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The bioavailability of different chemical forms of zinc in fattening lambs

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Abstract

The aim of the presented study was an estimation of the zinc bioavailability derived from amino acid complexes with methionine, lysine and glycine in growing lambs. 48 lambs, Polish Merino × Romanowski × Charolaise crossbreed, at age about 10 weeks and average body weight of 20 kg were randomly divided into 4 experimental groups. Animals were kept collectively and fed with concentrate and hay in amount: 0.7-1.2 and 0.3-0.5 kg/day/head, respectively. After rearing period 6 rams from each group with average body weight of about 30 kg were divided into digestibility-balance experiments. At the end of the experiment the blood samples were taken, then from each group 8 lambs were chosen, killed and during dissection tissue samples were taken (liver, pancreas, kidney and rib bone). In tissue samples as well as in fodders and excrements the content of zinc was determined. Obtained data in digestibility experiments allowed on calculation of apparent absorption and retention of zinc and the level of Zn determined in tissues' samples were used to the estimation of bioavailability of zinc from different ones applied in experiment sources.

Apparent absorption and retention of zinc were higher ($P \leq 0.01$) in lambs receiving in mixtures amino acid complexes of zinc than in animals from the control group. The higher content of zinc in soft and hard tissues of lambs which received organic forms of zinc might indicate better assimilation and bioavailability of zinc from these forms than from oxide. Among tested organic forms of zinc, the zinc-lysine complex was characterized as having the most advantageous property.

Keywords: zinc, amino acid chelates, lambs, apparent absorption, retention

Zusammenfassung

Die Bioverfügbarkeit der verschiedenen chemischen Formen von Zink bei Mastlämmern

Das Ziel der vorliegenden Studie war eine Abschätzung der Bioverfügbarkeit von Zinkaminosäurekomplexen mit Methionin, Lysin und Glyzin für wachsende Lämmer. 48 Mischlingslämmer (Polnisch Merino × Romanowski × Charolaise) im Alter von 10 Wochen und mit 20 kg Körpergewicht wurden nach dem Zufallsprinzip 4 Versuchsgruppen zugeteilt. Die Tiere wurden in Gruppen gehalten und mit Kraftfutter und Heu in den Mengen: 0,7-1,2 und 0,3-0,5 kg/Tag/Tier gefüttert. Sechs Böcke aus jeder Gruppe mit einem durchschnittlichen Körpergewicht von 30 kg wurden den Verdaulichkeit-Balance-Experimenten zugeteilt. Am

Ende des Versuchs wurden Blutproben entnommen. Dann wurden aus jeder Gruppe 8 Lämmer ausgewählt, geschlachtet und ihnen Gewebeproben (Leber, Bauchspeicheldrüse, Nieren und Rippenknochen) entnommen. In den Gewebeproben, im Futter und in den Exkrementen wurde der Zinkgehalt bestimmt. Die scheinbare Absorption und Retention von Zink und der Gehalt von Zink in den Gewebeproben, sowie die Abschätzung der Bioverfügbarkeit von Zink aus verschiedenen Quellen wurden ausgewertet.

Die scheinbare Absorption und Retention von Zink war höher ($P \leq 0{,}01$) bei Lämmern, deren Futtermischungen Zinkaminosäurenkomplexe enthielten, als bei den den Kontrolltieren. Der höhere Zinkgehalt im Weich- und Hartgewebe der Lämmer, die mit organischen Formen von Zink gefüttert wurden, könnte auf eine bessere Aufnahme und Bioverfügbarkeit dieser gegenüber Zinkoxiden hindeuten. Von den gestesteten organischen Zinkformen besaß der Zink-Lysin-Komplex die günstigsten Eigenschaften.

