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Research Article

Effect of Dietary Zinc (II) Chelate Complex and Zinc (II) Enriched Soybean Meal on Selected Parameters of *in vivo* Caecal Fermentation in Laying Hens (Lohman Brown) [1]

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Abstract

The study was conducted on 18 laying hens from Lohman Brown at 23 weeks of age. There were two research groups and one control group with six animals each. In the last four weeks of age, the animals were fed follows: Control group (Group C) complete diet; Group I diet containing zinc added via the biosorption process and; Group II: diet containing zinc as an organic chelate complex. The levels of short chain fatty acids (SCFAs) such as acetate, propionate, iso-butyrate, butyrate, iso-valeriate and valeriate were determined. It was observed that using a diet with additives obtained via biosorption does not have a negative influence on fermentation processes in the hen's caecum.

Keywords: *Laying hen, Caecum, Short-chain fatty acid, Biosorption, Soybean meal, Zinc*

Yumurtacı Tavuklarda (Lohman Brown) *in vivo* Körbarsak Fermentasyonunda Sindirilebilir Çinko (II) Şelat Kompleksi ve Çinko (II) İle Zenginleştirilmiş Soya Fasulyesi Küspesi İlavesinin Seçilen Parametrelere Etkisi

Özet

Çalışma 23 haftalık, 18 adet Lohman Brown ırkı yumurtacı tavuklarda yürütülmüştür. Araştırma altışarlı iki deney ve bir kontrol grubuyla yapılmıştır. Hayvanlar süreç içerisinde son dört haftada, aşağıdaki gibi beslenmiştir: Kontrol grubu (C) sadece yem; grupI çinko içeren biosorpsiyon prosesi ile, Grup II: organik şelat kompleksi olarak çinko içeren yemlerle beslenmişlerdir. Asetat, propiyonat, izo-bütirat, bütirat, izo-valeriat ve valeriat gibi kısa zincirli yağ asitlerinin düzeyleri belirlenmiştir. Burada katkı maddeleri ilave edilmiş yem kullanarak oluşturulan biozorpsiyonun tavuk körbarsağında fermantasyon prosesine olumsuz bir etkisinin olmadığı gözlemlenmiştir.

Anahtar sözcükler: *Yumurtacı tavuk, Körbağırsak, Kısa-zincirli yağ asidi, Biozorpsiyon, Soya fasulyesi küspesi, Çinko*

INTRODUCTION

The deficiency of micronutrients is a serious problem in human as well as veterinary science [1,2]. It is believed that zinc deficiencies impact 1/3 of the human population [3]. It seems that an integration of micronutrient-rich foods such as vegetables, fruits and animal products into the diets is the most practical and sustainable way to alleviate micronutrients deficiency [4,5]. In the recent years, research on the possibility of supplementing the micronutrients, including zinc, with the use of biofortified food were conducted [6,7]. In this way, the concentration of desired micronutrients in the food could be precisely controlled. Zinc is traditionally supplied as chelate complexes or

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inorganic salts. However, it is known that micronutrients supplied as inorganic salts have very little bioavailability and have a kind of a transit character [8-10]. The high supply of inorganic salts in diet causes their significant accumulation in the environment [11-14]. Conversely, using a much lower level of organically complexed minerals in diets, instead of inorganic forms, have no negative influence on haematological and biochemical parameters in broilers [15]. The chelate complexes have higher bioavailability than inorganic salts but lower than products obtained using biosorption. With respect to the above, studies on the use of biosorption in enriching the animals' diet with minerals were undertaken [16-18]. The products of animal origin obtained this way have a desired concentration of certain minerals with very high bioavailability that limits their emission into the environment [7]. Nonetheless, the influence of potential feed additives obtained with the biosorption on the processes occurring in the hens' caecum, which is of great importance to the health and productivity of the animals, is still not known. The studies conducted *in vitro* shows that feed additives obtained this way do not have a negative influence on fermentation processes occurring in the caecum of laying hens and should not have a negative influence on the animals' health [19-21]. In this study, we present the results of *in vivo* research on the fermentation process in the caecum of laying hens fed with the addition of zinc (II) brought in via the biosorption method, and compared it with the zinc (II) chelate complex.

