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THE INFLUENCE OF DIETARY MOLYBDENUM AND COPPER SUPPLEMENTATION ON THE CONTENTS OF SERUM URIC ACID AND SOME TRACE ELEMENTS IN COCKS

By

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KARRING, M., R. POHJANVIRTA, T. RAHKO and H. KORPELA: *The influence of dietary molybdenum and copper supplementation on the contents of serum uric acid and some trace elements in cocks.* Acta vet. scand. 1981, 22, 289—295. — The effect of molybdenum (Mo) and copper (Cu) supplementation on some blood parameters and trace elements of tissues of cocks was investigated. The animals were fed with commercial poultry feed and water ad libitum and the experimental groups received peroral supplementation of different amounts of Mo or Cu during 4 weeks. Lowered values of serum uric acid were established in animals receiving 400 μg supplementation of either Mo or Cu. In contrast, the cocks receiving 100 μg supplementation of Cu displayed elevated concentrations of uric acid in the serum. A very significant statistical difference was noted between the uric acid levels of the animals receiving either moderate (group IV) or excess (group V) supplementation of Cu. The cocks with an excess of Cu displayed lowered hemoglobin and hematocrit values but no signs of Cu-intoxication were found in macro- and microscopical studies. Atomic absorption spectrophotometry was employed in the determinations of the content of Mo, Cu, Zn, Mn, Fe and Se in the liver and kidneys of the cocks. The authors suggest that the results of the trace element analyses presented are to be considered as preliminary values. Furthermore, the effect of Mo and Cu on uric acid metabolism should be additionally clarified by applying histochemical studies on xanthine oxidase in different tissues of cocks in order to make conclusions on the significance of Mo and Cu in the etiology of avian gout.

molybdenum; copper; uric acid; poultry.

Xanthine oxidase is an iron and molybdenum-containing enzyme of the purine metabolism, whose function is to catalyze the formation of uric acid from xanthine. The increase in the activity of xanthine oxidase is followed by an elevation in the concentration of serum uric acid, thus causing disposition to gout (*Hall et al.* 1967, *Olson et al.* 1974, *Pohjanvirta et al.* 1979).

Previous studies have shown that changes of the copper and molybdenum ratio cause changes in the formation of uric acid in the rat (*Kovalsky & Vorotnitskaya* 1970). The purpose of this investigation was to clarify if the dietary supplementation of these trace elements influences the concentrations of serum uric acid and some trace elements in poultry.

MATERIAL AND METHODS

Thirty clinically healthy cocks aged 12 weeks of the white leghorn race were randomly divided into 5 groups with 6 animals in each group. They were fed commercial poultry feed (Kasvatus-Punaheltta, Vaasan Höyrymylly) and water ad libitum.

By a pharyngeal syringe a peroral supplementation of molybdenum (Mo) and copper (Cu) in the form of a water solution of ammonium molybdate and copper sulphate was given daily during 4 weeks as follows: 400 μg Mo in group I; 100 μg Mo in group II; 100 μg Cu in group IV; 400 μg Cu in group V, and group III served as control, without mineral supplementation.

After 4 weeks blood samples were taken from the carotid arteries and the cocks were killed and immediately necropsied. In addition to macroscopical studies, samples of tissue were selected for histopathological studies from the liver, kidneys, heart and lungs, fixed with 10 % buffered formaline solution for 2 days, embedded in paraffine, cut at 5 μm and routinely stained with haematoxylin and eosin (*McManus & Mowry* 1960).

The blood samples of the cocks were submitted for usual hematological and chemical studies, including hemoglobin (g/1000 ml), hematocrit, creatinine (μmol/l), aspartate aminotransferase (E.C. 2.6.1.1., ASAT μkat/l) and alanine aminotransferase (E.C. 2.6.1.2, ALAT μkat/l) determinations. The concentration of uric acid in the serum of the cocks was estimated according to the enzymatic method described by *Hyvärinen et al.* (1972). Atomic absorption spectrophotometry was employed in the determination of the content of trace elements (mg/kg) as follows: Mo and Cu of the liver and kidneys of all the animals, zinc (Zn), manganese (Mn) and iron (Fe) of the liver of all the animals and selenium (Se) of the liver in only groups I, III and V.

RESULTS AND DISCUSSION

The general condition of the cocks remained good during the experiment.

In the necropsy of the animals no differences were found in the appearance of the inner organs between the experimental and control groups. This was also the result in microscopical studies of the organs and it was concluded that the dietary supplementations of Mo and Cu to the animals did not cause observable morphological changes due to Cu-intoxication. This conclusion was additionally confirmed by the determinations of ASAT, ALAT and creatinine values shown in Table 1. However, hemoglobin and hematocrit values showed distinct differences between the experimental and control groups. The lowest hemoglobin ($P < 0.05$) and hematocrit ($P < 0.01$) values were encountered in the animals of group V which had the greatest supplementation of Cu. This finding might indicate that the excessive amount of Cu possibly produced a harmful effect on hematopoiesis of the cocks. However, further studies are needed to confirm this hypothesis as other results of the present investigation did not reveal changes typical of Cu-intoxication (*Gratzl & Köhler* 1968).

