

## METABOLIC AND PRODUCTIVITY EFFECTS OF HIGH FLUORIDE PHOSPHATE IN LAYING HENS

## METABOLIČNI I PROIZVODNI UČINCI VISOKOFLUORIDNIH FOSFATA U KOKOŠI NESILICA

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### SUMMARY

Effects of high fluoride phosphate (HFP) on egg production, metabolic state and fluoride concentration in bone, soft tissues and eggs were examined. High fluoride intake in the diet had no significant effect on metabolic state or fluoride concentration in bone and egg yolk. Egg production was 5.56% and 8.84%, respectively, higher in the experimental groups than in the control groups. Fluoride concentrations in soft tissues and egg shell were also significantly higher in the experimental groups. Mortality was significantly lower in the experimental groups.

Key Words: Fluoride, tissue, egg, metabolic state, mortality.

### INTRODUCTION

Poultry are known to be the most tolerant to fluoride of all domestic animals (Hahn and Guenter, 1986; Weber, *et al.*, 1984; Cakir, *et al.*, 1978). The usage of phosphorous supplement with fluoride content, or phosphorous with NaF added, was reported by Kuhl and Sullivan (1978) to result in increase of egg production up to 500 ppm fluoride, whereas Hahn and Guenter (1986) reported that continuous feeding with 400 ppm as NaF in the diet lowered egg production as well as feed efficiency. At the same time, due to the supplementation with 500 ppm as NaF, Kuhl and Sullivan (1978) reported an increase in egg weight. Nahorniak, *et al.*, (1983) reported that supplementation of the diet with NaF of broilers and laying hens reared in cages increased the ash content in bone and the strength

of bone. Hahn and Guenter (1986) contradicted this report, putting in evidence that 100 ppm fluoride as NaF lowered the ash content in bone.

Increase of fluoride content in bone as a function of fluoride level in the diet and the duration of its usage has been found by all investigators (Guenter and Hahn, 1986; Hahn and Guenter, 1986; Michel, *et al.*, 1984; Nahorniak, *et al.*, 1983; Suttie, *et al.*, 1984). Although fluoride content in soft tissues depends on the level and the duration of fluoride supplementation, levels that would be harmful to human health were never reached in

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chicken tissues (Jenkins, *et al.*, 1970; Clarke and Clarke, 1975). Hahn and Guenter (1986) suggested that a considerable amount of fluoride in bone might be added to the meat and bouillon by mechanical deboning, but they did not elaborate further. Fluoride content in egg yolk did not vary with fluoride in several different compounds in the feed, or with the duration of feeding, but its content in egg shell did reflect the amounts in feed and the duration of feeding (Kuhl and Sullivan, 1978; Hahn and Guenter, 1986). Total protein, calcium and inorganic phosphorus were not influenced by the level and the duration of feeding fluoride compounds in the diet, whereas alkaline phosphatase activity increased with fluoride in the diet (Weber, *et al.*, 1984).

The aim of this study was to determine the effect of HFP on the metabolic state, productivity mortality and fluoride deposited in the bone, soft tissues, egg shell and yolk for an entire production cycle. The final aim was to determine if HFP could be used as phosphorous supplement for layer hen diets.

## MATERIALS AND METHODS

The chemical characteristics of HFP are shown in Table 1. The HFP had much more Si, S, F, Al as dicalcium phosphate, and lower phosphorous. Fluoride was in the  $\text{CaF}_2$  form. Analytical methods used were: calcium by titrimetric method, phosphorous by spectrophotometric method, crude protein by Kjeldahl method, tibial ash by CEE method (Martillotti and Antongiovani, 1972), and fluoride by ion selective electrode (Villa, 1979). Semiquantitative spectral analysis was used to measure Ba, Be, Si, Al, Mn, Sr, Fe and S. Metabolic energy was calculated from tables (Scott, *et al.*, 1982).

Composition of the diets used is shown in Table 2.

In the first experiment, 270 eight-month-old hens were divided into three groups and kept in cages with three hens per cage. The experiment continued for nine months. Egg production and bird mortality were recorded daily. Biochemical blood parameters were determined every three months, and fluoride content in bone and soft tissues was determined at the end of the experimental period.