MATERIAL and METHODS

Methods of Manufacturing Diet Supplements with Microelements

The experiment was carried out according to Local Bioethic Comitee Permission No. 129/2010. The soybean meal (Vetos, Zębowice) was enriched with zinc (II) ions by biosorption using inorganic salt ($ZnSO_4{\cdot}7H_2O$) permitted by law as a feed additive (POCh, Gliwice). The biosorption was conducted using a column reactor with a bed of volume of 0.1 dm^3. Water of pH 5.0 demineralized using 0.1M NaOH/HCl (POCh, Gliwice) was applied to the process. The water reaction was controlled using Mettler-Toledo pH-meter (Seven Multi, Switzerland) equipped with an InLab413 electrode with temperature compensation [21]. The biosorption process was conducted in a temperature of 20°C until bed saturation, while also controlling the concentration of solution flowing out of the column. The enriched biomass was air dried for 48 h.

The zinc concentration in the samples taken was determined using ICP-OES plasma spectrometer (Varian Vista-MPX; Varian, PaloALto, USA) in the Chemical Laboratory of Multi-elemental Analysis at Wrocław University of Technology, accredited by ILAC-MRA and the Polish Accreditation Centre (PCA) (No AB 696) *(Table 1)* [22].

The control group (C) and two experimental groups (I and II) were distinguished. In the first group (I) the zinc assimilation from a product obtained using biosorption was studied, and in the second group (II) the zinc absorption from organic chelate complex was examined. The biological preparation and the zinc chelate complex were separately added to the prepared diet (NJT-214, Tasomix). Compounds of diet mix were the same for all the groups *(Table 2)*. The diet composition was established not to contain the zinc addition, and the requirement for other micronutrients was assured by the addition of inorganic salts *(Table 3)*. The requirement for zinc in group I was fulfilled in 100% with the biological preparation and in group II – in 100% with the zinc chelate complex (Glystar Forte, Agsol). The digestibility of both the biological prepared zinc group (I) and zinc chelate complex (group II) was compared to the Control group, which was fed with

Table 1. *The zinc content of soybean meal before and after biosorption*
Tablo 1. *Biozorpsiyondan önce ve sonra soya küspesinin çinko içeriği*

Parameter	Non-enriched Soybean Meal ($\bar{x}$±SD, N=3)	Enriched Soybean Meal ($\bar{x}$±SD, N=3)
Zn (mg/g)	0.054±0.005	14.088±0.403

Table 2. *The ingredients of experimental diet (Tasomix®)*
Tablo 2. *Deney yemin bileşenleri (Tasomix®)*

Ingredients	%
Ground corn	29.92
Triticale	15.00
Soybean meal	13.70
Wheat	12.00
Calcium carbonate	8.42
Decoction wheat-corn	6.00
Triticale mix	4.50
Sunflower meal	4.20
Fats	2.30
Vitamin-mineral premix	2.00
Dried full blood	1.80
Mycofix selected (mineral sorbents)	0.05
L-Lysine	0.02
Biosorbent addition for	0.07
Lucantin pigment – red	0.02