Table 1. Mean values ± standard deviations of hemoglobin (Hb), hematocrit (Hcr), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) and creatinine (Creat) in blood samples of groups of 6 cocks receiving dietary supplementation of either molybdenum (Mo) or copper (Cu) (Group I: 400 µg Mo, Group II: 100 µg Mo, Group III: Control, Group IV: 100 µg Cu and Group V: 400 µg Cu).

	Hb	Hcr	ASAT	ALAT	Creat
Group I	132 ± 11	33.3 ± 1.3	3.9 ± 0.2	1.3 ± 0.3	43.5 ± 4.1
Group II	138 ± 9	33.3 ± 0.4	4.4 ± 0.2	1.3 ± 0.3	45.0 ± 3.8
Group III	123 ± 8	31.5 ± 0.6	4.2 ± 0.1	1.3 ± 0.1	44.5 ± 0.9
Group IV	148 ± 10	33.3 ± 1.4	4.0 ± 0.5	**0.6 ± 0.2	47.6 ± 6.2
Group V	*114 ± 5	**29.0 ± 1.0	3.3 ± 0.5	1.2 ± 0.1	37.0 ± 6.4

Significant differences between the experimental and control groups are marked with asterisks: * $P < 0.05$; ** $P < 0.01$.

The concentration of uric acid in the serum of the cocks (Fig. 1) was distinctly affected by the dietary supplementation of Mo or Cu. In both marginal groups fed with excessive Mo or Cu, clearly lower values of serum uric acid were encountered, with the lowest values being in the animals of group V which received the highest Cu supplementation. The highest contents of uric acid, on the contrary, were found in group IV receiving

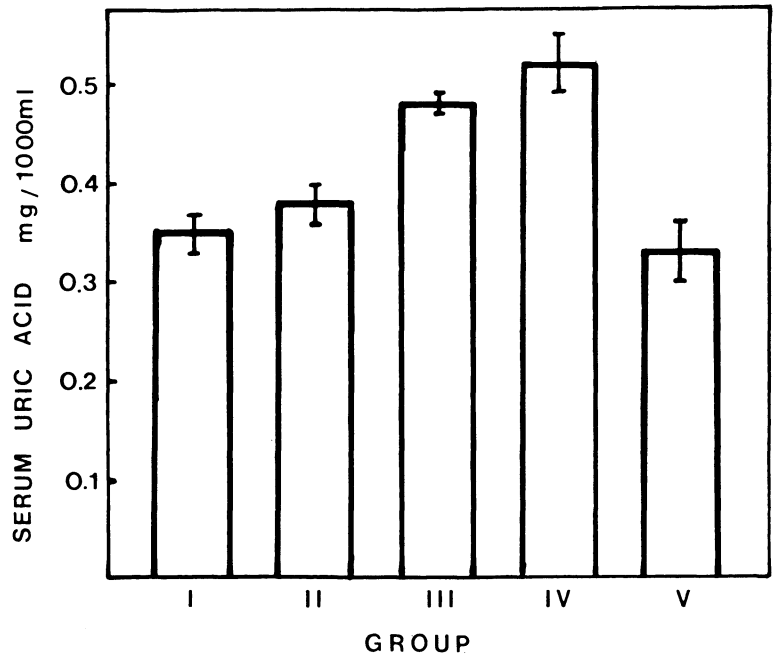

Figure 1. Mean values ± standard deviations of uric acid (mg/1000 ml) in serum samples of groups of cocks receiving dietary supplementation of either molybdenum (Mo) or copper (Cu) (see text of Table 1).

a moderate Cu addition. Statistical analyses confirmed a highly significant difference in the concentration of uric acid between the groups IV and V ($P < 0.001$).

Previous studies in the rat have shown that the activity of xanthine oxidase is affected by changes in the dietary Mo:Cu ratio (*Kovalsky & Vorotnitskaya* 1970). This is supposed to result from the presence of Cu and Mo in xanthine oxidase in this species of animal. Bovine animals are also known to possess both Mo and Cu in xanthine oxidase, but in cocks the composition of trace elements of the enzyme has not been previously clarified as far as the authors know. The relationship between Mo and Cu is antagonistic (e.g. *Davies* 1972, *Kovalsky et al.* 1973, *Goodhart & Shils* 1976) and apparently therefore Cu may inhibit the activity of xanthine oxidase.

Experimental studies on the effect of Mo and Cu supplementation have dealt with mammalian animals but not fowls. Owing to differences in uric acid metabolism between these animal spe-

cies, the result of the present study cannot be adequately compared with other studies. However, many similarities are to be noted in the metabolic response of the rat (*Kovalsky & Vorotnitskaya* 1970) and of the cocks to the dietary minerals studied.

