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UTILISATION OF IRON METHIONATE IN BROILER CHICKENS COMPARED TO IRON SULPHATE

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Abstract

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35-day tests with broiler chickens treated with Bulgarian iron methionate administered through the food compared to the iron sulphate (heptahydrate) in doses of 60 ppm and 300 ppm were conducted. The test involved 55 broiler chickens aged 10 days, divided into 5 groups of 11 chickens. The tests started on May 21st, 2012 and continued 35 days. The basic mixed feed was prepared by using a recipe for growing broiler chickens and an average content of 85.6 ± 2.4 mg Fe/kg. The appetite, health status (clinical one) and individual weight of the chickens were controlled. On the 15th day samples of the liver from three euthanized chickens of each group were taken for histological and chemical studies. On the 35th day four more chickens of each group were subjected to the same studies. The liver samples intended for chemical analysis were frozen at-18°C and after 22 days were thawed out and tested for iron content by optical emission spectrophotometer ICP-OES 715-S. Samples of the cloacal content were taken from the chickens euthanized on the 15th and 35th day. They were also frozen and then thawed out, dried and analysed for iron content by using atomic absorption spectrophotometer equipped with graphic cuvette, model Spectra AA 800. The statistical results were processed by three different methods - parametric (ANOVA one-way), non-parametric (Mann-Whitney U-test) method and by using the tables of Student-Fisher. During the test period no clinical symptoms and signs of disease or mortality were found in all treated chickens; there were no pathomorphological changes in the liver of the chickens. In general, the utilisation was more favourable for the iron methionate compared to the iron sulphate. It was better expressed in the low concentration (60 ppm) - a steady growth, trend for better deposition in the liver and significantly smaller amount (up to two times) of iron in the cloacal content (beneficial for the environment). The iron deposited in the liver of the treated chickens was from 40 to 60% more than that in the control ones.

Key words: iron methionate, iron sulphate, chickens, utilisation

Introduction

The iron is a nutrient with high demand, especially in the fast-growing organisms (Dilov, 1985). In respect of the broiler chickens, it is important for their productivity. Despite their rapid growth, they rarely suffer from iron deficiency anaemia, since the protein components of their feed contain high amount of iron (Marinov, 2011). The aplastic (viral) anaemia, which the chickens suffer from, does not depend on the iron (Miller et al., 2005). In 1929, Elvehjem and Hart first discussed the chickens' need for iron. Later on, in 1968, Davis et al. presented scientific evidence that the chicken need for iron reached from 75 to 150 mg / kg feed. In 1987, Vahle and Van Klooster suggested that the broiler chicken feed should con-

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tain 80 ppm iron and an additive of 20 ppm or 100 ppm iron. In 1994, NRC concluded, based on multiple studies, that the need for iron in the broiler chicken feed was 80 ppm throughout the fattening period. The most commonly recommended amount of any additive of the iron inorganic salts in the broiler chicken feed ranges from 25 to 40 ppm (Surodzhiyska, 1996; Lyons 2006). The increased dose of iron in the oral administration reduces its absorption (defensive response) (Pollack et al., 1964 and Edwarts and Washburn, 1968). In the broiler chickens, the iron is deposited mostly in the liver, but also in the spleen, tibia, thoracic and thigh muscles (Shinde et al., 2011). The iron, which is not absorbed, is excreted in the cloacal content.

The disadvantages of the inorganic salts of the iron (iron sulphate, ferric oxide, etc.) in the feed additives, such as low absorption, poor bioactivity, low adaptability in the homogenisation with the mixed feed, contamination of the environment by the excrements, etc., are well known. Options to replace them by organic iron compounds have been searched for. Products (chelate complexes) containing iron and one or more amino acids (US Patent # 4,067, 994-Fe-methionine complex, US Patent # 5,698, 724-Fe-amino acid complex) have been produced. The scientific data about their advantages in the broiler chickens compared to that of $FeSO_4.7H_2O$ are incomplete (Cao et al., 1996 and Shinde et al., 2011).

In Bulgaria, methionine iron complex was synthesized. It was tested and showed positive results in sows and pigs (Petrichev, 2006). Moreover, pharmacokinetic study in broiler chickens was conducted and some rheological methods were applied (Arnaudova-Matey et al., 2013).

