52**        | **4.80**        |
| **Crude fibre, %**                | **2.21**     | **1.60**       | **2.00**        | **2.24**        |
| **Crude ash, %**                  | **3.75**     | **4.67**       | **4.45**        | **4.59**        |
| **ADF, %**                        | **5.66**     | **4.74**       | **4.44**        | **5.36**        |
| **NDF, %**                        | **10.19**    | **9.26**       | **9.60**        | **10.27**       |
| **ME, MJ/kg**                     | **12.60**    | **13.20**      | **13.33**       | **13.09**       |

Table 1. Chemical composition of diets for broiler chickens.

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| Crude fat, %                | 5.41         | 5.45           | 5.52            | 4.80            |
| Crude fibre, %              | 2.21         | 1.60           | 2.00            | 2.24            |
| Crude ash, %                | 3.75         | 4.67           | 4.45            | 4.59            |
| ADF, %                      | 5.66         | 4.74           | 4.44            | 5.36            |
| NDF, %                      | 10.19        | 9.26           | 9.60            | 10.27           |
| ME, MJ/kg                   | 12.60        | 13.20          | 13.33           | 13.09           |

DM, dry matter; CP, crude protein; ADF, acid detergent fibre; NDF, neutral detergent fibre; ME, metabolizable energy.

Table 3. Microbial profile of EM probiotics.

| Determination target            | Cell count (cfu/ml) |
|---------------------------------|---------------------|
| Lactobacillus spp.              | 1.5 × 10⁷           |
| Bifidobacterium spp.            | 1.0 × 10⁷           |
| Lactococcus spp.                | 8.0 × 10⁶           |
| Streptococcus thermophilus       | 8.0 × 10⁶           |
| Bacillus subtilis               | 3.0 × 10⁹           |
| Rhodopseudomonas spp.           | 3.0 × 10⁹           |
| Saccharomyces cerevisiae         | 1.0 × 10⁷           |

Determination target Pro-Biotyk (Em-15) EMFarma

Lactobacillus spp. 1.5 × 10⁷ 5.0 × 10⁹
Bifidobacterium spp. 1.0 × 10⁷ 2.0 × 10⁹
Lactococcus spp. 8.0 × 10⁶ 4.0 × 10⁹
Streptococcus thermophilus 8.0 × 10⁶ 6.0 × 10⁹
Bacillus subtilis 3.0 × 10⁹ 1.1 × 10⁹
Rhodopseudomonas spp. 3.0 × 10⁹ <10³
Saccharomyces cerevisiae 1.0 × 10⁷ 2.0 × 10²
organs (including proventriculus, gizzard, heart, liver, spleen and lungs). The length of duodenum, jejunum, ileum, both caeca and colon was tape measured to the nearest 1 mm. The length of duodenum was measured from the pylorus to pancreatic loop, the length of jejunum was the distance from the pancreatic loop to Meckel’s diverticulum and the length of ileum was the distance from Meckel’s diverticulum to the ileocaecal junction. The length of the colon was measured as the distance from the mouth of the caeca to the cloaca, while the length of both caeca was measured as total distance of the mouth of the ileum to the apex of the right and left caeca. The diameters of individual intestinal segments were measured with callipers to an accuracy of 0.01 mm (three measurements at the beginning, in the middle and at the end of each intestinal segment); they were used to calculate the mean measurement of the diameter of a given segment. In addition, the internal organs – gizzard (without digesta), proventriculus (without digesta), liver (without gallbladder), heart and spleen were separated and weighed on a Medicat M160 balance (Medicat, Zurich, Switzerland) with an accuracy of 0.001 g and their percentage in pre-slaughter weight was calculated.

On day 42 of rearing, the dimensions of femoral and tibial bones were determined according to the method described by Driesch (1976). The following was measured with an electronic calliper with an accuracy of 0.01 mm: greatest length, medial length, greatest breadth of proximal end, greatest depth of proximal end, smallest breadth of the corpus, greatest breadth of the distal end, greatest depth of the distal end of femur bone. The following measurements of the tibia were also carried out: greatest length, axial length, greatest diagonal of the proximal end, smallest breadth of the corpus, smallest breadth of the distal end, depth of the distal end.

