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Haematological and biochemical indicators in the serum of weaned piglets fed on fodder mixture contaminated by ZEN, with the addition of clinoptilolite

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Summary

The effect of zeolite clinoptilolite (CLIN) on some metabolic parameters in blood serum (total protein, albumin, glucose, triacylglycerols (TRI), cholesterol (CHOL), bilirubin, urea, creatinine, Fe, ALT, AST, GGT, ALP, CK, LDH), and haematological values (RBC, MCW, MCH, MCHC, Hb, hematocrit, thrombocyte, WBC) in weaned piglets fed with increased levels of zearalenone (ZEN) was investigated over a 14-day period. The trial was conducted with 3 groups of animals, each group comprising 10 female piglets (fed with starter mixture 20% CP; 29.95 MJ ME/kg). Group C was supplied with food containing 0.2 g/kg CLIN (ZEN level< 5.1 ng/g), group E1 3 mg/kg ZEN and 0.2 g/kg CLIN, and group E2 3 mg/kg ZEN. Blood samples were collected on the 8th and 14th days of the trial. Group C was characterized by the highest body weight and daily gain (P<0.01). Values of CHOL were the lowest on the 8th day, while values of TRI were the highest on the 14th day (E1: C, P=0.058; E1:E2, P=0.0006) in E1 and the lowest on the 14th day in E2 (E2: C, P=0.0096). Serum Fe was the lowest on the 8th day (P=0,002) and on the 14th day (P=0.15) in E2. The highest activity of AST and CK was on the 8th day in E1 (P<0.05) and on the 14th day in E2 (P<0.05). MCV was the lowest on the 8th day (P=0.02) and on the 14th day (C:E1, P=0.02) in E1. The estrogenic metabolic effect of ZEN was determined to be in E2, with added CLIN, ZEN action was reduced .
Key words: clinoptilolite, zearalenone, biochemical parameters, haematological parameters, weaned piglets

Introduction

The presence of mycotoxins has been established all over the world (JURJEVIĆ at al., 1999; WOOD, 1992; PLACINTA et al., 1999) but in varying concentrations depending on climatic and meteorological conditions, as well as on the conditions of cattle feed production. Research has shown that 25% of cereals in the world are contaminated with mycotoxins (LAWLOR and LYNCH, 2001). Zearalenon, produced by fungi of the species *Fusarium*, is a non-steroid oestrogen found on grains of maize, oats, barley, wheat as well as in cattle feed mixtures (BAUER et al., 1980). The grains are contaminated in the field at an optimum temperature of 18-24 ^{o}C, and at a relative humidity of over 71%. The toxin is introduced

orally and its metabolic activity is complex. Biotransformation takes place in the liver and digestive system and is the result of the activity of tissue enzymes and microflora (GALTIER, 1999; YIANNIKOURIS and JOUANY, 2002), and it shows an affinity for oestrogen receptors (POWELL-JONES et al., 1981; MUELLER et al., 2004). Consequently, its effect is primarily linked to reproductive organs, although its influence on hypothalamus and pituitary gland has also been established (KITAGAWA et al., 1982). It is secreted through the gall bladder (65%), urine (21%), faeces (GAUMY et al., 2001) and milk (HAGLER et al., 1980). Following the competitive binding to oestrogen receptors (ER) the synthesis of proteins is increased and proliferation of cells is induced, resulting in an increase of organ mass (anabolic effect). It is believed that its effect may not be restricted to oestrogen receptors alone (MURATA et al., 2002), since it has been established that oestrogen, as well as phytoestrogen genestein and quercetin, are able to stimulate gene expression independently of classical ERs (MAGGIOLINI et al., 2004). Acute toxicity was not found but at lower concentrations cause macroscopic changes in ovaries (GAUMY et al., 2001; ZÖLLNER at al., 2002; YIANNIKOURIS and JOUANY, 2002); changes in the external parts of sex organs of new-born and suckling pigs (DACASTO at al., 1995; ALEXOPOULOS, 2001) and is the primary cause of rectum prolapse in pigs (PERFUMO at al., 2002), and results in unfavourable effects on maturing of oocytes and the culture of embryos in pigs (ALM et al., 2001). The development of early mastopathy in children is linked to the effect of zearalenone present in cereals (SZUETS et al., 1997). Mycotoxins reduce the activities of T and B lymphocytes, indicating a carcinogenic effect (GUERRE et al., 2000; BEREK et al., 2001; GAUMY et al., 2001), which prompts some researchers to stress that the requirement should be for a zero level of contamination (GUERRE et al., 2000).