[a] The composition of standard feed was established by the producer. Provides in kg of diet: vitamin A (retinyl acetate), 10.000 IU; cholecalciferol, 2.000 IU vitamin E (DL-α-tocopheryl acetate), 20.000 IU, vitamin K (bisulfite menadionsodium) 1.5 IU, vitamin B_1 (thiamine mononitrate) 3.99 IU, vitamin B_2 (riboflavin) 4.0 IU, Vitamin B_3 (nicotinic acid) 20.0 IU, Vitamin B_5 (D-calcium pantothenate) 8.0 IU, Vitamin B_6 (pyridoxine hydrochloride) 1.5 IU, Vitamin B_{12} (cyanocobalamin) 15.0 IU, biotin (D-biotin) 50.0 IU, folic acid (folic acid) 0.8 IU, choline (choline chloride) 999.6 IU. IU-international unit; [a] Provides (mg/kg of diet): Cu, 8 (as $CuSO_4{\cdot}5H_2O$); Fe, 45 (as $FeSO_4{\cdot}H_2O$); Mn, 85 (as MnO_2); Zn, Control group 60 mg/kg of diet (as ZnO); Group I Zinc (II) enriched soybean meal - 4.259 g/kg of diet; Group II Zinc (II) enriched chelate complex - 0.375 g/kg of diet

Table 3. *The chemical composition of the experimental diet (Tasomix®)*
Table 3. *Deneme yeminin kimyasal kompozisyonu (Tasomix®)*

Chemical Composition of Diet	g/kg
Dry matter	889.5
Crude ash	123.7
Crude protein	166.9
Crude fiber	32.00
Crude fat	46.3
Calcium	36.5
Total phosphorus	4.7
Metabolic energy (kcal/kg)	2821.45

a diet containing all of the micronutrients given as inorganic salts. In Group I, a preparation of 4.259 g of Zn (II) was used, and in the group II 0.375 g of zinc organic chelate complex was added.

Animals

The research material consisted of the caecum content collected from 18 hens from Lohman Brown at 23 weeks of age. The animals were held in the furnished Battery Cage System (one hen per cage) under microclimate controlled conditions. The animals from, 19 to 23 weeks of age, were fed with obtained diet mixes *(Table 3)*. Three groups of animals were selected: group C – the Control group fed with complete diet (n=6), group I – fed with diet containing zinc added with the biosorption process (n=6), group II – fed with diet containing zinc as an organic chelate complex (n=6).

SCFAs Analysis

The caecum content from each animal was collected during autopsy. It was subsequently mixed with the buffer of pH=7.3 in the ratio of 1 to 8, and underwent homogenisation. The pH of obtained suspension was measured with CP-401 pH-meter (ELMETRON, Zabrze, Poland) equipped with EPP-3 electrode and temperature sensor. The solution was centrifuged for 15 min with 13.000 rpm. The concentrated formic acid was added to the obtained liquid (0.1ml per 2ml of the solution) to stop the fermentation processes.

The samples of liquid were analysed using gas chromatograph (Agilen Technologies 7890A GC System) with FID detector to determine the total concentration of SCFAs and the percentage of particular acids: acetate, propionate, iso-butyrate, butyrate, iso-valeriate and valeriate. The identification and level of production of volatile fatty acids in the analysed samples was carried out by comparing to the retention time and the under-peak-area from the Supelco standard in ChemStation programme. The relationship between concentration of acetate and propionate and between concentration of propionate and butyrate were also evaluated.

Statistical Analysis

Data were tested for normality (Shapiro–Wilk's test). Statistical analysis was done by multivariate analysis of variance using Statistica 9.0 software (StatSoft Poland, Krakow, Poland) [23]. Significant differences were determined using Duncan's test. Differences with probability of $P \leq 0.01$ were considered significant.

RESULTS

The higher production of all the volatile fatty acids in the caecum content from hens fed with the zinc organic chelate complexes (II) was observed. The total amount of the produced volatile fatty acids was 20% higher than in the control group *(Table 4)*.

The results obtained in the samples from animals fed with the addition of biological preparation (I) were similar to the results from the group C, excluding acetic, propionic and isovaleric acids. Additionally, a lower concentration of acetic acid in the caecum content from hens fed with zinc added as a biological preparation (I) compared to the caecum content from hens fed with zinc added as an organic chelate complex (II) was noted. The lowest production of propionic acid (8.98 µmol/g) and butyric acid (5.91 µmol/g) was observed in hens from the group C.