**Caecal microflora analysis**

After slaughter and evisceration, pooled faecal samples were collected from the caeca of 48 birds within groups. Total fungal count, aerobic bacteria count and lactic acid bacteria count were determined in 1 ml of faeces. The determinations were made at the Veterinary Laboratory by quantitative culture (Petri dish method). The determinations were made in accordance with Polish standards.

**Statistical analysis**

The numerical data were subjected to statistical analysis. Arithmetic means and pooled standard error (SE) for both groups were calculated for the analysed traits. Normal distribution of the morphometric characteristics of the digestive system and leg bones was verified with the Shapiro–Wilk test. Two-way analysis of variance was used to determine the effect of genotype and sex on the above meat characteristics of the ducks. To this end, the following linear model was used: \( y_{ijk} = \mu + a_i + b_j + (a \times b)_{ij} + e_{ijk} \), where \( y_{ijk} \) – value of the analysed trait, \( \mu \) – overall mean of the analysed trait, \( a_i \) – effect of ith duck genotype, \( b_j \) – effect of jth sex, \( (a \times b)_{ij} \) – genotype by sex interaction, \( e_{ijk} \) – random error. Significant differences between the means of the traits between the groups and between males and females in a group were analysed by means of Tukey’s test.

The level of significance was at \( P < 0.05 \). Statistics were analysed with SAS Software, ver 9.4. (SAS Institute Inc 2014).

**Results and discussion**

The mean body weight of 42-day-old broiler chickens from the compared groups exceeded 3100 g in males and 2600 g in females, which may be indicative of their normal development resulting from the genetic makeup, proper microclimate parameters and good quality feeds (Table 4). Ross 308 broiler chickens, supplemented with EM probiotic preparations, had non-significantly higher body weight at 42 days of age when compared to control chickens that received no probiotics. These results are consistent with the findings of Mutus et al. (2006), Olnood et al. (2015) and Malik et al. (2016), who reported no statistically significant effect of supplemental probiotics on body weight of broiler chickens at 35 or 42 days of rearing. The body weight of 6-week-old broiler chickens from our study was greater than that reported by Radu-Rusu et al. (2008), Marcus et al. (2012) and Malik et al. (2016). In both groups, sex of the birds had a significant effect on the body weight of the chickens aged 42 days. Control males were 507 g (16.3%) heavier than females from the same group, whereas experimental males weighed 510 g (16.3%) more than experimental females. The group × sex interactions for body weight were not significant (Table 4).

The data listed in Table 4 demonstrate that the use of EM probiotic preparations did not have a significant effect on body length, and a significant effect on total intestinal length, and intestine:body length ratio. In both groups, body weight, total intestinal length and intestine:body length ratio were significantly \( (P < 0.05) \) greater in males than in females. The group × sex interaction was not significant for the above traits. In an earlier study, Kokoszynski et al. (2017) observed smaller total intestinal length (251.4 cm) in Ross 308 chickens aged 42 days, and Gabriel et al. (2008) reported shorter small intestine in 44-day-old Ross PM3 males.

Our results revealed that the EM probiotic preparations had no significant effect on the length and diameter of individual intestinal segments. Regardless of the treatment, males had significantly longer individual segments of the small intestine and significantly smaller diameter of duodenum compared to females (Tables 5 and 6). The group × sex interactions for