In an effort to reduce mycotoxic contamination, pigs are increasingly being fed with mixtures containing a variety of adsorbents (HUWIG et al., 2001). Attempts are being made to utilize the observed ability of zeolite to bind mycotoxins and heavy metals (POND and YEN, 1983; POND, 1985; LINDEMANN et al., 1993). Other properties it possesses, i.e. not to bind amino acids, vitamins and minerals, as well as having no detrimental effect on the composition of the serum (PAPAIOANNOU et al., 2002), combined with a possible anti-carcinogenic effect (PAVELIĆ et al., 2002) make it suitable for administration in the diet of domestic animals.

A positive effect of the addition of organozeolite to the feed of piglets has been established (STOJIĆ et al., 1998), sows and gilts (KYRIAKIS et al., 2002; PAPAIOANNOU et al., 1988), as well as an additive to the ZEN-contaminated feed of feeder pigs (POND et al., 1988; ZÖLLNER et al., 2002), dairy cows (ENEMARK et al., 2003), lambs (STOJŠIČ, 2004), broiler chickens (DWYER et al. 1997; OGUZ et al., 2000), and fish, where increased levels of lead are present in water (TEPE et al., 2004).

Also available, however, is information on the lack of effect of clinoptilolite as an additive to the feed of pigs, particularly feed contaminated with different mycotoxins and heavy metals (POULSEN and OKSBJERG, 1995).

The overall effect of zearalenone, which is regarded as a strong phytoestrogen and the effect of which is the result of chemical composition, which ranks it among selective oestrogen modulators, has not been explained. The aim was to assess its influence on the general condition of organs and the organism as a whole, as well as on the metabolic status of pigs as the species most susceptible to the effects of zearalenone. Interest also focused on the possible reduction of its detrimental effect through the application of clinoptilolite, based on certain biochemical and haematological values and histological results.

Due to contradictory results regarding the effectiveness of clinoptiolite, the neutralizing effect that zearalenone has on organs and the general condition of the organism, as well as on the metabolic processes of the pig as the most sensitive species, was investigated on the basis of certain biochemical and haematological tests and histological results.

Materials and methods

Animals and feeding

The research involved three groups of weaned piglets aged 40 to 54 days. All groups were fed on fodder mixture for growing pigs containing 20% crude protein and 29.95% MJ ME/kg. Each group comprised 10 female piglets bred from four sows and two boars. The quantity of feed consumed per group was monitored throughout the period of rearing. The first group (C) was fed on fodder mixture containing 0.2% of modified clinoptilolite (Min-a-Zel Plus®; levels of zearalenone >5.1 ng/g). This group was used as control the – natural zeolite clinoptilolite used as a dietary supplement did not relevantly affect blood count parameters (Ivković et al., 2004). Likewise, with regard to serum chemistry, POND and YEN (1983) found no effect of zeolite on plasma potassium, sodium and magnesium levels in swine. Piglets in the second group (E1) were fed on fodder mixture containing and addition of 3 mg/kg concentration of zearalenone (Sigma-Aldrich Co) and 0.2% of Min-a-Zel Plus® preparation. The third group (E2) was fed on fodder mixture to which 3 mg/kg of zearalenone was added, but with no addition of Min-a-Zel Plus®. Composition of Min-a-Zel Plus® is shown in Table 1; cation exchange capacity was 160 +/- 10 meq/100g.

