DECREASED DIETARY Cu, Fe, Mn AND Zn LEVELS IN THE DIETS FOR WEANED PIGLETS WHICH INCLUDE WILD FLORA PLANTS

Eugenia Mircea[1*], Arabela Untea[1], Rodica Diana Criste[1], Nicoleta Lefter[1]

[1]National Research-Development Institute for Animal Biology and Nutrition - IBNA Baloteşti, Romania

Abstract

Part of the dietary inorganic salts was replaced by wild flora plants with the purpose to decrease the excreted Cu, Fe, Mn and Zn. Two experiments were conducted on three groups each (C, E1 and E2) of Landrace × Large White weaned piglets. The piglets received the same basal diet (64% corn, 14% soybean meal); the difference was the addition of either Echinacea (Echinacea purpurea), or stinging nettle (Urtica dioica), phytase and the vitamin-mineral premix. In the Echinacea experiment, the diet for group C included 1% vitamin-mineral premix, E1 received 3% Echinacea and 1% vitamin-mineral premix with 50% less Cu, Fe, Mn and Zn salts than C; E2 received 0.1% phytase in addition to E1. In the stinging nettle experiment E1 used 3% stinging nettle instead of Echinacea, and E2 received 0.2% phytase in addition to E1. The phytase was added in order to enhance the bioavailability of the plant minerals. In the Echinacea experiment, both the weight gains and the feed intakes displayed significant differences between group C and experimental groups, while the level of excreted Cu, Fe, Mn and Zn was significantly lower in the faeces from groups E1 and E2. In the stinging nettle experiment, the average daily weight gain was significantly higher in C than in E2, but the concentration of trace minerals in faeces was significantly lower in E1 and E2.

Key words: piglets, echinacea, stinging nettle, trace elements, faeces

INTRODUCTION

Even though the physiological requirements of the pigs are known [11], the issue of the mineral supplements in amounts and forms that support productions of proper amounts, of proper quality and under proper economic and ecologic conditions is still debated. In practice, the trace elements supplementation to animal diets is done according to the recommendation of the mineral premix (concentrates, mixtures) manufacturer. These recommendations are 2-3 times higher than the requirement determined by the scientific community. This approach, lead in time to the build-up of metals in the soil, some of them having toxic potential [10].

The ban on growth promoters directed the research towards alternative solutions to maintain animal health and productions. The interest for the plants and plant products not just as traditional remedies, but as dietary supplements for animals too, increased over the past three decades [1]. The use of botanical additives, essential oils and botanical mixtures is highly promoted by the producers, but the scientific support is limited.

Among the plants traditionally used in human nutrition and as possible feed additives are the Purple coneflower (*Echinacea purpurea*) and the stinging nettle (*Urtica dioica*). Many Echinacea species are known, but the traditional medicine uses just three of them (*Echinacea angustifolia*, *Echinacea pallida*, *Echinacea purpurea*) from the *Asteraceae* (*Compositae*) family. Purple coneflower used previously mainly in folk medicine now is the subject of research and its immunologic activity is well known [2]. Purple coneflower has anti-inflammatory effect (it inhibits the cyclooxygenase), antiviral effect (it stimulates the interferon synthesis) and bacteriostatic effect (it inhibits the hyaluronidase and blocks the bacterial replication. Echinacea has been used in piglet

*Corresponding author: mirceaeugenia@gmail.com
The manuscript was received: 05.02.2013
Accepted for publication: 11.06.2013

feeding and it improved feed conversion ratio [8]. The administration of Echinacea extracts to fat pigs resulted in high weight gains [3] and in a higher meat proportion in the carcass [5].

The effects of stinging nettle (*Urtica dioica*) consumption have been examined as far as in the antiquity. Nassiri-AslMet. [9] noticed that *Urtica dioica* extract has hypo-cholesterolemic effects in the animal model. The stinging nettle contains glucydes-like substances, amines, sterols, volatile oils, fat matter, acids (formic, acetic, pantothenic, folic), vitamins (A, C, B2and K), chlorophyll, Ca, Mg and Fe salts and other.

In the attempt to reduce the level of Cu, Fe, Mn and Zn in piglets' excreta by decreasing the dietary level of salts, while not hindering animal performance, two studies were conducted on the use of phytoadditives (purple coneflower and stinging nettle) in weaned piglets diets.