The relationship between concentrations of particular volatile acids in the caecum content samples from hens from different groups varied. A reduced ($P \leq 0.01$) percentage of acetic acid in the caecum content from hens receiving zinc added by biosorption (I) as compared to other groups (C and II) was noted. The opposite dependence was observed for propionate and iso-valeriate, where was noticed a statistically significant differences between groups C and I ($P \leq 0.01$). The use of zinc as an organic chelate complex (II) caused the increase of percentage of propionate compared to the group C. The lowest level of butyrate production was observed in the caecum content from hens fed with zinc (II) organic chelate complex compared to group C and group I ($P \leq 0.01$).

The reduction of the ratio between the concentration of acetate and the concentration of propionate (A:P) in the group of hens fed with zinc added as a biological preparation (I) compared to group C ($P \leq 0.01$) was observed *(Fig. 1)*. The opposite tendency was observed in the ratio between the concentration of propionate and the concentration of butyrate (P:B) in the caecum content from hens fed with zinc added as an organic chelate complex (II) compared to other groups. The highest A:P ratio and the lowest P:B ratio was noted in the caecum content from hens in the Control group compared to hens fed with zinc enriched diet (I and II).

The pH of the caecum contents *(Fig. 2)* increased ($P \leq 0.01$) when using zinc obtained via biosorption and zinc organic chelate complex (adequately: 0.72 and 0.52 of pH value)

***Table 4.** Effects of zinc supplementation on caecal SCFAs production (μmol/g and mol %) in laying hens (mean± SD, n=6)];[a] statistically significant differences (P≤0.01*

***Tablo 4.** Seçilmiş mikroelementlerin ilavesinin tavuklarda (SD±, n=6) körbarsak içeriğinde kısa zincirli yağ asidi (μmol/g ve % mol) üretimi üzerine etkisi;[a] istatiksel açıdan önemli farklılık (P≤0.01*

SCFAs	Statistical	Group		
		C	I	II
μmol/g				
Acetate	x̄ ± SD	31.89±11.04	24.79±7.46	40.15±17.02
Propionate	x̄ ± SD	8.98±3.71	10.05±3.48	13.15±5.90
izo-Butytrate	x̄ ± SD	0.77±0.70	0.74±0.63	1.05±0.56
n-Butytrate	x̄ ± SD	5.91±1.27	6.05±2.65	5.92±2.91
iso-Valeriate	x̄ ± SD	0.26±0.10	0.39±0.23	0.33±0.23
n-Valeriate	x̄ ± SD	0.62±0.13	0.53±0.21	0.68±0.21
Total	x̄ ± SD	48.43±15.38	42.55±14.20	61.29±25.81
mol%				
Acetate	x̄ ± SD	65.41±2.46[a]	58.78±3.72[a]	65.57±4.45[a]
Propionate	x̄ ± SD	18.34±2.48[a]	23.66±1.84a	21.41±2.15
izo-Butytrate	x̄ ± SD	1.42±0.78	1.58±2-0.86	1.72±0.58
n-Butytrate	x̄ ± SD	12.95±3.58	13.83±1.88[a]	9.57±2.49[a]
iso-Valeriate	x̄ ± SD	0.54±0.08[a]	0.89±0.29[a]	0.55±0.24
n-Valeriate	x̄ ± SD	1.33±0.27	1.25±0.18	1.19±0.36