| Table 4. Body weight, body and intestine length and their ratio in 42-day-old broiler chickens. |
| --- |
| Group | Sex (n = 12) | Body weight (g) | Length (cm) | Intestine: Body length ratio |
| --- | --- | --- | --- | --- |
| Control | Male | 3120 | 29.9 | 282.5 \( ^c \) | 9.4 |
| | Female | 2613 | 29.7 | 244.8 \( ^b \) | 8.2 \( ^b \) |
| Experimental | Male | 2866* | 29.8 | 263.7 \( ^* \) | 8.8 \( ^* \) |
| | Female | 3135 | 30.2 | 291.3 \( ^b \) | 9.6 |
| | Mean | 2635 | 30.0 | 279.7 \( ^* \) | 9.3 \( ^* \) |
| Pooled SE | 45.4 | 0.2 | 3.9 | 0.1 |
| Group | Sex | 0.804 | 0.743 | 0.037 | 0.025 |
| | Sex | <0.001 | 0.088 | 0.001 | 0.022 |
| | Group × sex | 0.990 | 0.397 | 0.402 | 0.763 |

*Statistically significant differences determined between males and females in columns within group \( (P < 0.05) \).
length and diameter of the intestinal segments were not significant (P > 0.05). Sato et al. (2002) observed that dietary addition of probiotic had no significant effect on intestine length and percentage in broiler chickens. Pelicia et al. (2004) found no significant differences in the length of individual segments of the small intestine (duodenum, jejunum, ileum) and the ratio of their weight to preslaughter body weight in 84-day-old free-range broiler chickens in response to a probiotic and prebiotic mixture of bacterial or/and yeast origin.

Tables 7 and 8 show that the use of EM probiotic preparations did not have a significant effect on the liver, heart, gizzard, and spleen weight and percentage, and on the proventriculus weight in preslaughter body weight. A significant effect of EM preparations was observed for proventriculus percentage. Regardless of the treatment, males had significantly higher weight of spleen and lower percentage of liver in body weight compared to females. For the weight and percentage of internal organs, the group × sex interactions were not significant (P > 0.05). An earlier experiment (Malik et al. 2016) found that commercial probiotics containing Bacillus subtilis powder had no significant effect on the percentage of liver, gizzard, heart, spleen, intestine and abdominal fat in 42-day-old broiler chickens. However, Mahajan et al. (1999) observed significantly higher giblets values in probiotic-supplemented broiler chickens. In turn, Kokosynski et al. (2017) reported lower liver, heart, proventriculus and spleen percentage, and higher gizzard percentage in preslaughter body weight of 42-day-old Ross 308 chickens compared to the results of the present study. Higher liver percentage in preslaughter body weight was also obtained by Hernandez et al. (2004), Sharifi et al. (2012) and Hussain et al. (2012).

The administration of EM probiotic preparations to broiler chickens had no significant effect on the measurements of femur and tibia bones. Regardless of the group, males

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**Table 5. Length of intestine segments in 42-day-old broiler chickens.**

| Group   | Sex (n = 12) | Duodenum (cm) | Jejunum (cm) | Ilum (cm) | Caecum (cm) | Rectum (cm) |
|---------|--------------|---------------|--------------|----------|-------------|-------------|
| Control | Male         | 34.5          | 97.8         | 96.5     | 45.7        | 8.0         |
|         | Female       | 31.8          | 80.2         | 82.6     | 42.5        | 7.7         |
|         | Mean         | 33.2*         | 89.0*        | 89.5*    | 44.1        | 7.9         |
| Experimental | Male    | 37.0          | 99.0         | 99.7     | 47.8        | 7.8         |
|         | Female       | 32.7          | 88.8         | 95.2     | 43.1        | 8.3         |
|         | Mean         | 34.9*         | 93.9*        | 97.4     | 45.5        | 8.0         |
| Pooled SE |             | 0.5           | 1.6          | 1.6      | 0.9         | 0.3         |
| Group   |              | 0.141         | 0.160        | 0.055    | 0.592       | 0.839       |
| Sex     |              | 0.004         | 0.005        | 0.027    | 0.133       | 0.950       |
| Group × sex |         | 0.452         | 0.279        | 0.237    | 0.757       | 0.678       |

*Statistically significant differences determined between males and females in columns within group (P < 0.05).