The purpose of these studies was to contribute to the assessment of the Bulgarian iron methionate by determining the iron deposited in the liver and its presence in the cloacal content in broiler chickens treated with iron methionate and iron sulphate (heptahydrate) included in feed.

Materials and Methods

Iron methionate (Fe-met.) synthesized by the Organic Chemistry Department at the University of Chemical Technology and Metallurgy – city of Sofia was studied. It contained 13.3% iron and 34% methionine. The product was analysed by using atomic absorption method. Its molecular weight was 426. Ferrous sulphate ($FeSO_4.7H_2O$) produced by the company Merck, containing 20% iron, was used for comparison.

Fifty five broiler chickens (four of them linear hybrid ROSS-IKOV) of both genders at the age of 10 days were involved in this test, which began on May 21st, 2012. The chickens were randomly selected, divided into 5 groups of 11 chickens and then set in cages with galvanized net and automatic feeders and drinkers. They were treated during 35 days, as follows: I Group - 60 ppm iron methionate, II Group - 300 ppm iron methionate, III Group - 60 ppm iron sulphate, IV Group - 300 ppm iron sulphate, V Group – control one (without additive of iron). The basic feed (with no added iron), used for feeding the treated and control chickens, was prepared by using a recipe for growing broiler chickens and contained an average of 5 randomly selected samples 85,6 ± 2,4 mg Fe / kg. The iron containing products were added and mixed with the feed manually. The appetite, health status (clinical one) and individual weight of the chickens were controlled daily. On the 15th day after the beginning of the test 3 chickens of each group were euthanized and bled to death and on the 35th day four more chickens of each group were euthanized and bled to death. The chickens were subjected to post-mortem tests and samples were taken from the liver (after removal of the gallbladder) for histological and chemical studies. The liver samples intended for chemical analysis were frozen at -18^{0}C, after 22 days were thawed out, and tested for iron content through optical emission spectrophotometer ICP-OES 715-S (Varian Medical Systems, Palo Alto, Canada). The samples taken from the basic feed were analysed for iron content by using the same method. Samples of the cloacal content were taken from the chickens euthanized on the 15th and 35th day. They were also frozen and then thawed out, dried and analysed for iron content by using atomic absorption spectrophotometer equipped with graphic cuvette, model Spectra AA 800 (Varian Medical Systems, Palo Alto, Canada).

The individual weights of the treated and control chickens were processed by two different statistical methods – the parametric (ANOVA one way) and non-parametric (Mann-Whitney U test) methods. The results of the analyses of the liver and cloacal content were processed by using variations and statistical methods; the tables of Student-Fisher and the confidence limits determined the significance of the differences – through the method described by Belenykiy (1963).

Results and Discussion

The observations did not show any clinical symptoms and signs of disease of the chickens from the treatment and control groups. Mortality was not established during the period of the experiment. The chickens had no weakness in the limbs due to the high concentrations of the iron (300 ppm) in both compounds, which was observed by Vahl et al. (1987) in high doses of iron. Other authors, however, did not find any evidence of toxic signs and manifestations in the chickens treated with feed containing from 500 to 1600 ppm iron (Southern and Baker, 1982). During the first 15 days of the

test (Table 1) the best growth was shown in the chickens from the control group (statistically significant difference at P<0.05, compared to the chickens from I, II, III and IV Groups). Obviously, the 85.6 ppm content of iron in the basic food was sufficient to maintain adequate productivity in the broiler chickens during their growth period, which was confirmed by the studies and requirements of NRC (1994), (Davis et al., 1968 and Vahl et al., 1987). During this period of the test, the most rapid growth was observed in the chickens treated with feed containing 60 ppm $FeSO_4$ (III Group), which was significantly different from that observed in the chickens from I Group (60 ppm iron methionate) - 1115 ± 37 g versus 901 ± 23 g - and insignificant compared to the growth of the chickens from the control group (P>0.05). No significant difference in the weight was observed in the chickens, administered 60 and 300 ppm iron methionate. It is known that the increase in the dose of iron in the chicken food does not contribute to the higher productivity (Mcghee et al., 1965; Vahl et al., 1987 and Cao et al., 1996). This was what we observed in the assessment of the chicken growth on the 35th day of the test in terms of $FeSO_4$ - 1604 ± 76 g at 60 ppm iron versus 1300 ± 83 g at 300 ppm iron. In the opinion of Vahl et al. (1987) if the iron content in the basic feed is 107 ppm the weight of the chickens increases after adding up to 60 ppm $FeSO_4.7H_2O$. No effect of the high doses was detected in the chickens administered iron methionate. The chickens from the control group maintained steady growth but lagged in weight compared to the chickens from II and III Groups. The data about the weight of the chickens included in Table 1 give rise to discussion that when adding iron sulphate the weight is more unevenly distributed at the end of the test compared to that of the chickens treated with iron methionate - Sx 76 and 83 versus 55. This manifestation of the iron sulphate may be explained with the poorer rheological parameters set by us through technology testing and with the advantages of the iron methionate in terms of some pharmacokinetic parameters (Arnaudova-Matey et al., 2013).