**Table 1. Composition of high fluoride phosphate.**

**Tablica 1. Sastav visokofluoridnog fosfata**

Barium	Barij	0.07
Beryllium	Berilij	0.0002
Silica	Silicij	6.0
Phosphorous	Fosfor	8.3
Magnesium	Magnezij	4.5
Aluminium	Aluminij	1.0
Manganese	Mangan	0.1
Sodium	Natrij	0.5
Strontium	Stroncij	0.06
Iron	Željezo	1.0
Sulfur	Sumpor	9.1
Calcium	Kalcij	20.3
Fluorine	Fluor	1.1

In the second experiment, 26000 twenty-five-week-old White Leghorn hens were divided into two groups and housed in 30x40 cm cages. The experiment lasted 12 months. Egg production and bird mortality were recorded daily. Biochemical parameters in the blood were determined every three months. Determination of fluoride content in bone, soft tissues, egg shell and yolk was carried out at the end of the experimental period.

The fluoride content was obtained using ion selective electrode method as elaborated by Villa (1979) and the Journal of Association of Official Analytical Chemists (Anon, 1975), and the blood biochemical parameters were determined by methods described in *Biohimiceskij Metodi v Klinike* (1969).

## RESULTS AND DISCUSSION

The blood biochemical parameters of the experimental groups at the end of the experimental period (Table 3) were not significantly different from the control groups ( $P < 0.05$ ). The data are in agreement with Weber, *et al.* (1969) and Scott, *et al.* (1982).

**Table 2. Composition of feed****Tablica 2. Sastav hrane**

Sastojci - Ingredients (%)	Year 1986 - Godina 1986			Year 1987 - Godina 1987	
	Control Kontrola	Test 1	Test 2	Control Kontrola	Test
Corn - Kukuruz	60	60	60	58	58
Soybean meal - Sojina sačma	8	8	8	10	10
Sunflower meal - Suncokretova sačma	9	9	9	9	9
Ground flour - Stočno brašno	8	8	8	7	7
Fish meal - Riblje brašno	5.5	5.5	5.5	7	7
Limestone - Vapnenac	7	7	7	7	7
Dicalcium phosphate - Dikalcijfosfat	1.5	0.75	nil	1	nil
High fluoride phosphate - Visokofluoridni fosfat	nil	0.75	1.5	nil	1
Vitamin-mineral premix - Vitaminskomineralni premiks <sup>1</sup>	1	1	1	1	1
NaCl - Sol	0.3	0.3	0.3	0.3	0.3
Chemical analysis - Kemijska analiza					
Crude protein - Sirove bjelančevina, g%	15.55	15.55	15.55	14.84	14.84
Metabolic energy - Metabol. energija, Kcal/kg	2585	2585	2585	2616	2616
Calcium - Kalcij, g%	3.02	3.02	3.02	3.01	3.01
Phosphorous - Fosfor, g%	0.58	0.51	0.44	0.68	0.59
Fluoride - Fluor, ppm	45	138	231	45	156

<sup>1</sup> The vitamin-mineral premix supplied per kg of ration - Vitaminsko-mineralni sastav po kilogramu mješavine: Vit. A, 12000; Vit. D, 1500; Vit. E, 10; Vit. K, 2 mg; Thiamin, 2.2 mg; Riboflavin, 5 mg; Pyridoxine, 5 mg; Cobalamine, 0.015 mg; Nicotinic acid, 35 mg; Pantothenic acid, 15 mg; Folic acid, 1.5 mg; Fe, 25 mg; Cu, 4 mg; Mn, 60 mg; Zn, 50 mg; Co, 1 mg.

The percentage of tibia ash and calcium per cm<sup>3</sup> of fresh tibia increased with fluoride content in the diet. Nahorniak, *et al.* (1983) pointed out that there was no change in the percentage of tibia ash when 200 ppm fluoride was added to the diet as NaF, whereas Guenter and Hahn (1986) noticed no significant decrease until 100 ppm fluoride was added as NaF.

Egg production (Fig. 1 and Table 5) varied with the level of Fluoride in the diet and was slightly higher in the experimental groups. The increase was relatively small, 5.56% and 8.84% in two of the experiments, and negligible in another. This agrees with Kuhl and Sullivan (1978) who found significant

increases in egg production up to 500 ppm fluoride as NaF. It is partly in agreement with Guenter and Hahn (1986) who found that when 100 ppm fluoride was added to the diet as NaF, egg production increased 6.4%, but it differs from their finding that there was a marked fall in egg production at higher fluoride levels.