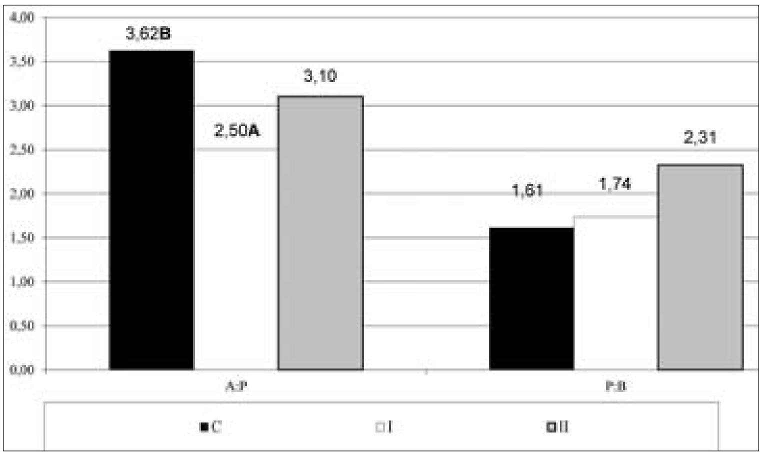

Fig 1. Concentration ratio between the acetate-propionate-butyrate
[A-B] statistically significant differences (P≤0.01)

Şekil 1. Asetat-Propionat-Butirat arasındaki konsantrasyon oranı
[A-B] istatiksel açıdan önemli farklılık (P≤0.01)

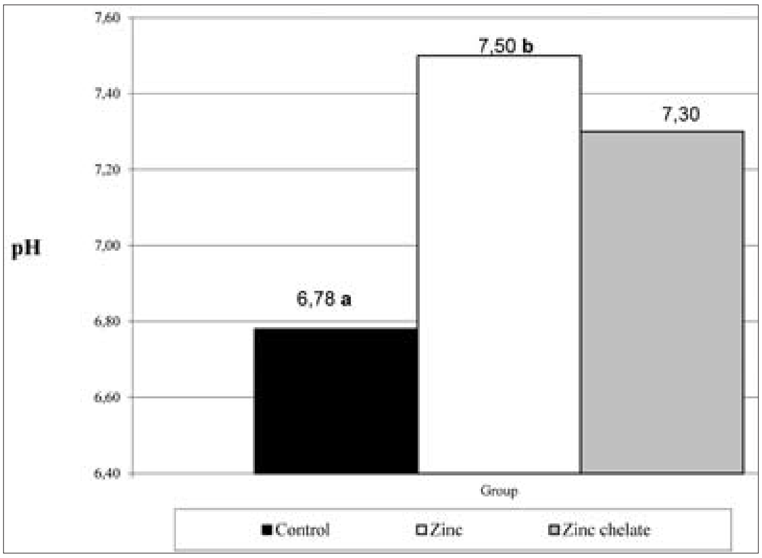

Fig 2. The level of the active acidity in caecal content
[a, b] highly statistically significant differences (P≤0.01)

Şekil 2. Körbarsak içeriğinin aktif asit seviyesi
[a, b] istatiksel açıdan yüksek önemli farklılık (P≤0.01)

as compared to the control group. The highest pH level of caecum contents was observed in the group of hens fed with zinc obtained via the biosorption method (7.5) and the lowest level was found in the Control group (6.78).

DISCUSSION

The fermentation processes in the hen's caecum are influenced by various factors. The use of reduced calcium level (800 mg/kg) and increased zinc content (110 mg/kg) in the diet decreases the level of production of volatile fatty acids (SCFAs) in the hens' caecum [24]. Because of their acid properties, SCFAs cause a decrease in the pH value in ceacum contents, therefore, the increased level of zinc in diet can cause the increase in the caecum content pH value [25]. In the analysed samples, zinc was added to the diet as an inorganic salt in the control group and as a preparation after biosorption (I) or as an organic chelate complex (II) in the research groups (60 mg/kg). An increase of pH value in the caecum content from hens receiving organic forms of zinc along with a simultaneous increase of about 12.89 µmol/g of the level of SCFAs (II) was noted, however, a decrease of about 5.88 µmol/g of produced SCFAs in Group I as compared to the Control group was observed. The obtained results can show a better digestibility of zinc given as a part of preparation obtained via biosorption. Nonetheless, the organic chelate complex causes an increase in the level of SCFAs, what is beneficial to the poultry. The diets used did not in negatively influence the pH value of the hens' caecum content, which reached the level of 6.78 to 7.50 in all of the research groups. Other studies obtained similar pH values [26-28].