MATERIAL AND METHOD

Experiment 1

The experiment ran for 28 days, used 27 Landrace × Large White half-brothers, male, castrated pigs with an average initial weight of 9.47 ± 0.1 kg. Throughout the experimental period the piglets were randomly assigned to 3 groups (C, E1, E2) raised under farm conditions in 3 collective pens with an area of 2 m^2/pen. The piglets were fed the respective diets daily, at 8.00 a.m., by using a conventional open-front feeding area. Water was supplied via drinking nipples.

The piglets were fed ad libitum on 3 experimental isoprotein and isocaloric diets (Table 1). The diet of the control group C had 1% mineral vitamin premix Zoofort P1+2 produced by IBNA Balotesti. Compared to group C, group E1 included 3% Echinacea, while the Cu, Fe, Mn and Zn salts from Zoofort P1+2 were reduced by 50% compared to C; group E2 included 3% Echinacea, 0.1% phytase, and 50% less Cu, Fe, Mn and Zn salts compared to group C. One phytase unit is the amount of enzyme necessary to release 1 μmol inorganic P / minute from the sodium phytate, at pH 5.5 and 37°C.

Experiment 2

The experiment ran for 21 days, used 24 Landrace × Large White half-brothers, male, castrated pigs with an average initial weight of 12 ± 2.69 kg. The piglets were fed ad libitum on 3 experimental isoprotein and isocaloric diets (Table 1). Throughout the experimental period the piglets were randomly assigned to 3 groups - 8 animals per group (C*, E1*, E2*). Animal housing and all feed management criteria were as described for Experiment 1.

Table 1 Compound feed structure and quality indices

Ingredients		**Experiment 1**		**Experiment 2**	
	C, C* %	**E1 %**	**E2 %**	**E1* %**	**E2* %**
Corn	64.15	61.15	61.05	61.15	60.95
Sunflower meal	8.00	8.00	8.00	8.00	8.00
Soybean meal	14.00	14.00	14.00	14.00	14.00
Gluten	2.00	2.00	2.00	2.00	2.00
Powder milk	5.00	5.00	5.00	5.00	5.00
Oil	1.80	1.80	1.80	1.80	1.80
Monocalcium phosphate	1.40	1.40	1.40	1.40	1.40
Calcium carbonate	1.75	1.75	1.75	1.75	1.75
Sale	0.20	0.20	0.20	0.20	0.20
Methionine	0.10	0.10	0.10	0.10	0.10
Lysine	0.50	0.50	0.50	0.50	0.50
Choline	0.10	0.10	0.10	0.10	0.10
Premix Zoofort P1+2	1.00	-	-	-	-
Premix Zoofort P1+2 with 50% less Cu, Z, Fe, Mn	-	1.00	1.00	1.00	1.00
Phytase	-	-	0.10	-	0.20
Echinacea	-	3.00	3.00	-	-
Stinging nettle	-	-	-	3.00	3.00
Total	**100**	**100**	**100**	**100**	**100**

The diet of the control group C* had 1% mineral vitamin premix Zoofort P1 + 2 produced by IBNA Balotesti. Compared to group C*, group E1* included 3% stinging nettle, while the Cu, Fe, Mn and Zn salts from Zoofort P1+2 were reduced by 50% compared to C; group E2* included 3% stinging nettle, 0.2% phytase, and 50% less Cu, Fe, Mn and Zn salts compared to group C*.

Purple coneflower and stinging nettle were produced by SC Hofigal SA Bucharest from vegetal material grown without any chemical stimulators.

The piglets were weighed individually at the beginning, weekly and at the end of experiment. The feed given to animals was weighed on a daily basis and the data were used to calculate the average daily feed intake. The data on pig weight and on feed intake were used to calculate the average daily weight gain, the average daily feed intake and the feed conversion ratio (feed/gain).

Blood samples were collected from piglets at the end of experiments. The blood samples were collected from the jugular vein, in tubes with EDTA for hematologic determinations performed on a MINDRAY BC 2800 VET, AUTO HEMATOLOGYANALYZER (China).

Faeces samples were collected from each pen on a daily basis. The daily samples were pooled to form average weekly samples per pen and prepared for the determination of mineral concentration. The faeces samples were weighed, homogenized, dried at 65°C and ground.