Schlüsselwörter: Zink, Aminosäuren Komplexe, Lämmer, scheinbare Absorption, Retention

Introduction

The bioavailability of minerals from diet depends on its content, chemical form, solubility and interactions with other components of ratio (Ammerman 1995, Ammerman *et al.* 1998, House 1999, Underwood & Suttle 1999). Kirchgessner *et al.* (1993), Skrivan *et al.* (2005) affirm that, in adapted conditions, with adequate for animals' requirement of trace elements quantity both the apparent and true absorption are closely correlated with bioavailability. To better reflect on this process the accumulation of particular elements in the target tissues is needed (Miles & Henry 2000), although the continuous circulation of minerals as well as homeostasis mechanisms make the determination of bioavailability difficult (Fredrich 2002). The physiological functions of zinc are numerous. It is required for the functional and structural integrity of more than 300 zinc-depended enzymes and great number of functional zinc proteins. Consequently, worth mentioning is the fact that almost every signalling and metabolic pathway depends on one or more zinc-requiring protein. Zinc plays role in gene expression, appetite control, fat absorption or antioxidant defence. In all species zinc deprivation is characterized by inappetence and retardation of growth, skeletal or reproductive disorders. The late signal of zinc deprivation is thickening, hardening and fissuring of the skin (*parakeratosis*) (Suttle 2010).

The dread of zinc deprivation is being exploited commercially by feed industry, which offers wide variety of mineral additives, including organically bounded trace elements, as compounds for the enhanced bioavailability for numerous »organic« zinc supplements. So far the obtained data, regarding the bioavailability of elements from these forms, is vague and even controversial (Acda & Chae 2002, Suttle 2010).

Therefore, the attempted researches using organically bounded trace elements in animal nutrition are justified, taking into account not only humans and animals safety but also environment protection.

The aim of the presented study was the estimation of zinc bioavailability from amino acid complexes with methionine, lysine and glycine in growing lambs.

Material and methods

Animals, diet and experimental design

The experiment was conducted in Zootechnical Experimental Institute in Pawlowice near Leszno, which belongs to National Research Institute of Animal Production in Krakow. Experimental material constituted of 48 lambs, Polish Merino (25 % of genes) × Romanowski (25 % of genes) × Charolaise (50 % of genes) crossbreed, which at age about 10 weeks and average body weight of 20 kg were randomly divided into 4 experimental groups (12 heads per each – 6 ♀ and 6 ♂). These animals were kept collectively (group-pen) and fed with concentrate mixture and meadow hay. Concentrate mixture was prepared with the usage of barley meal (36.7 %), wheat meal (30.0 %), extracted soybean meal (10.0 %), extracted rapeseed meal (10.0 %), wheat bran (10.0 %), pasture salt (0.3 %), pasture chalk (1.0 %) and mineral-vitamin premix (2.0 %). Experimental groups were diversified considering zinc forms included in premixes and added to concentrates.

The zinc source in control group was zinc oxide, while in experimental groups – organic forms of this element: zinc-glycine (gr. II), zinc-lysine (gr. III) and zinc-methionine (gr. IV) were added. The content of basic nutrients and minerals in fodders was determined according AOAC (2000) (Table 1).

Table 1
The chemical composition of concentrate mixture and meadow hay used in experiment

Item	The content of individual constituent	
	Concentrate mixture	Meadow hay
Dry matter, g	886.5	921.1
Crude protein, g (g/DM)	172.9 (195.0)	94.4 (102.5)
Crude fat, g (g/DM)	23.4 (25.9)	12.5 (13.6)
Crude fibre, g (g/DM)	59.0 (66.5)	341.3 (370.5)
Crude ash, g (g/DM)	48.7 (54.9)	39.8 (43.2)
NFE, g (g/DM)	755.4 (852.1)	433.1 (470.2)
Minerals		
Phosphorus (P), g (g/DM)	4.4 (4.9)	1.2 (1.3)
Calcium (Ca), g (g/DM)	5.5 (6.2)	3.8 (4.1)
Magnesium (Mg), g (g/DM)	2.4 (2.7)	2.0 (2,2)
Zinc (Zn), mg (mg/DM)	66.4 (74.9)	23.5 (25,5)
Copper (Cu), mg (mg/DM)	10.5 (11.8)	8.7 (9.4)