Table 1 **Chemical composition of the Min-a-Zel Plus®, organic modification of the zeolitic mineral-clinoptilolite with a long chain quaternary amonium salt**

Compound	wt. (%)
SiO_2	63-68
Al_2O_3	11-14
Fe_2O_3	0.8-2.5
MnO	0.01-0.03
CaO	2.5-4.5
MgO	0.8-1.5
Na_2O	0.8-1.5
K_2O	1.0-2.0
L.I.	10.5-14.5

Taking blood samples, biochemical indicators and blood count

Blood samples were taken from the animals on the 8th and 14th days in order to determine haematological values and biochemical indicators. Five millilitre samples were obtained from the *v. Cavae cranialis* using a Venoject (R) vacuutainer into a test tube containing an anticoagulant (EDTA), and 5 ml for biochemical tests. The levels of metabolites (glucose, urea, creatinine, cholesterol, bilirubin, total proteins, albumins, triglycerides), enzyme activities (AST, ALT, ALP, GGT, CHE, CK, LDH and amilase), and mineral levels (Fe, P, Na, CA, K, C1) were established using automatic analyzer Olimpus AU 640.

Table 2 **Body weight, daily gain and feed conversion of weaned piglets fed on fodder mixture contaminated by zearalenone with addition of clinoptilolite Min-a-Zel Plus®* (n=5)**

Indicators	Statistical size	Groups of piglets		
		C	E1	E2
Body weight, kg				
Start	$\bar{x}$	13,30	12,93	12,71
	s	0,85	1,13	1,22
Final	$\bar{x}$	16,93**[2,3]	15,39	15,06
	s	1,05	1,32	0,80
Daily gain, g				
Total	$\bar{x}$	259,00**[2,3]	175,57	168,00
	s	44,88	66,72	57,69
Feed conversion, kg				
	$\bar{x}$	1,15	1,32	2,10

** P<0,01

The number of erythrocytes, leukocytes, trombocytes, levels of haemoglobin and haematocrit were established using the Symex SF-3000 automatic counter. Blood smears were prepared and stained according to Pappenheim and investigated under a microscope in order to arrive at the differential blood count. The relative ratio of individual cells of leukocytes is given in percentages in relation to their total number.

Pathophystological tests

Upon the completion of the experiment the animals were euthanized by an intracardial injection of 0.3 ml/kg of T61$^{(R)}$ (Hoechst, Munich, Germany) preparation, and organ samples were taken (ovaries, kidneys, liver, spleen) for pathophystological investigations. Samples were fixed in a 4% solution of paraformaldehyde in a phosphate puffer at room temperature for 48 hours prior to processing. They were dehydrated by immersion into 70%, 96% and 100% alcohol (twice for a period of one hour) and stored overnight in chloroform at 56 ^{o}C. The tissues were then placed into a mixture of chloroform and paraplast (1:1) for one hour at 56 ^{o}C, and then embedded in paraplast I and paraplast II (for one hour at the same temperature). A microtome was used to cut 6 μm-thick sections, which were then fixed onto slides with 2% APES (3-aminopropil-trietoxilene: Sigma, St. Louis, U.S.A.) in acetone. The sections were then cleaned of paraffin by immersion into xilol (2x 10min.), a succession of alcohol concentrations (5 minutes each in 100%, 96%, 80% and 70% concentration), and into distilled water (2 x 5minutes). The next phase was staining with hemalaun-eosin and embedding into Canadian balsam. Thus prepared histological slides were studied using a light microscope.

Statistical processing

The values obtained from studied indicators were processed using computer programme Statistica (StatSoft Inc., 2001). The significant differences between groups of animals were established on the basis of the Student T-test of independent variables and the LSD *post hoc* test of independent variables.