Laboratory Analyses

The samples of the control and experimental diets, as well as the faeces samples were analysed for mineral concentrations applying flame atomic absorption spectrometry (FAAS) as described by Untea [12], after microwave digestion. All reagents were supplied by Merck (Darmstadt, Germany) and they were of analytical purity.

The analytical equipment included: Atomic absorption spectrometer Thermo Electron – SOLAARM6 Dual Zeeman Comfort (Cambridge, UK); Microwave digestion system with remote temperature measurement, BERGHOF, SpeedwaveMWS-2 Comfort (Eningen, Germany); Analytical scale Sartorius balance (Gottingen, Germany); Water distiller Milli-Q Ultrapure Water Purification System, Millipore (Billerica, USA).

Statistics: The analytical data were compared performing analysis of variance (ANOVA), using STATVIEW for Windows (SAS, version 6.0). The differences between mean values in the groups were considered significant at $P<0.05$.

RESULTS AND DISCUSSIONS

Purple coneflower and stinging nettle were included in the compound feed of the experimental diets at a level of 3%. Table 2 shows the concentration of minerals in the phytogenic additives.

The trace mineral concentrations in feed are shown in table 3.

Table 2 Mineral composition of phytogenic additives

Parameter	Purple coneflower	Stinging nettle
Ca (%)	2.25	1.02
P (%)	0.14	0.98
Cu (mg/Kg)	8.23	12.52
Fe (mg/Kg)	384.8	285.48
Mn (mg/Kg)	30.83	75.35
Zn (mg/Kg)	19.36	67.66

Table 3 Trace mineral concentrations in feed

Trace element	C, C*	E1	E2	E1*	E2*
Cu (ppm)	38.33	24.50	24.50	24.54	24.54
Fe (ppm)	271.22	233.01	233.01	229.05	229.05
Mn (ppm)	62.13	44.78	44.78	45.97	45.97
Zn (ppm)	161.26	107.78	107.78	109.08	109.08

The 50% of the inorganic salts from the mineral premix resulted in 15-36% lower concentrations of the trace elements compared to group C / C*.

The sharpest decrease was for Cu and the lowest for Fe.

Table 4 shows animal performance within the two trials.

Table 4 Animal performance

Group	Initial weight, kg	Final weight, kg	Feed consumption, g	ADG, g
	Experiment 1			
C	9.48 ± 0.91	23.63 ± 1.32[bc]	1033.40 ± 35.50[bc]	506.84 ± 16.10[bc]
E1	9.50 ±1.31	21.38 ± 1.64[a]	879.25 ± 32.02[a]	424.11 ± 14.53[a]
E2	9.43 ±1.57	21.75 ± 1.63[a]	902.51 ± 23.73[a]	440.00 ± 12.75[a]
	Experiment 2			
C*	12.00 ± 2.69	17.81 ± 3.34	833.33 ± 1.87	415.23 ± 14.12[c]
E1*	12.00 ±2.62	17.52 ± 2.82	843.48 ± 1.67	377.10 ± 10.58[a]
E2*	12.15 ±1.26	17.43 ± 1.53	862.22 ± 1.82	394.28 ± 12.53

Where: a – significantly different from C/C* (p≤0,05); b, c - significantly different from E1/E1*, E2/E2* (p≤0,05)

For experiment 1, Table 4 shows significant (P≤0.05) differences existed between group C and groups E1 and E2 both for the weight gain for the average daily feed intake. No differences were noticed in experiment 2, however. Although the trace elements (Cu, Fe, Mn, Zn) concentrations were reduced to half in the experimental diets, animal growth and performance were not affected.

The literature also reported other positive results of Purple coneflower use in pig diets. Hanczakowska [3] noticed that 500 mg Purple coneflower/kg feed increased the weight gain and improved the feed conversion rate. In 2004, Krusiński [6] found that 2.5% supplement of coneflower seeds to fatteners feed improved body weight gains, feed utilization and carcass meatiness. Kolesnyk M. [4] has shown the multilateral systemic effect of the Purple coneflower on the efficiency and economic feasibility using pigs of different ages and sex.