The digestibility-balance experiment

During the experiment the same fodders were used but the amount of concentrate mixtures and meadow hay given to animals depended on the age of lambs and their feed intake and ranged: 0.7-1.2 and 0.3-0.5 kg/day/head, respectively. After rearing period six rams from every group, with average body weight of about 30 kg were divided into digestibility-balance experiments and fed with concentrate mixture and meadow hay in amounts: 1.2 and 0.2 kg/day/head, respectively. The content of zinc in lambs rations was similar and was about 85 mg/kg of DM. Rams were kept individually in digestible cages, without the possibility of movement but at the same time they were able to see each other. The preliminary period

of digestible-balance experiments was 5 days and the collection period was 7 days. During collection period the amounts of fodders intake and the remains of feeds as well as amounts of excrements (faeces and urine) were controlled (Ziołecka 1969). Daily collected excrements were weighted and the 5 % of them were taken to storage sample. Faeces were frozen and urine preserved with 5 % sulphuric acid and kept in refrigerator. After digestible-balance experiment rams got back to their pens-groups.

Blood and tissues' samples collection

At the end of the experiment, when animals achieved average body weight of about 35 kg the blood samples were taken, and in the blood serum, after previous wet mineralization, the zinc content was determined (AOAC 2000) with the usage of atomic absorption spectrophotometer Varian. From each group 8 lambs (4 ♀ and 4 ♂) were chosen, killed and during dissection tissue samples were taken (liver, pancreas, kidney and rib bone). According to the AOAC methodology, in tissue samples as well as faeces and urine, the content of zinc was determined after previous wet mineralization with atomic absorption spectrophotometer Zeiss (AAS-3).

Obtained data, in digestibility experiments, allowed on calculation of apparent absorption and retention of zinc. The level of Zn determined in tissues' samples was used to estimate the bioavailability of zinc from different ones applied in experiment sources.

Statistical analysis

All obtained data was statistically analysed with one-factorial variance analysis and the significance of differences was estimated by multiple range Duncan test using of software Statistica 7 (StatSoft 2004).

Results

During rearing period the average daily gains of lambs were similar and achieved about 250 g (Table 2), the feed utilization for 1 kg of body mass gain was similar in all experimental groups.

Table 2
Daily body weight gain of sheep during experiment period and feed utilization

Item		Feeding groups			
		I - ZnO	II – Zn-Gly	III – Zn-Lys	IV – Zn-Met
Average daily gain, g	$\bar{x}$	250^{a}	240^{a}	250^{a}	250^{a}
	SD	±20	±20	±30	±30
Feed utilization, kg/	$\bar{x}$	5.5^{a}	5.6^{a}	5.3^{a}	5.5^{a}
1 kg body weight gain	SD	±1.43	±1.16	±1.27	±1.71

^{A,B}Values in the rows with different letters differ significantly ($P \leq 0.01$). ^{a,b}Values in the rows with different letters differ significantly ($P \leq 0.05$).

The zinc amount intaken by lambs during digestible-balance experiment in all groups was similar (Table 3), however the level of zinc excreted by animals from experimental group (II-IV) differed significantly ($P \leq 0.01$). The animals receiving zinc in organic forms excreted

less zinc in faeces ($P \leq 0.05$) and in urine ($P \leq 0.01$) than animals that received mineral-vitamin mixture with zinc oxide. Thus, the apparent absorption and retention of zinc were higher ($P \leq 0.01$) in lambs receiving mixtures containing amino acid complexes of zinc than in animals from the control group (Table 3). The highest apparent absorption and retention of zinc were stated in lambs receiving mineral mixture with zinc-lysine (70.2 % and 56.94 mg/day/head, respectively), whereas in animals receiving mixtures with zinc-glycine and zinc-methionine these values were lower.