The deposition of the iron in the liver of the chickens from all treatment groups significantly exceeded in amount (P> 0.05) that of the chickens from the control group (Table 2). The difference was best expressed in the chickens from I Group (60 ppm Fe-meth.) compared to those from V Group (plus 65.8%). Moreover, in the other treatment groups, the iron content deposited in the liver exceeded that in the control chickens by over 40%. On the 15th day of the test higher values of the iron in the liver were found in the chickens treated with feed containing iron methionate, compared to the chickens administered iron sulphate. On the 35th day, this difference was removed (high dose effect). Similar results concerning the iron deposition in liver were also observed by other authors when comparing inorganic compounds with iron organic complexes (Vahl et al., 1987 and Cao et al., 1996). On the 35th day, the values of the iron in the cloacal content (Table 2), only in the chickens from I Group (60 ppm Fe-meth.) were similar to those found in the control chickens (P>0.05). In the other three treatment groups the iron in the cloacal content

Table 1
Changes in the weight of the broiler chickens, kg

Groups	Beginning n=8 x±Sx	15th day n=8 x±Sx	35th day n=8 x±Sx
I Group 60 ppm Iron methionate	0.182±0.007	0.901±0.023 $u_2;a_3;b_1$	1.377±0.055 b_1
II Group 300 ppm Iron methionate	0.178±0.006	0.903±0.026 $u_1;a_2$	1.528±0.055 u_1; b_2
III Group 60 ppm Fe SO_4	0.181±0.007	1.115±0.037	1.609±0.076
IV Group 300 ppm Fe SO_4	0.177±0.007	0.976±0.064 a_1	1.300±0.083
Control group	0.165±0.008	1.126±0.058	1.488±0.057

Statistically significant difference compared to the control group calculated through the non-parametric method Mann-Whitney U test;
u_1 at p< 0.05; u_2 at p< 0.01.
Statistically significant difference compared to the control group calculated through the parametric method one way ANOVA;
a_1 at p< 0.05; a_2 at p< 0.01.
b_1 - statistically significant difference between I Group and III Group;
b_2 - statistically significant difference between II Group and V Group

was significantly elevated (P<0.05); in the chickens administered iron sulphate the iron in the cloacal content was also increased. These values were, as follows: chickens from III Group compared to chickens from I Group + 101.9%; chickens from IV Group compared to chickens from II Group + 21.6%. The lowest rate was observed in the chickens from I Group compared to that from V Group - 12.8% and the highest one – in the chickens from IV Group compared to that from V Group -221.3%.

The studies of some authors that in high doses, upon reaching the maximum capacity of the liver, the deposition of the iron is limited and its excretion from the bile and excrements is increased (Cao et al., 1996 and Nollet et al., 2005), were confirmed. It is known that in the high doses of iron its deposition in the spleen, thoracic and thigh muscles also increases (Seo et al., 2008). The discussed results of the deposition and excretion of the iron in the chickens treated with the comparable products lead to the conclusion that the iron methionate somewhat exceeds in absorption the iron sulphate in case of prolonged administration. This effect is probably related to smoother absorption of the iron methionate, which we found in the previous pharmacokinetic studies (Arnaudova-Matey et al., 2013). The digestive tract should reabsorb the comparable products in different manner. $FeSO_4.7H_2O$ easily dissolves in water (1:2.2) and has relatively little molecular weight (278.2), allowing more rapid diffusion through the intestinal mucosa.