Results

Production indicators and clinical picture

In view of the production results (Table 2), weaned piglets of control group (C) had a better body mass as early as the 8th day of the experiment in comparison to the groups of piglets which were administered zearalenone (E1 and E2) At the end of the experiment control group (C) had a significantly ($P<0.01$) higher body mass than the other two groups which were fed on food contaminated with ZEN (E1 and E2). Total daily increase during the observed period was also higher in group C, which also had the best food conversion ratio. The worst food conversion ratio was found in the group fed on feed contaminated with ZEN (E2).

Biochemical parameters

According to data presented in Table3, glucose values in all groups during the observed period were within the parameters of physiological values (Kaneko, 1997). However, a significantly ($P<0.05$) higher value was established in the control group on the 8th day, while on the 14th the value was higher in the group which had been given the toxin and clinoptilolite (E1). Comparing the values between groups it was found that on the 14th day piglets in the control group had lower levels of urea, creatinine and bilirubin. The values of triglicerydes were significantly lower in test group E2 on day 14 in relation to group E1. The level of iron in serum was significantly lower ($P<0,05$) in the group of piglets given feed mixture with an increased level of ZEN as early as day 8, and this trend continued to the end of experiment.

Table 3 **Biochemical values in blood serum of weaned piglets on the 8th and the 14th day of trial fed on zearalenone contaminated feed in addition of clinoptilolite Min-a-Zel Plus®(n=5)**

Indicators	Statistical size	8 th day			14th day		
		C	E1	E2	C	E1	E2
Glucose, mmol·L^{-1}	$\bar{x}$	4,30	3,92	3,72	3,58	4,17	3,66
	s	0,66	1,46	1,53	0,37	0,55	0,96
Urea, mmol·L^{-1}	$\bar{x}$	5,38	6,17	5,22	3,60	4,80	4,35
	s	0,49	2,03	0,52	0,40	1,30	0,58
Creatinine, µmol·L^{-1}	$\bar{x}$	69,80	63,75	70,40	45,80	50,50	62,50
	s	15,41	22,69	12,99	28,27	31,96	19,67
Bilirubin-total, µmol ·L^{-1}	$\bar{x}$	4,40	4,75	4,80	3,60	3,75	3,80
	s	0,54	0,95	0,44	0,54	0,95	0,83
Total protein,g·L^{-1}	$\bar{x}$	62,20	58,25	61,50	58,38	54,92	64,42
	s	4,50	9,33	7,71	5,78	5,94	7,85
Albumin, g·L^{-1}	$\bar{x}$	28,84	30,00	27,80	27,88	29,27	29,86
	s	3,10	13,10	2,62	10,16	7,77	13,38
Cholesterol, mmol ·L^{-1}	$\bar{x}$	1,93	1,53*^{E2}	1,99*^{E1}	2,55	2,71	2,96
	s	0,14	0,34	0,38	1,61	1,93	3,33
Triacylglycerols , mmol· L^{-1}	$\bar{x}$	0,46	0,52	0,33	0,47	**^{E2} 0,57	**^{E1} 0,33
	s	0,23	0,16	0,06	0,01	0,05	0,06
Fe, µmol ·L^{-1}	$\bar{x}$	**^{E2} 12,08	**^{E2} 13,10	**$^{C, E1}$ 4,80	8,33	7,66	4,67
	s	4,07	1,11	2,33	1,72	0,75	3,36
Feritin, µg ·L^{-1}	$\bar{x}$	9,60	10,33	5,25	12,60	11,00	7,25
	s	7,26	1,52	2,06	5,98	3,74	4,78
P, mmol·L^{-1}	$\bar{x}$	3,34	2,85	3,37	3,84	4,03	3,57
	s	0,25	0,35	0,54	0,89	1,53	0,98
Ca, mmol·L^{-1}	$\bar{x}$	2,57	2,56	2,47	1,78	1,97	1,89
	s	0,04	0,13	0,08	0,50	0,56	0,62