Table 5 Haematological parameters (average values/ group)

Haematological parameter	Experiment 1			Experiment 2		
	C	E1	E2	C*	E1*	E2*
White blood cells (109 / L)	22.05	19.02	19.32	15.56	16.16	15.22
Red blood cells (mil/mm3)	6.90	6.76	6.83	6.10	6.68	6.82
Haemoglobin (g/dL)	9.7	8.87	8.83	9.08	9.20	9.23
Hematocrit (%)	34.47	33.05	33.25	30.66	33.64	33.45
Mean cell volume (fL)	50.06	48.98	48.26	50.66	50.40	48.63
Mean cell hemoglobin (pg)	13.79	13.07	13.27	13.74	13.58	13.23
Mean corpuscular hemoglobin concentration (g/L)	276	268	269	273.20	270.20	266
Red blood cell distribution width (%)	17.12	18.03	17.72	17.18	15.96	15.82
Platelet (109 / L)	375	378	375	410.20	501.40	478

The haematological parameters (Table 5) observed for experiment 1 didn't show significant differences (P≤0.05) between groups, but haemoglobin (HB) concentration was lower in the experimental groups (in absolute value). Actually, HB concentration below 9 g/dL shows a slight anaemia, which means that the dietary Fe and Cu level may not be enough in these groups. In experiment 2, the values of HB concentration in the experimental groups were higher than in group C* due to high Fe concentration in the dietary nettle; however, no significant differences were noted for any studied parameter.

Table 6 shows the results of Cu, Fe, Mn and Zn determination in piglet manure.

Table 6 Trace elements concentration in piglet manure (average values/group)

Trace elements	Experiment 1			Experiment 2		
	C	E1	E2	C*	E1*	E2*
Cu (ppm)	175.7 ± 17.6^{bc}	100.5 ± 11.0^{a}	102.2±10.5^{a}	168.7±10.2^{bc}	103.61 ±12.53^{a}	113.27 ± 8.26^{a}
% din M	100	57.18	58.16	100	61.41	67.13
Fe (ppm)	1834.8±150.3^{bc}	1632.4±84.9^{a}	1696.2±64.2^{a}	1069.8±127.1	897.81 ± 24.45	889.72 ±21.23
% din M	100	88.96	92.44	100	83.92	83.16
Mn (ppm)	307.2 ± 32.0^{bc}	204.9±36.5^{a}	208.4 ± 16.1^{a}	270.4±13.0^{bc}	210.53 ±16.27^{a}	223.48 ± 5.68^{a}
% din M	100	66.71	67.83	100	77.84	82.62
Zn (ppm)	627.8 ± 26.0^{bc}	493.7±28.7^{a}	498.2 ± 15.6^{a}	578.6±21.0^{bc}	438.98 ±12.24^{a}	442.23±10.51^{a}
% din M	100	78.63	79.35	100	75.86	76.42

Where: a – significantly different from C/C* ($p \leq 0.05$); b - significantly different from E1/E1* ($p \leq 0.05$); c - significantly different from E2/E2* ($p \leq 0.05$).

The trace elements concentration in the faeces from the experimental groups in both experiments decreased significantly (in average by 39% for Cu; 12.8% for Fe; 26.2% forMn; 22.4% for Zn). This was an expected outcome, as the literature shows that trace elements elimination through the manure is directly proportional to trace elements ingestion [7]. The trace elements concentration in the faeces from the experimental groups was comparable (P>0.05) which shows that phytase, in the amount used in these experiments, could not mobilize the trace elements from the dietary ingredients.

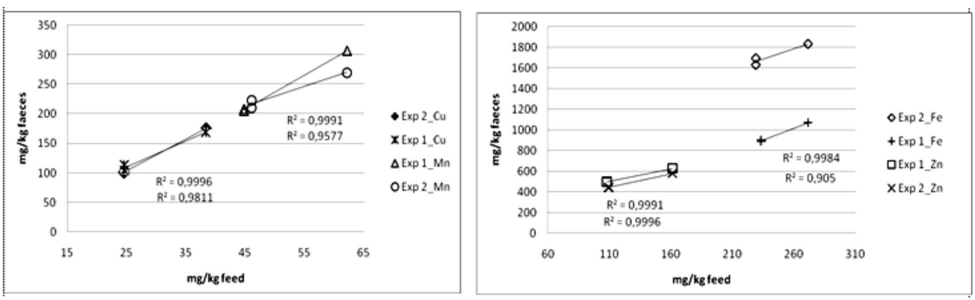

Fig. 1 The correlation between the mineral concentrations in the ingesta and excreta

The graphical representation of the correlation between the mineral concentrations in the ingesta and excreta, show a direct proportionality between the ingested level and the excreted level for all the studied groups (Fig. 1), as also reported by Zielinska [13]. The correlation factors exceed the value of 0.9.