The iron methionate dissolves much more difficult in water and has higher molecular weight (426), which prevents its rapid diffusion through the tissue membranes. It is an amino acid complex and, as other authors suggests (Lyons, 2006), the chelate complexes are reabsorbed with the help of the bearing amino acid and the complex is decomposed in the liver. The data entered and the confidence limits in Figures 1 and 2 show a larger spread in the individual results. Similar to the weight, this result may be explained as by the process of absorption, as well as by both products rheological parameters (their ability to mix with the feed), which is more favourable for the iron methionate (Arnaudova-Matey et al., 2013). Moreover, the iron sulphate, which is an ionizing agent, has a higher reactivity compared to the other products contained in the feed and the digestive tract, which could lead to adverse effects (incompatibility with B vitamins, etc.).

In the post-mortem and histological tests of the liver no visible changes and pathomorphological alterations were observed in the chickens from the five treatment groups (Figure 3).

These observations and the clinical monitoring of the health status and growth of the treated and control chickens showed that the utilisation of the comparable products was not influenced by health factors and other side effects.

Conclusion

According to the objective of our research, we aimed not to trace effects important for chickens' productivity of these comparable products, but to report their side effects, which may affect their utilisation. The results concerning chicken growth, the deposition of the iron in the chicken liver and its presence in the cloacal content demonstrated that the utilisation was generally more favourable for the iron methionate compared to the iron sulphate and better expressed in the low concentration (60 ppm). By the 15th day of the test the iron

Table 2
Concentrations of Fe in the liver and cloacal content of broiler chickens

Groups	Fe in the liver, ppm			Fe in the cloacal content, ppm		
	15th day n=3 x±Sx	35th day n=4 x±Sx	15th day + 35th day n=7 x±Sx	15th day n=2-3* x±Sx	35th day n=4 x±Sx	15th day + 35th day n=6-7* x±Sx
I Group 60 ppm Iron methionate	168.5	224.8±16.7	200.7±42.8	152.2	211.15±11.7	190.8±23
II Group 300 ppm Iron methionate	147.9	199.2±10.1	173.6±13.7	666.6	459±73	446.5±80
III Group 60 ppm Fe SO_4	159.8	188.2±17.8	172±20	501.32	427±85	385.3*±65
IV Group 300 ppm Fe SO_4	139.0	208.2±28.6	173.6±27.5	491.0*	505.8±83	543*±84
V control group	116.0	132.7±8.2	121±11.8	67.64	219.5±23	169*±36

35th day: I, II, III, IV compared to V - $p < 0.05$; II, III, IV compared to I and V - $p < 0.05$; I compared toV – $p>0.05$
I compared to III and II compared to IV – $p >0.05$
15th day + 35th day: I, II, III, IV compared to V - $p < 0.05$ II, III, IV compared to I and V - $p < 0.05$; I compared to II – $p<0.05$;
I compared to II and III compared to IV – $p > 0.05$. III compared to IV– $p > 0.05$

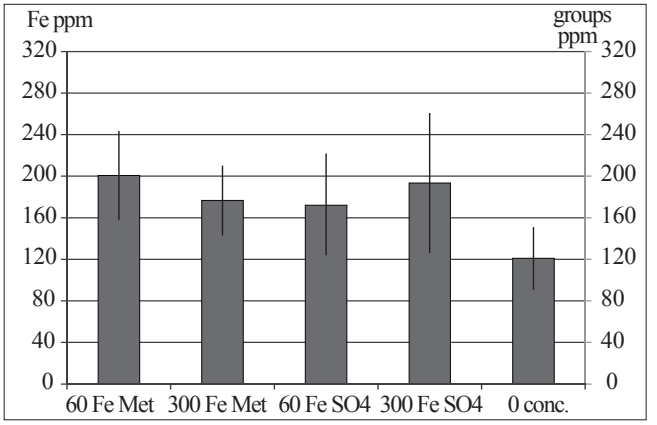

Fig. 1. Summary data about Fe (15th - 35th day in the liver)

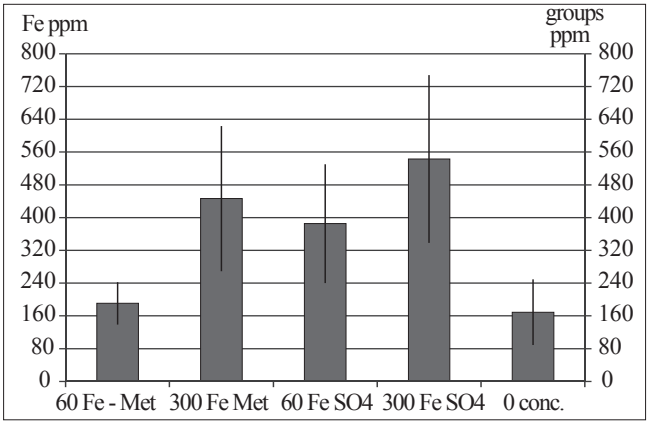

Fig. 2. Summary data about Fe (15th - 35th day in the cloacal content)