Mortality was decreased by increased fluoride in the diet (Table 4). Guenter and Hahn (1986) arrived at the same conclusion, whereas Kuhl and Sullivan (1978) and Nahorniak, *et al.* (1983) did not observe any variation until 500 ppm fluoride as NaF.

**Table 3. Effect of dietary fluoride on blood and bone parameters.****Tablica 3. Učinak fluorida u hrani na parametre krvi i kostiju**

Parameters - Parametri	Year 1986 - Godina 1986			Year 1987 - Godina 1987	
	Control Kontrola	Test 1	Test 2	Control Kontrola	Test
Total calcium - Ukupni kalcij, mg% (n=60)	32.7	33.7	33.1	26.2	25.44
Inorganic phosphorous - Anorganski fosfor, mg% (n=60)	5.15	5.60	5.60	6.78	6.30
Alkaline Phosphatase <sup>1</sup> - Alkalna fosfataza (n=60)	0.28	0.29	0.32	0.27	0.30
Tibia ash - Pepeo tibije % (n=6)	46.77	48.55	49.5	45.4	46.7
Ca in fresh tibia - Ca u svježoj tibiji mg/cm <sup>3</sup> (n=6)	125.9	126.1	119.6	121.4	120.8

<sup>1</sup> Alkaline phosphatase activity is given as  $\mu\text{mol}$  4-nitrophenol liberated by 0.05 ml blood plasma in 30 minutes at 37°C.

Aktivnost alkalne fosfataze daje se kao  $\mu\text{mol}$  4-nitrofenola oslobođenog sa 0.05 ml krvne plazme u 30 minuta na 37 °C.

Means in experimental groups were not significantly different from control groups ( $P>0.05$ ).

Srednje vrijednosti u pokusnim skupinama nisu se značajno razlikovale od kontrolnih skupina ( $P>0.05$ )

**Table 4. Effect of high fluoride phosphate on mortality.****Tablica 4. Učinak visokofluoridnog fosfata na smrtnost.**

Parameters - Parametri	Year 1986 - Godina 1986			Year 1987 - Godina 1987	
	Control Kontrola	Test 1	Test 2	Control Kontrola	Test
Hens at start of period - Kokoši na početku razdoblja	90	90	90	13249	13305
Hens at end of period - Kokoši na kraju razdoblja	82.4	86.40*	89.3**	12648	13114*
Mortality - Smrtnost, % <sup>1</sup>	8.9	4.05*	0.8**	5	1.5*

<sup>1</sup> Means in experimental groups were significantly different from control groups at \* $P<0.05$  and \*\* $P<0.01$ .

Srednje vrijednosti pokusnih skupina značajno su se razlikovale od kontrolnih skupina pri \* $P<0.05$  i \*\*  $P<0.01$ .

Increasing fluoride in the diet was followed by an evident increase of fluoride in the bone (Table 5). This corresponded to the data given by Nahorniak, *et al.* (1983), Suttie, *et al.* (1984), Michel, *et al.* (1984) and Hahn and Guenter (1986). On the other hand, fluoride content in the soft tissues (Table 6) was not influenced by the fluoride level in the diet. This observation is in agreement with conclusions reached by Hahn and Guenter (1986), Muhler, *et al.* (1970), and Clarke and Clarke (1975). At the same time, the fluoride found in bone at the high level of fluoride did not come out into the bouillon after mechanical deboning and boiling (Table 5). These results affirm the general conclusion arrived at by Clarke and Clarke (1975), Jenkins, *et al.*

(1970) and Armstrong, *et al.* (1970) that animal meat, even with fluorosis, does not constitute a medical problem for human health.

Fluoride content in egg yolk was 2.4 to 3.97 ppm. Our data showed a dependence of fluoride level in the egg yolk to the amount taken in the diet, whereas Kuhl and Sullivan (1978) and Hahn and Guenter (1986) did not find such a link. Fluoride content in the egg shell was influenced by fluoride in the diet. This was in agreement with the data given by Hahn and Guenter (1986). This fluoride content varied from 36.5 ppm in the control groups to 201,18 ppm in the experimental. As for the bone tissues, the differences were statistically significant ( $P<0.05$ ).