*, $P<0,05$
**, $P<0,01$

Peripheral blood count

Results of blood count monitored in the middle and at the end of the experiment show that there was no deviation from physiological values (Table 4). Nevertheless, on the 8th day the animals in control group C showed an increased level of leucocytes. On the same day the values of hemoglobinoglobin and hematocrit were highest in control group C. MCV was also significantly higher ($P<0.01$) in the control group (C) in relation to the second test group E2. The value of MCV was significantly higher ($P<0.05$) in the test group which was given ZEN contaminated food in comparison with the group fed on feed containing Min-a-Zel Plus® and ZEN (E1). Of the total number of leucocytes on the 8th day of the experiment the participation of segmented leukocytes was lowest in group E2, as was the level of non-segmented leukocytes. On day 8 the participation of lymphocytes and monocytes was higher in group E2, but no significant differences were established.

Enzymatic activities

On day 8 AST activity was significantly higher ($P<0.05$) in group E2 in relation to the control group, and on the 14th day it was significantly higher in relation to groups C and E1. ALT activity was significantly lower in the group fed on feed with a higher ZEN content, but only for the 8 days of the experiment; on the 14th day there was no difference observed between groups. CK activity was at its lowest in the control group, and on day 8 the difference was statistically significant in comparison with both test groups. At the end of the experiment the CK value was significantly higher in group E2 in relation to group E1 (table 5).

Table 4 **Haematological values of weaned piglets on the 8th and 14th day of trial fed on zearalenone contaminated feed in addition of clinoptilolite Min-a-Zel Plus®* (n=5)**

Indicators	Statistical size	8 th day			14th day		
		C	E1	E2	C	E1	E2
WBC, $10^9 \cdot L^{-1}$	$\bar{x}$	24,36	22,02	23,78	22,36	21,27	22,24
	s	6,22	9,52	8,45	3,97	11,54	6,87
RBC, $10^{12} \cdot L^{-1}$	$\bar{x}$	6,35	6,59	6,36	5,71	5,44	5,73
	s	0,29	0,90	0,40	0,44	1,25	0,72
Hemoglobin, $g \cdot L^{-1}$	$\bar{x}$	115,20	110,25	112,80	104,6	100,7	105,2
	s	7,32	17,57	5,49	8,11	16,19	9,80
Hematocrit, $L \cdot L^{-1}$	$\bar{x}$	0,42	0,41	0,42	0,36	0,31	0,33
	s	0,02	0,06	0,02	0,03	0,09	0,05
MCV, fL	$\bar{x}$	**E1 66,38	**C,*E2 61,67	*E1,**C 65,68	*E1 63,80	*C 56,52	58,80
	s	2,05	3,00	1,42	3,96	4,62	3,03
MCH, pg	$\bar{x}$	*E1 18,14	*C, E2 16,70	*E1 17,76	18,32	18,80	18,46
	s	1,08	0,68	0,76	1,07	1,90	1,37
MCHC, $g \cdot L^{-1}$	$\bar{x}$	273,60	270,50	270,20	287,00*E1	335,50*C	315,80
	s	9,60	4,79	8,92	9,66	58,68	37,41
Thrombocytes, $10^9 \cdot L^{-1}$	$\bar{x}$	330,80	239,75	299,40	448,40	446,25	423,20
	s	100,75	216,67	213,54	227,83	68,20	228,77
Neseg, %	$\bar{x}$	5,20	6,00	6,40	5,80	5,00	6,20
	s	1,78	2,16	0,89	2,28	1,63	2,94
Seg, %	$\bar{x}$	50,80	55,00	46,80	49,00	47,25	49,20
	s	7,98	7,70	4,43	6,67	4,64	6,76
Ly, %	$\bar{x}$	36,40	33,25	40,00	40,20	42,75	40,80
	s	11,97	6,84	6,44	5,06	4,99	5,40
Mono, %	$\bar{x}$	5,60	5,75	6,80	5,00	5,00	5,80
	s	1,67	2,36	1,64	1,87	1,41	2,58