Table 3
Apparent absorption, % and retention of zinc, mg/day/head

Item	Experimental groups			
	I – ZnO-control	II – Zn-Gly	III – Zn-Lys	IV – Zn-Met
Zn - intaken, mg	84.40^{a}	81.17^{a}	81.07^{a}	80.06^{a}
Zn - excreted, mg				
in faeces	40.25^{a}	23.63^{b}	21.82^{b}	24.08^{b}
in urine	9.80^{Aa}	6.21^{Bb}	2.33^{Cc}	3.88^{Cd}
total	50.01^{A}	29.84^{B}	24.15^{C}	27.96^{CB}
Retention of Zn, mg/day/head	34.39^{A} ±1.55	51.33^{B} ±8.45	56.94^{B} ±5.38	52.10^{B} ±6.61
Apparent absorption, %	40.7^{Aa} ±1.84	63.2^{Bb} ±6.42	70.2^{Bc} ±3.69	65.0^{Bbc} ±4.18

^{A,B}Values in the rows with different letters differ significantly ($P \leq 0.01$), ^{a,b}Values in the rows with different letters differ significantly ($P \leq 0.05$)

These applied zinc compounds had an influence, however statistically insignificant, on the content of this trace element in blood serum. Lambs from the control group receiving mineral-vitamin mixture with zinc oxide had a lower zinc level (17.30 µmol/l) in blood serum, than animals from groups which received organic forms of zinc – amino acid complexes (Table 4).

Table 4
The concentration of zinc in blood serum and selected tissues of experimental lambs

Item	Experimental groups			
	I-ZnO	II – Zn-Gly	III – Zn-Lys	IV – Zn-Met
Blood serum, µmol/l	17.30^{a}±1.82	17.81^{a}±±2.27	18.92^{a}±1.29	17.81^{a}±1.89
Liver				
mg/kg dry matter	153.0^{a}±18.23	153.8^{a}±15.23	156.8^{a}±20.16	153.1^{a}±14.14
mg/kg fresh matter	47.4±5.65	47.7±4.72	48.6±6.25	47.4±4.38
Pancreas				
mg/kg dry matter	141.2^{a}±5.56	142.7^{a}±9.06	144.1^{a}±9.43	141.3^{a}±8.15
mg/kg fresh matter	38.0±1.50	38.4±2.44	38.8±2.54	38.0±2.19
Kidney				
mg/kg dry matter	97.9^{a}±4.8	99.1^{a}±18.76	101.2^{a}±7.87	98.0^{a}±7.81
mg/kg fresh matter	20.5±1.01	20.8±3.94	21.3±1.65	20.6±1.64
Rib bone				
mg/kg ash	172.7^{a}±16.01	184.8^{a}±6.59	187.2^{a}±12.03	174.9^{a}±10.98
mg/kg dry matter	155.5±6.53	166.3±15.85	168.5±11.91	157.4±10.87

^{a}Values in the rows with different letters differ significantly ($P \leq 0.05$).

The highest zinc level in blood serum of lambs which received zinc-lysine (18.92 µmol/l) was stated; whereas the zinc level in blood serum of lambs receiving zinc glycine and methionie was similar (about 17.81 µmol/l).

The organic forms of zinc – amino acid complexes had a positive effect on accumulation of this microelement in selected soft and hard tissues; however these differences were statistically insignificant. The higher content of zinc was stated in liver, pancreas, kidney and rib bone from those lambs which obtained mineral-vitamin mixtures with amino acid complexes of zinc (groups II – IV), than in tissues of lambs which obtained zinc as oxide. The content of zinc in lambs' tissues was diversified and depended on the kind of applied in mixtures amino acid complexes. The highest level of zinc in all examined tissues in lambs which obtained zinc-lysine was stated (Table 4). The highest apparent absorption and retention of zinc as well as the level of zinc in blood serum and tissues of lambs which obtained mineral-vitamin mixtures with amino acid complexes of zinc indicated on the trend of slightly better utilization of zinc from organic forms than from oxide.