It is believed that during the fermentation process in the caecum, the ratio between the acetate, propionate and butyrate should amount to 3-5:2:1 [29-32]. It is known that oversupply of zinc in diet causes an increase in acetate and propionate values and a decrease in the butyrate value [24]. In our study, a negative influence of given diet on the ratio between mentioned acids was not noted. The most beneficial ratio between the acetate and propionate (A:P) and between the propionate and butyrate (P:B) was noted in the caecum content from hens fed with zinc as an organic chelate complex (II) and amounted to 3:1 and 2:3.

The diversity of bacteria in a hen's caecum is relatively stable [24]. The bacterial fermentation leads to the formation of SCFAs, which are necessary in the metabolism of the intestine epithelium [32]. The acetic acid is characterised by the lowest energy value (0.876 MJ/mol) as compared to the propionate (1.536 MJ/mol) and the butyrate (2.194 MJ/mol), and their mutual ratio plays a role in determining the amount of energy delivered to the organism [33]. In our study the highest level of acetate, propionate and butyrate was noted in the caecum content from hens fed with zinc given as an organic chelate complex (II), which suggest the highest level of energy production.

It was shown that volatile fatty acids such as propionate and butyrate have an influence on the inhibition of pathogenic bacterial flora development [24,25,31,32,34-36]. A higher concentration of those acids was noted in the caecum content from hens fed with zinc as an organic and biological preparation as compared to the inorganic form. With reference to above, we surmise that using zinc in diet as an organic chelate complex or as a preparation obtained via biosorption can have an influence on limiting the pathogenic bacteria population and increasing the amount of energy obtained during fermentation processes. The influence of SCFAs on a microbial population requires further investigation.

The *in vitro* studies shown that using zinc as an organic chelate complex or as a preparation obtained via biosorption have a better influence on fermentation products in a hen's caecum than inorganic salts [20,21]. Our results confirm studies conducted by other authors who noted that micronutrients fed to hens as organic compounds have better digestibility than inorganic salts [37]. In *in vivo* studies zinc used as an organic chelate complex had the best influence on the level and profile of volatile fatty acids compared to other forms of this mineral, though it should be mentioned that using biological preparation obtained via biosorption did not have a negative influence on the processes studied. Taking into consideration the benefits following the use of supplements obtained via biosorption, it should be deemed appropriate to introduce them into livestock production, as this could have a significant influence on the possibilities of supplementing micronutrients deficient in human nutrition.

REFERENCES

1. Welch RM, Graham RD: Breeding for micronutrients in staple food crops from a human nutrition perspective. *J Exp Bot,* 55, 353-364, 2004.

2. Kania M, Mikolajewska D, Marycz K, Kobielarz M: Effect of diet on mechanical properties of horse's hair. *Acta Bio and Biom,*11 (3): 55-57, 2009.

3. Hotz C, Brown KH: Assessment of the risk of zinc deficiency in populations and options for its control. *Food Nutr Bull*, 25, 94-204, 2004.

4. Mubarik A, Tsou SCS: Combating micronutrient deficiencies through vegetables - A neglected food frontier in Asia. *Food Policy*, 22, 17-38, 1997.

5. Prakash S: Incorporating nutritional considerations when addressing food insecurity. *Food Secur,* 1, 431-440, 2009.

6. Michalak I, Chojnacka K, Dobrzański Z, Górecki H, Zielińska A, Korczyński M, Opaliński S: Effect of macroalgae enriched with microelements on egg, quality paramateres and mineral contents of eggs, eggshell, blood, feathers and droppings. *J Anim Physiol Anim Nutr,* 95, 374-387, 2011.

7. Janeczek M, Chojnacka K, Chrószcz A, Zielińska A: New generation of dietary supplements with microelements for livestock: Possibilities and prospects. **In,** Javed MT (Ed): Livestock: Rearing, Farming Practices and Diseases. 17-44, NOVA Science Publisher, New York, 2011.