On completion of the present experiment the amounts of Mo and Cu in samples of the commercial feed were analyzed. The analysis showed the presence of about 0.51 mg/kg Mo and 9.8 mg/kg Cu in the samples. Due to the unexpected great difference in the contents of these elements in the feed, the supplementation of Mo and Cu of the same quantities did not produce grave changes between the Mo:Cu ratio of the experimental groups. It appears that in future studies the Cu spplementation should be greater if commercial feed is used instead of an experimental diet devoid of these substances.

The contents of Mo, Cu, Zn, Mn, Fe and Se in the organs of the cocks in the different experimental groups are presented in Table 2. Because of the small size of the groups, the results are to be considered as only directional values.

Table 2. Mean values ± standard deviations of Mo, Cu, Zn, Mn and Se (mg/kg) in tissue samples of the liver and kidneys of groups of 6 cocks receiving dietary supplementation of either molybdenum (Mo) or copper (Cu) (see the text of Table 1).

	Mo liver	Mo kidney	Cu liver	Cu kidney
Group I	3.0 ± 0.5	4.0 ± 0.3	14.5 ± 0.9	12.5 ± 0.3
Group II	3.0 ± 0.0	3.8 ± 0.2	14.7 ± 1.1	12.0 ± 0.3
Group III	3.3 ± 0.5	3.8 ± 0.2	14.2 ± 1.1	10.8 ± 0.4
Group IV	2.5 ± 0.3	4.5 ± 0.3	13.5 ± 0.4	11.5 ± 0.3
Group V	2.7 ± 0.4	4.0 ± 0.3	13.2 ± 1.3	11.3 ± 0.3
	Zn liver	**Mn liver**	**Fe liver**	**Se liver**
Group I	104.3 ± 5.3	13.2 ± 0.7	805 ± 141	2.8 ± 0.2
Group II	103.0 ± 4.4	14.3 ± 0.9	870 ± 79	— —
Group III	104.3 ± 9.7	15.3 ± 2.0	648 ± 71	2.9 ± 0.3
Group IV	102.5 ± 3.0	13.8 ± 0.9	837 ± 171	— —
Group V	97.2 ± 7.8	12.7 ± 0.7	805 ± 94	4.0 ± 0.3

Nevertheless, the preliminary observations made should be thoroughly studied. The significance of the high values of Se in cocks with high Cu amounts in their diets especially need further studies in order to be elucidated.

CONCLUSIONS

The studies described in this paper on the influence of dietary molybdenum (Mo) and copper (Cu) supplementation on the concentration of uric acid in the serum of cocks showed a dependance between the degree of supplementation of the trace elements and the uric acid level. The experiments revealed lowered values of serum uric acid in groups receiving 400 μg addition of either Mo or Cu but elevated values in the cocks receiving 100 μg Cu supplementation in the diet. A statistical difference ($P < 0.001$) existed between the uric acid concentrations of animal groups receiving moderate or excess Cu supplementation.

In hematological studies lowered hemoglobin and hematocrit values were established in animal groups with excess Cu supplementation, but otherwise the results concerning the usual hematological determinations and macro- and microscopical studies appeared normal. The determinations of Mo, Cu, Zn, Mn, Fe and Se in some organs of the experimental animals are to be considered as preliminary results, due to the small size of the groups, but give rise to further studies.

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SAMMANFATTNING

Om inverkan av dietärt molybden och koppar tillskott på halten av serums urinsyra och några spårämnen hos tuppar.

Effekten av molybden (Mo) och koppar (Co) tillskottet på ett antal blodparametrar och vävnaders spårämnes halt hos tuppar undersöktes. Djuren utfodrades med kommersiellt hönsfoder och vatten ad libitum och experimentgrupperna erhöll peroralt särskilda Mo och Cu tillskott under 4 veckor. Sänkta värden av serums urinsyra noterades hos djur, som erhöll ett 400 µg stort tillskott antingen av Mo eller Cu. Däremot uppvisade tupparna, som erhöll ett Cu tillskott på 100 µg ökad konsentration av urinsyra i serumet. Statistiskt mycket signifikant skillnad noterades mellan urinsyra nivåerna hos djuren, som erhöll antingen moderat eller ökat tillskott av Cu. Tupparna med överskott av Cu uppvisade sänkta hemoglobin och hematokrit värden, men inga tecken på Cu förgiftning kunde påvisas medelst makro- och mikroskopiska undersökningar. Atomabsorption spektrofotometri användes för bestämning av halten av Mo, Cu, Zn, Mn, Fe och Se i tupparnas lever och njurar. Författarna antyder att resultaten av spårämnes analyserna i tabell 2 måste beaktas som preliminära värden. Dessutom måste verkan av Mo och Cu på urinsyreämnesomsättningen ytterligare utredas genom att bruka histokemiska undersökningar av xantin oxidas i olika vävnader hos tuppar för att dra slutsatser om Mo:s och Cu:s betydelse i giktens etiologi hos fåglar.

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