*, P<0,05

**, P<0,01

Discussion

In our experiment the increased levels of ZEN in food had a negative influence on daily growth and food conversion in piglets, which resulted in a statistically significant difference ($P<0.01$) in body mass and daily growth between groups C and E2 at the end of the experiment (Table 2), nor was that negative influence significantly reduced by the addition of clinoptiolite (group E1). The negative effect that ZEN has on the growth and conversion of food was established by HORUGEL and VERGARA (2203), and KALIAMURTHY et al., (1997), while other authors regard it as a strong anabolic (Pfaffl *et al*.). The obtained results on the effect of clinoptiolite in pigs vary – according to some authors the effect was positive (POND et al., 1988; WARD et al., 1991; PAPAIOUNNOUE et al., 2002), while for other authors it had no effect (POULSEN and OKSBJERG, 1995). Improved food conversion and better daily growth was established in lambs (POND, 1984), broiler chickens (FETHIERE et al., 1994; DWYER et al., 1997), and laying hens (OLVER, 1997).

Research involving lambs has found that the compound had a positive effect on the absorption of mycotoxins, greater when the level was 0.5% than when it was 0.2% (STOJŠIĆ et al., 2004). PAPAIOUANNOU et al., (2002) have established that clinoptiolite had a positive effect on sows and their piglets. The low levels of triacylglycerols in group E2 on day 8, and a significant level on day 14 ($P<0.01$) would be a typical consequence of the agonistic oestrogenic effect of zearalenone. Notably, the main site of triacyglycerols synthesis in pig is fatty issue and, and for this site specificity for a new fatty acid synthesis lipoprotein lipase activity plays a major role in the eventual storage of triacyglycerols from both diet and liver in adipose tissue. Hormones increase the influx of blood triacyglycerols into adipose tissue (BEITZ, 2004). Oestrogens also increase synthesis of triacylglycerols and fat depositions (GOLDFEIN and SCOTT, 1997). Consequently, larger dosages of zearalenone (group E2) would have a significant influence on depositions of triacylglycerols in fatty tissue, thereby stimulating the activity of lipoprotein lipase outside a fatty cell or, like insulin, the activity of the esterification enzyme in cells. At the same time, such activity would mask the results of its activity in liver cells, causing a summary decrease of triacylglycerols in blood. That this activity is indeed so is supported by data on food conversion which was poorer in group E2 in relation to groups C and E1 (the ratio being 0.55 and 0.62, respectively), with a significant difference in body weight at the end of the experiment and the daily gain ($P<0.01$).

The effect with the added apsorbens, i.e., in low concentration, led to a fall in the cholesterol level in group E1 on day 8 (C:E1, $P<0.0766$) and to an insignificant increase in triacyglycerols on day 14 (C:E1, $P=0.058$). This was a characteristic response of liver to the effect of oestrogen the activity (model such as raloxifene, a selective oestrogen receptor modulator, HERINAGA, 2003). Oestrogens lower the circulating levels of cholesterol and increase the level of very low-density lipoproteins, which results in an increase of the circulating levels of triacylglycerols (OJEDA, 2000). Such agonistic oestrogen effect would be characteristic for humans, whose liver is the main site of tgriacylglycerol synthesis, but this is questionable with regard to pigs. Bearing in mind that on day 14 group E1 had the highest urea level (C:E1, $P=0.0559$), and the lowest level of total proteins (E1:E2, $P=0.0568$), it can be assumed that increased triacylglycerol levels in group E1 were accompanied by intensified lipogenesis from carbohydrates. On day 8 group E1 had the highest urea level, with a concurrent increased activity of AST and ALT which, in combination with the highest glucose value on day 14, would indicate increased glucogenesis and increased synthesis of fats in liver. This kind of effect would be similar to the effect of the coumestrol phytoestrogen (NOGOWSKI, 1999; NOGOWSKI et al., 2002) and could not be attributed to the oestrogen activity pattern of zearalenone.