8. Kaya A, Kaya H, Macit M, Çelebi Ş, Esenbûga N, Yörük MA, Karaoğlu M: Effects of dietary inclusion of plant extract mixture and copper into layer diets on egg yield and quality, yolk cholesterol and fatty acid composition. *Kafkas Univ Vet Fak Derg,* 19 (4): 673-679, 2013. DOI: 10.9775/kvfd.2013.8644

9. Michalak I, Chojnacka K, Marycz K: Using ICP-OES and SEM-EDX in biosorption studies. *Microchimica Acta,* 172 (1-2): 65-74, 2011.

10. Witkowska Z, Chojnacka K, Korczyński M, Świniarska M, Saeid A, Opaliński S, Dobrzański Z: Soybean meal enriched with microelements by biosorption - A new biological feed supplement for laying hens. Part I. Performance and egg traits. *Food Chem,* 151, 86-92, 2014.

11. Nicholson FA, Chambers BJ, Williams JR, Unwin RJ: Heavy metal contents of livestock feeds and animal manures in England and Wales. *Bioresour Technol,* 70, 23-31, 1999.

12. Mosenthin R, Broz J: Mineral digestibility and environmental issues. Efficacy and interactions of phytases. *Livestock Scien*, 134, 258-260, 2010.

13. Aksu T, Özsoy B, Saripinar Aksu D, Yörük MA, Gül M: The effects of lower levels of organically complexed zinc, copper and manganese in broiler diets on performance, mineral concentration of tibia and mineral excretion. *Kafkas Univ Vet Fak Derg,* 17 (1): 141-146, 2011.

14. Kania M, Mikolajewska D, Marycz K, Kobielarz M: Effect of diet on mechanical properties of horse's hair, *Acta Bioeng Biomech*, 11 (3): 53-57, 2009.

15. Sarıpınar Aksu D, Aksu T, Özsoy B: The effect of lower supplementation levels of organically complexed minerals (zinc, copper and manganese) versus inorganic forms on haematological and biochemical parameters in broilers. *Kafkas Univ Vet Fak Derg,* 16 (4): 553-559, 2010.

16. Chojnacka K: Biosorption of Cr (III) ions by eggshells. *J Hazardous Materials,* B121: 167-173, 2005.

17. Chojnacka K: Using biosorption to enrich the biomass of seaweeds from the Baltic Sea with microelements to produce mineral feed supplement for livestock. *Bio Eng J,* 39, 246-257, 2008.

18. Witek-Krowiak A, Podstawczyk D, Chojnacka K, Dawiec A, Marycz K: Modelling and optimization of chromium (III) biosorption on soybean meal, *Central European J Chem*, 11 (9): 1505-1517,2013.

19. Janeczek M, Chojnacka K, Nezir YT, Pecka E, Czerski A, Witkowska Z, Chrószcz A, Korczyński M: The Effect of Cu^{+2}, Fe^{+2} and Cr^{+3} in Mineral Additives Enriched with Biosorption Process Form on Chosen Parameters of *in vitro* Caecal Fermentation in Laying Hens (Lohman Brown). *J Anim Vet Adv,* 21, 3991-3998, 2012.

20. Kosiorowska A, Puggaard L, Hedemann MS, Sehested J, Jensen SK, Kristensen NB, Kuropka P, Marycz K, Vestergaard M: Gastro-intestinal development of dairy calves fed low- or high-starch concentrate at two milk allowances. *Animal* 5 (2): 211-219, 2011.

21. Starosta R, Brzuszkiewicz A, Bykowska A, Komarnicka UK, Bazanow B, Florek M, Gadzala L, Jackulak N, Krol J, Marycz K: A novel copper(I) complex, [CuI(2,2 ‘-biquinoline)P(CH2N(CH2CH2)(2)O)(3)] - Synthesis, characterisation and comparative studies on biological activity. *Polyhedron* 50 (1): 481-489, 2013.

22. Kalinski K, Marycz K, Czogala J, Serwa E, Janeczek W: An application of scanning electron microscopy combined with roentgen microanalysis (SEM-EDS) in canine urolithiasis. *J Electron Microsc (Tokyo)*, 61 (1): 47-55, 2012.