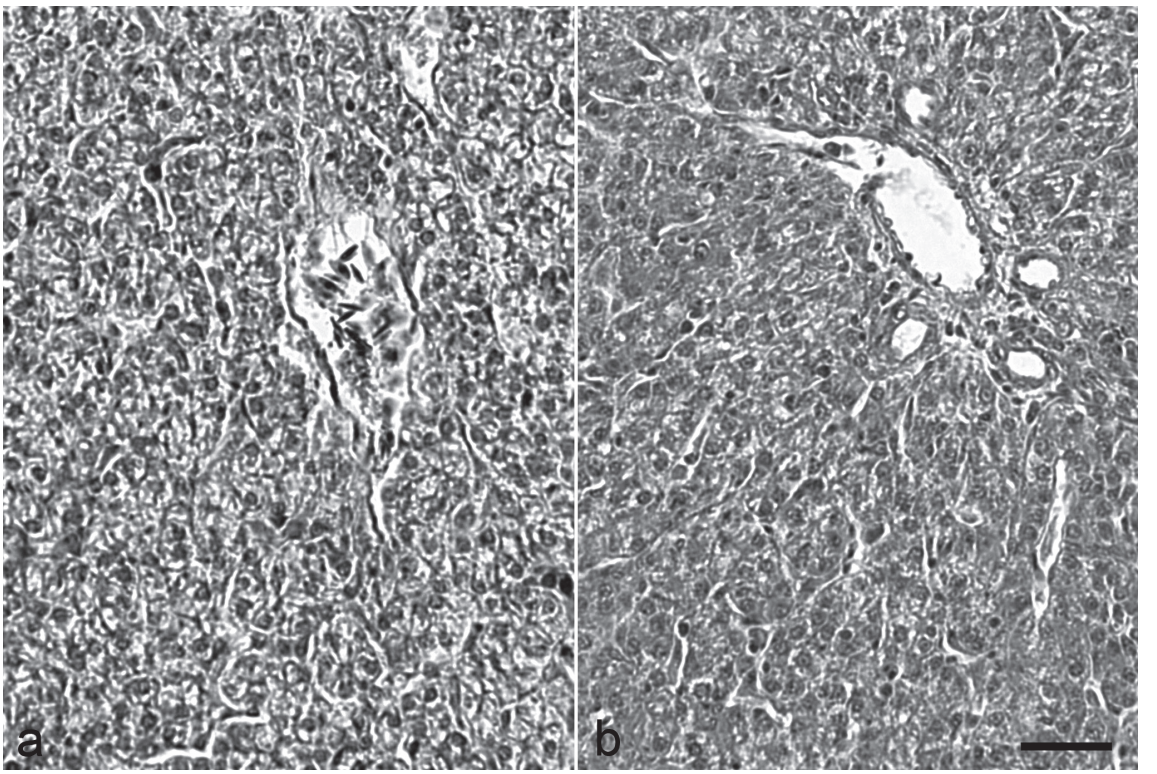

Fig. 3. Chickens' livers, 35th day: (a) 300 ppm Fe- sulfate in feed; (b) 300ppm Fe- met. in feed. Normal appearance of the livers. H&E. Scale bar 30μm.

content in the basic feed (85.6 ± 2.4 ppm) was enough to meet the requirements for productivity in the control chickens, but in the later period their growth slowed. The chickens treated with iron methionate gained weight on a regular basis, including in a dose of 300 ppm Fe, while in the chickens treated with iron sulphate this effect was observed ountil the 15th day. The iron deposited in the liver of the treated chickens was from 40 to 60% more than that in the control ones; the differences between the two treatment groups, however, were not statistically significant (the trends were in favour of the iron methionate). Two times more iron was found in the cloacal content of the chickens treated with iron sulphate (risk

of environmental pollution). The treated and control chickens showed no clinical symptoms and signs suggestive of any disease. There were no pathomorphological changes in the liver of the chickens.

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