Discussion

The influence of different form of zinc applied in mixtures for lambs was not proven on gaining their body weight. The average daily gains for lambs during rearing period were similar – about 250 g/day. According to available literature it could be stated that the zinc level in the rations had no influence on the live body mass gain or feed consumption (Pond 1983). The zinc deprivation or inadequate balance only could lead to loss of appetite and decrease of body mass gain which are result of insufficient zinc amounts for normal growth and development (Suttle 2010). There was stated that application of different forms of zinc source had no influence on feed utilization, it was similar in animals from all groups. However, Haryanto *et al.* (1994) in experiments on sheep indicated that application in their daily rations of zinc-methionine had a positive effect on feed utilization. Such effect could be explained by the influence of zinc on fat and protein utilization (Suttle 2010).

The application in rations for fattening lambs organic forms of zinc, especially zinc-lysine, positively influenced the utilization of this element by animals. Apparent absorption and retention of zinc in lambs which obtained organic forms were higher in comparison to animals received this trace element as oxide. On the contrary, Spears (1989) in experiments on fattening lambs indicated that apparent absorption of zinc from zinc-methionine and oxide was similar. However, the zinc excretion in urine was lower for lambs which received zinc-methionine, what caused the increase of zinc retention. Kinal *et al.* (2003) and Kinal (2005b) substituted in mineral mixtures for lambs salts of zinc and magnesium with amino acid chelates of these elements indicated that those elements were better utilized from organic forms. The above was confirmed by higher apparent absorption and retention of those elements. Moreover, the comparative research of Słupczynska *et al.* (2007) indicated that the more efficient source of zinc for growing lambs was zinc-glycine, than zinc oxide, what was confirmed by higher apparent absorption and retention of this element from the organic form.

The higher zinc level in blood serum and selected tissues of lambs which received the organic forms of zinc stated better zinc availability from this form than from oxide. Ryan *et al.* (2002) used as indicator of availability of zinc's level in blood serum, stated that zinc-bioplex was

better available zinc source in comparison with sulphate. The higher content of zinc in blood serum lambs and cows after application of organic forms of this element (bioplexes) in comparison with inorganic source of zinc indicated Kinal *et al.* (2003, 2005a,2005b) and Strusińska *et al.* (2003). On the other hand, Spears (1989) had not indicated the influence of zinc form (Zn-methionine vs. zinc oxide) in lambs on its level in blood serum. As Spears (1989) stated the increase of zinc amount in tissues with application of organic forms of this element did not depend only on zinc source but also on zinc level in ratio. Rojas *et al.* (1995) used in rations for lambs zinc oxide and zinc sulphate and zinc complexes with methionine and lysine indicated that concentrations of zinc in soft tissues of lambs (kidney, liver, pancreas) which received organic forms of this element were higher than in animals which received sulphate and oxide. Kincaid *et al.* (1997) indicated that application in calves' nutrition of zinc-lysine and zinc-methionine in amount of 300 mg of Zn/kg of feed caused the increase of content of this element in liver in comparison with animals received zinc oxide. The higher zinc content in calves tissues (Wright & Spears 2001) and lambs (Cao *et al.* 2000) indicated also comparison the availability of zinc from zinc-proteinate and zinc sulphate. The application of different forms of zinc changed the pathways of absorption of this element. Zinc bounded with amino acids is absorbed in amino acid absorption pathways, and as this complex it could be deposited together with amino acid into the proteins of tissues increasing the element concentration. The increase of minerals concentration in tissues could be considered in aspects of functional food meaning »minerals-enriching«.

In conclusion, the application in mineral-vitamin mixtures amino acid complexes of zinc caused the increase of apparent absorption and retention of zinc, and improved the mineral status of lambs - which was indicated by higher zinc level in blood serum. The higher zinc content in soft and hard tissues of lambs which received organic forms of zinc could indicate the better assimilation and bioavailability of zinc from these forms than from oxide. Among tested organic forms of zinc, the zinc-lysine complex was characterized by the most advantageous property.

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