**Table 6. Diameter of intestine segments in 42-day-old broiler chickens.**

| Group   | Sex (n = 12) | Duodenum (mm) | Jejunum (mm) | Ilum (mm) | Caecum (mm) | Rectum (mm) |
|---------|--------------|---------------|--------------|----------|-------------|-------------|
| Control | Male         | 11.8          | 11.4         | 9.0      | 10.1        | 8.2         |
|         | Female       | 13.6          | 10.4         | 9.0      | 9.3         | 7.8         |
|         | Mean         | 12.7*         | 10.9*        | 9.0      | 9.7         | 8.0         |
| Experimental | Male    | 12.3          | 11.1         | 10.6     | 10.4        | 8.7         |
|         | Female       | 13.6          | 10.7         | 9.5      | 10.4        | 7.3         |
|         | Mean         | 13.0*         | 10.9*        | 10.1*    | 10.4        | 8.0         |
| Pooled SE |             | 0.2           | 0.2          | 0.2      | 0.2         | 0.3         |
| Group   |              | 0.599         | 0.937        | 0.059    | 0.236       | 0.989       |
| Sex     |              | 0.010         | 0.222        | 0.305    | 0.514       | 0.177       |
| Group × sex |         | 0.620         | 0.566        | 0.259    | 0.465       | 0.509       |

*Statistically significant differences determined between males and females in columns within group (P < 0.05).

**Table 7. Weight of main internal organs in 42-day-old broiler chickens.**

| Group   | Sex (n = 12) | Liver (g) | Heart (g) | Proventriculus (g) | Gizzard (g) | Spleen (g) |
|---------|--------------|-----------|-----------|--------------------|-------------|------------|
| Control | Male         | 51.6      | 10.5      | 8.4                | 48.8        | 3.0        |
|         | Female       | 47.5      | 9.8       | 7.9                | 46.7        | 2.7        |
|         | Mean         | 49.6      | 10.2      | 8.2                | 47.8        | 2.9*       |
| Experimental | Male    | 50.4      | 11.8      | 10.6               | 56.4        | 3.7        |
|         | Female       | 52.4      | 9.9       | 9.1                | 44.7        | 2.5        |
|         | Mean         | 51.4      | 10.9      | 9.9*               | 50.6*       | 3.1*       |
| Pooled SE |             | 0.9       | 0.2       | 0.2                | 1.4         | 0.1        |
| Group   |              | 0.431     | 0.328     | 0.101              | 0.448       | 0.327      |
| Sex     |              | 0.636     | 0.058     | 0.056              | 0.067       | 0.004      |
| Group × sex |         | 0.217     | 0.371     | 0.207              | 0.196       | 0.098      |

*Statistically significant differences determined between males and females in columns within group (P < 0.05).
showed significantly higher measurements for greatest length, medial length, greatest breadth of proximal end, smallest breadth of the corpus and greatest breadth of the distal end of femur bone (Table 9). The group × sex interactions were not significant for the analysed femur bone measurements except for the measurement of the smallest breadth of the corpus of this bone. The analysis of tibia bone dimensions in the analysed broiler chickens (Table 10) showed a non-significant effect of administering EM probiotic preparations on tibia bone measurements. Regardless of the treatment, males had significantly greater tibia measurements compared to females. The group × sex interactions were not significant except for the interaction for the greatest diagonal of the proximal end, smallest breadth of the corpus and smallest breadth of the distal end of the tibia.

The results of microbiological determinations of caecal faeces are listed in Table 11. The analysis of the results showed decreases in the number of aerobic bacteria and lactic acid bacilli and an increase in total fungal count in
caecal faeces from 42-day-old chickens following the application of EM probiotic preparations.

Concluding, the use of EM probiotic preparations had a positive effect on the body weight and length, total intestinal length, and the length and diameters of intestinal segments in 42-day-old Ross 308 chickens. The EM probiotics had a significant effect on total intestine length, intestine–body length ratio and proventriculus percentage in body weight. Sex of birds had a significant effect on body weight, total intestinal length, length of the small intestine segments, intestine–body length ratio, spleen weight, liver percentage and almost all the measurements of femur and tibia bones.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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