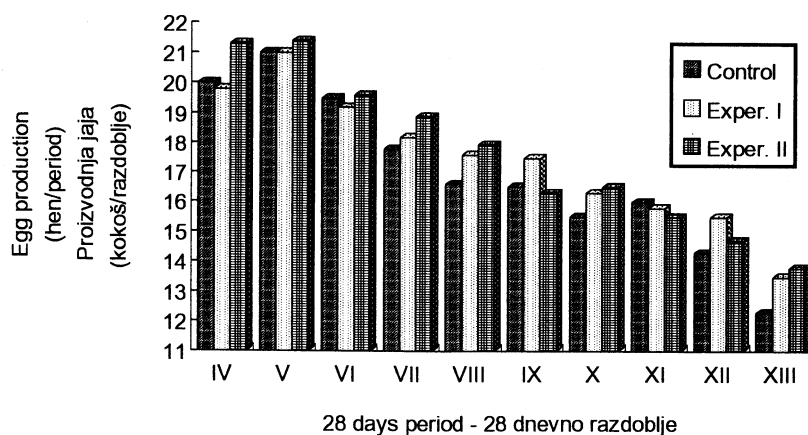


Figure 1. Egg production for 28 day period in laying hens fed diets supplemented or not supplemented with high fluoride phosphate.

Slika 1. Proizvodnja jaja u razdoblju od 28 dana kokoši nesilica hranjenih hranom s dodatkom visokofluoridnog fosfata ili bez dodatka.

Table 5. Effect of high fluoride phosphate on egg production.

Tablica 5. Učinak visokofluoridnog fosfata na proizvodnju jaja.

Parameters - Parametri	Year 1986 - Godina 1986			Year 1987 - Godina 1987	
	Control Kontrola	Test 1	Test 2	Control Kontrola	Test
Eggs per hen - Jaja po kokoši	170.37	175	176.56*	192.93	193.81
Production - Proizvodnja, %	100	105.6	108.84*	100	100.4

\*Significantly different from controls at  $P < 0.05$ .

Značajna razlika od kontrole pri  $P < 0.05$ .

Table 6. Concentration of fluoride in soft tissues, egg shell and yolk, and tibia ashes (ppm) (n=10).

Tablica 6. Koncentracija fluorida u mekim tkivima, ljusci i žumanjcu jajeta i pepela tibije (ppm) (n=10).

Parameters	Year 1986 - Godina 1986			Year 1987 - Godina 1987	
	Control Kontrola	Test 1	Test 2	Control Kontrola	Test
Liver - Jetra	2.1	2.9	3.7	2.9	3.5
Kidney - Bubrezi	2.4	2.6	3.03	1.93	3.32
Sternal muscle - Prsni mišić	1.97	2.3	2.9	1.73	3.1
Tibial muscle - Mišić tibije	1.71	1.90	2.02	1.82	3.3
Bouillon - Juha	1.0	0.86	1.05	1.52	1.06
Egg yolk - Žumanjak	1.7	2.4	3.2	1.65	3.97
Egg shell - Ljuska jajeta	36.5	152.3***	201.18***	28.6	176.9***
Tibia ash - Pepeo tibije	3970	5850**	8100***	4200	6800**

Means in experimental groups were significantly different from control groups at \*\* $P < 0.01$  and \*\*\* $P < 0.001$ .

Srednje vrijednosti pokusnih skupina bile su značajno različite od kontrolnih skupina pri \*\* $P < 0.01$  i \*\*\* $P < 0.001$ .

## CONCLUSIONS

We conclude that HFP in poultry feed is beneficial for chickens, and meat and eggs from chickens fed HFP are safe for human consumption.

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## SAŽETAK

Ispitivani su učinci visokofluoridnog fosfata na proizvodnju jaja, stanje metabolizma i koncentraciju fluorida u kostima, mekim tkivima i jajima. Unošenje visokih količina fluorida hranom nije imalo značajnijeg učinka na stanje metabolizma ili koncentraciju fluorida u mekim tkivima i žumanjku jajeta. Proizvodnja jaja bila je 5.56%, odnosno 8.84% viša u pokusnim skupinama nego u kontrolnim skupinama. Koncentracije fluorida u koštanim tkivima i ljusci jajeta bile su isto tako značajno više u pokusnim skupinama. U pokusnim je skupinama smrtnost bila značajno niža.

Ključne riječi: Fluorid, tkivo, jaje, stanje metabolizma, smrtnost.