As expected, the 50% reduction of the trace elements (Cu, Fe, Mn, Zn) included in the premix for the experimental groups, compared to the usual amount used for the piglets from groups C/C*, decreased the concentration of minerals in the excreta of these piglets (in average by 39% for Cu; 12.8% for Fe; 26.2% for Mn; 22.4% for Zn).

CONCLUSIONS

These experiments show that the inclusion of two types of phytoadditives, in piglet diets which had also inorganic compounds Cu, Fe, Mn and Zn in halved proportion compared to the commercial premix didn't affect drastically the bioproductive performances or the haematological parameters which were according to the species and category.

REFERENCES

[1] CFT/EFSA/FEEDAP/2005/01; Assessment of plants/herbs, plant/herb extracts and their naturally or synthetically produced components as "additives" for use in animal production.

[2] Goel V., Lovlin R., Chang C., Slama J.V., Barton r., Gahler R., Bauer R., Goonewardene L., Basu R., 2005: A proprietary extract from the echinacea plant (Echinacea purpurea) enhances

systemic immune response during a common cold. Phytother. Res., 19, 689-694.
[3] Hanczakowska E., Świątkiewicz M., 2007: Effect of feed supplementation with the purple coneflower (echinacea purpurea) extract on fatty acid profile and quality of pig meat, Pol. J. Food Nutr. Sci., Vol. 57, No. 4(B), pp. 229-233.
[4] Kolesnyk M., 2005: Use of purple cone-flower (Echinacea purpurea Moench) in feed additives, Bulletin of Agrarian science, (no.7) p. 26-28.
[5] Krusinski R., 2000: Influence of some herbs on performance of pigs end their meat quality, Annales Universitatis Mariae Curie-Sklodowska. Sectio EE Zootechnica, ISSN0239-4243, v. 18 p. 269-277.
[6] Krusiński R., 2004: The level of herb content in feed mixture for pigs. Ann. UMCS, EE, 22, 123-127 (in Polish).
[7] Lee S. H., Shinde P., Choi J., Park M., Ohh S., Kwon I. K., Pak S. I., Chae B. J., 2008: Effects of Dietary Iron Levels on Growth Performance, Hematological Status, Liver Mineral Concentration, Fecal Microflora, and Diarrhea Incidence in Weanling Pigs, Biological Trace Element Research, 126 (1), 57-68.
[8] Maass N, Bauer J, Paulicks BR, Böhmer BM, Roth-Maier DA., 2005: Efficiency of Echinacea purpurea on performance and immune status in pigs, J Anim Physiol Anim Nutr (Berl), 89(7-8): 244-52.
[9] Nassiri-Asl M, Zamansoltani F, Abbasi E, Daneshi MM, Zangivand AA, 2009 May: Effects of Urtica dioica extract on lipid profile in hypercholesterolemic rats. Zhong Xi Yi Jie He Xue Bao.; 7(5):428-33. doi: 10.3736/jcim20090506.
[10] Nicholson F., Chambers B.J., 2008: Livestock manure management and treatment: implications for heavy metals inputs to agricultural soils, Trace elements in animal production systems, ed. P. Schlegel, S. Durosoy, A.W. Jongbleod Wageningen Academic Publishers. The Netherlands. 55-63.
[11] NRC, 1998: Nutrient Requirements of Swine (10th Ed).. National Academy Press. Washington, DC.
[12] Untea A., Criste R.C. & Vladescu L., 2012: Development and validation of a microwave digestion – FAAS procedure for Cu, Mn and Zn determination in liver. Revista de Chimie. 63, 341-346.
[13] Zielińska A., Chojnacka K. and Simonič M., 2009: Sustainable Production Process of Biological Mineral Feed Additives, American Journal of Applied Sciences, 6, 1093-1105.