It was found that zearalenone was a potent estrogen and activated the preferentially oestrogen receptor alpha and was antagonistic on both oestrogen receptor alpha and beta at high doses (MUELLER et al., 2004). The opposite effect of zearalenone is also visible in the increase of

total proteins in E2 in relation to E1, caused by a direct increase of the globulin fraction on day 14 of the experiment (E1:E2, P=0.0568).
The powerful influence that ZEB has on the lowering of serum iron, which was found to be significantly lower ($P<0.01$) in group E2 as early as day 8, can be explained through the inhibition of transferrin synthesis, the major Fe-transport protein in the plasma that is synthesized in hepatocytes. Oestrogens increase the plasma proteins that bind iron (OJEDA, 2000). The human breast cancer cell line MCF-7 secretes a factor which is immunologically identical to transferrin, and its secretion is enhanced by 17-β-oestradiol and reduced by the anti-oestrogen 4-hydroxy-tamoxifen (VANderWALLE et al., 1989). The ZEN influence on Fe metabolism is also manifested in the fall of the ferritine protein observed on day 14. In the group administered the apsorbens, the said parameters related to transfer of iron did not differ from those found in group C. However, the MCV and MCH values established in group E1, where the MCV value was lower on day 8, but also on day 14, in group E1 which in group C (P=0.0082 and P=0.0169, respectively), indicate that ZEN does in some way interfere with transfer of iron. Large quantities of absorbed ZEN, such as those in E2, are regarded, have reduced the MCV values in relation to group C after 14 days (P=0.065). On day 8 the MCH value in group E1 was the lowest (E1:C, P=0.0327; E1:E2, P=0.0997). Although PARENTMASSIN and PARCHMENT (1998) do not regard ZEN as a haematoxic toxin, certain other authors point out that ZEN has an influence on haematocrit, MCV, WBC and the number of platelets (MAAROUFI et al., 1996). And it is the values of MCV, MCH and MCHC that are used for the early detection of the anaemia-causing process (TYLER and COWEL, 1996).
ZEN has affected CK activity, causing its increase. Its significant increase in relation to both group C and group E2 manifested itself on day 8 in group E1, and on day 14 the same occurred in group E2. Based on the distribution of oestrogen receptors a high ERa expression could be observed in the uterus, udder and liver, but also in muscles (PFAFFL et al., 2001). Analyses of muscle tissue revealed relatively high amounts of nonglucocuronidated zeranol and α-zearalenol, together with traces of taleranol and zearalenone, indicating that the metabolism of zearalenone and its metabolites is not restricted to hepatic and gastrointestinal metabolic pathways (ZÖLLNER et al., 2002). Early uterotrophic uterine responses also include the oestrogen-induced expression of creatine kinase (PENTECOST et al., 1990. Brain (BB) isoenzymes od CK represented 73% of total activity of skinned guinea-pig uterus (CLARK, J.F. et al., 1993).
The rapid stimulation of the specific activity of the brain-type isozyme of creatine kinase is an almost universal marker of cell stimulation. The increase in its activity is closely correlated with the biochemical and morphological parameters, and the increase in CK activity has been used to demonstrate specific stimulation by oestrogens in the skeletal-derived cells (KAYE, A.M. et al., 1997), uterus adipose tissue (SOMJEN et al., 1996), vascular smooth muscle cells (SOMJEN et al., 2002; SOMJEN et al., 2004). Phytoestrogens have oestrogen-mimetic effects on cell growth and CK in the cultural human vascular cells and on the CK in rat vascular tissues in vivo, and the effects on replication are highly dependent on CK concentration (SOMJEN et al., 2001). The metabolic antioestrogen effect of ZEN was established in group E2, while group E1 manifested the agonistic effect due to the ZEN reduction by way of clinoptiolite.
Amylase activity was reduced proportionally to the assessed quantity of zearalenone absorbed. Severe liver damage may cause the low level of serum pancreatic isoamylase (MARUYAMA et al., 2003). The activity of enzymes determined in our experiment indicate the opposite, although a histological test in E2 did establish changes.

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