23. StatSoft Polska: Kraków, Statistica 9.0 software.

24. Ricke SC, Park SY, Moore RW, Kwon YM, Woodward CL, Byrd JA, Nisbet DJ, Kubena LF: Feeding low calcium and zinc molt diets sustains gastrointestinal fermentation and limits *Salmonella Enterica* serovar *enteritidis* colonization in laying hens. *J Food Safety,* 24, 291-308, 2004.

25. Van Der Wielen PWJJ, Biesterveld S, Notermans S, Hofstra H, Urlings BAP, Van Knapen F: Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth. *Am Soc Micr,* 66 (6): 2536-2540, 2000.

26. Józefiak D, Rutkowski A, Martin SA: Carbohydrate fermantation in the avian ceca: A review. *Anim Food Sci Tech*, 113, 1-15, 2004.

27. Lan Y, Williams BA, Verstegen MWA, Patterson R, Tamminga S: Soy oligosaccharides *in vitro* fermentation characteristics and its effect on caecal microorganisms of young broiler chickens. *Anim Food Sci Tech*, 133, 286-297, 2006.

28. Chotikatum S, Kramomthong I, Angkanaporn K: Effects of medium chain fatty acids, organic acids and fructo-oligosaccharide on Cecal *Slmonella enteritidis* colonization and intestinal parmeters of broilers. *Thai J Vet Med,* 39 (3): 245-258, 2009.

29. Marunek M, Rada V: Age effect on *in vitro* fermentation pattern and methane production in the caeca of chickens. *Physiol Res*, 47, 259-263, 1998.

30. Saengkerdsub S, Woo-Kyun K, Robin C, Anderson DJ, Nisbet S, Ricke C: Effects of nitrocompounds and feedstuffs on *in vitro* methane production in chicken cecal contents and fluid. *Anaerobe,* 12, 85-92, 2006.

31. Donalson LM, Kim WK, Chalova VI, Herrera P, McReynolds JL, Gotcheva VG, Vidanović D, Woodward CL, Kubena LF, Nisbet DJ, Ricke SC: *In vitro* fermentation response of laying hen cecal bacteria to combinations of fructooligosacharide prebiotics with alfalfa or a layer ration. *Poul Sci*, 87, 1263-1275, 2008.

32. Meimandipour A, Shuhaimi M, Soleimani AF, Azhar K, Hair-Bejo M, Kabeir BM, Javanmard A, Muhammad Anas O, Yazid AM: Selected microbal groups and short-chain fatty acids profile in a simulated chicken cecum supplemented with two strains of *Lactobacillus*. *Poul Sci,* 89, 470-478, 2010.

33. Kirgchgessner M, Eder K, Müller HL, Jamroz D: Zur energetichen bewertung von nichtstärke polisacchariden beim geflügel. *J Anim Nutr,* 81, 8151-8155, 1999.

34. Mead GC: Bacteria in the gastrointestinal tract of birds. **In,** Mackie RJ, White BA, Isaacson RE (Eds): Gastrointestinal Microbiology. 216-240, Chapman and Hall, New York, 1997.

35. Tsukahara T, Ushida K: Effects of animal or plant protein diets on cecal fermentation in guinea pigs *(Cavia porcellus)*, rats *(Rattus norvegicus)* and chicks *(Gallus gallus domesticus)*. *Com Bioch Phys Part A*, 127, 139-146, 2000.

36. Bou R, Guardiola FA, Barroeta C, Codony R: Effect of dietary fat sources and zinc and selenium supplements on the composition and consumer acceptability of chicken meat. *Poul Sci,* 84, 1129-1140, 2005.

37. Bao YM, Choct M, Iji PA, Bruerton K: The digestibility of organic trace minerals along the small intestine in broiler chickens. *Asian-Aus J Anim Sci,* 23 (1): 90-97, 2010.