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THE SERUM LEVELS OF INSULIN AND IGF-I IN NEWBORN PIGLETS TREATED WITH CLINOPTILOLITE

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The aim of this study was to investigate the influence of the natural mineral adsorber clinoptilolite on the serum levels of insulin and insulin-like growth factor-I in newborn piglets in the first 30 hours postnatally. A total number of 40 crossbred Landrace x Duroc newborn piglets from 4 litters was used. Five piglets from each litter were randomly assigned to the treated group and another five piglets to the control group. The treated animals received 10 ml of 15% clinoptilolite suspension three times: immediately after birth and subsequently at 12 and 24 hours after birth.

Mean serum insulin level in the treated newborn piglets was almost 20% higher at both time intervals after treatment but the increases were not statistically significant, due to the high individual variation (44.40±6.33:36.40±5.14 and 17.54±2.61:14.02±1.14 mIU/L, treated vs. control at 10 and 30 hours postpartum). Serum levels of IGF-I were also increased in the treated newborn piglets, and the differences between means were statistically significant (18.20±0.63: 13.70±1.02 and 17.61±0.173:12.48±0.64 nmol/L, $p<0.001$, treated vs. control at 10 and 30 hours postpartum). Our results indicate that clinoptilolite treatment could effectively increase serum IGF-I and possibly also insulin levels in newborn piglets.

Key words: insulin, IGF-I, clinoptilolite, newborn piglets

INTRODUCTION

Maybe the most critical time in their whole life for the survival of animals is the period immediately after birth. Until that period the young animal has depended entirely upon the placenta for the supply of oxygen and all other nutrients, and then it starts on independent life. One of the characteristics of growth in neonatal pigs is highly efficient nutrient utilization associated with high rates of skeletal muscle protein synthesis and deposition (Susenbeth and Keitel, 1987; Skjaerlund *et al.*, 1994). Possible factors responsible for the rapid postnatal development include genetic programming, effects of dietary nutrients and actions of colostrum-derived growth factors or hormones (Wang and Hu, 1996). Artificial feeding trials showed that newborn pigs fed porcine colostrum had heavier intes-

tines than those fed on mature milk or milk formula (Reinhart *et al.*, 1992). Insulin like growth factor-I (IGF-1) has been detected in the mammary secretions of various species including pigs and humans (Baxter *et al*., 1984; Simmen *et al*., 1988). Porcine colostrum contains 10- to 500-fold higher levels of IGF-I than mature milk (Donovan *et al.*, 1994). Insulin has also been reported in human, porcine and bovine milk, and its level in bovine colostrum is 3- to 10-fold higher than in mature milk (Kulski *et al.*, 1983; Jaeger *et al.*, 1987; Malven *et al.*, 1987). Insulin is readily taken up from the maternal circulation by the lactating bovine mammary gland and absorbed intact from the digestive tract of newborn piglets and calves (Schams *et al.*, 1991; Burin *et al.*, 1992).

Clinoptilolite is a naturally occurring mineral adsorbent obtained by technological preparation of the zeolite tuff from Zlatokop (south Serbia). Made of a special crystalline structure that is porous but remains rigid in the presence of water, it can be adapted for a variety of uses. It has been reported that clinoptilolite can increase the rate of colostral immunoglobulin G absorption in newborn calves and piglets (Stojić *et al.*, 1995; Stojić *et al.*, 1998). The aim of this work was to determine the effects of clinoptilolite on the blood serum concentrations of IGF-I and insulin in newborn piglets during the first 30 hours of life.

MATERIAL AND METHODS

Experimental design. A total of 40 crossbred Landrace x Duroc newborn piglets from 4 litters was used in the present study. Five piglets from each litter were randomly assigned to the treated group and another five piglets were used as a control group. The treated animals received 10 ml of 15% clinoptilolite suspension three times: immediately after birth and subsequently at 12 and 24 hours after birth. Due to technical difficulties such as lipidemia from the control group of piglets one serum sample for insulin analysis was omitted at 10h and three serum samples were omitted at 30h for both insulin and IGF-I analyses.

***Blood serum collection*.** Venous blood samples were obtained from the retroorbital sinus at 10 and 30 hours after birth. After coagulation and centrifugation the blood serum was aspirated and stored at -20°C until analysed.

***Preparation of clinoptilolite suspension*.** Clinoptilolite (Minazel-S, ITNMS, Belgrade, Serbia and Montenegro) suspension was prepared according to the producer's instructions. The chemical composition of Minazel-S is given in table 1. as determined on an ARL 94000 X-ray spectrometer.

Table 1. Chemical composition of the mineral adsorbent (%).

Component	SiO_2	Al_2O_3	Fe_2O_3	TiO_2	CaO	MgO	Na_2O	K_2O	L.I.
Content	66.46	12.77	2.66	0.12	3.22	1.11	0.78	1.21	9.15

The cation exchange capacity (CAC) and type of exchangeable cations were determined by the ammonium acetate method (Table 2).

Table 2. CEC and exchangeable cations of the mineral adsorbent.

Exchangeable cation	Ca^{++}	Mg^{++}	Na^{++}	K^{+}	Total
CEC mmol/100g	121	25	25	2	173

Determination of serum insulin and IGF-I concentrations. Blood serum insulin concentrations were determined by radioimmunoassay in accordance with the instructions (INEP Diagnostics, Zemun). The mean intraassay coefficient of variation of duplicate samples was 3-4%. Total serum IGF-I concentrations were determined after separation of binding proteins by acid-ethanol extraction with cryoprecipitation (Daughaday *et al.*, 1982; Breier *et al.*, 1991). The radioimmunoassay has been validated for swine serum (Nikolić *et al.*, 1996). Mean (SD) recovery of reference IGF-I (WHO/518) added to porcine serum samples was 92(9.5)% (n=3) and the mean intraassay coefficient of variation of duplicate determinations was 5.9%.

Statistical analysis. The results are expressed as mean (M), standard deviation (SD), standard error (SE) and CV for each group of piglets. Probability and statistical significance of differences between mean values were calculated using Student's t-test.

RESULTS

Blood serum insulin concentrations. The results for blood serum insulin concentrations are presented in Table 3.

Table 3. Blood serum insulin concentrations (mIU/L) in the control and treated group of neonatal piglets.

	Blood serum sampling time			
	10 hours postpartum		30 hours postpartum	
	Treated	Control	Treated	Control
	N = 20	N =19	N =20	N =17
M	44.40	36.40	17.54	14.02
SD	28.30	22.40	11.66	4.71
SE	6.33	5.14	2.61	1.14
CV(%)	63.74	61.54	66.48	33.59

It can be seen that clinoptilolite treatment tended to increase the blood serum insulin concentration in neonatal piglets during the first 30 hours of postnatal life. This increase of almost 20% in the mean serum insulin concentration in the treated group of piglets was evident at 10 as well as at 30 hours postpartum (44.40±6.33:36.40±5.14 and 17.54±2.61:14.02±1.14 respectively). Due to the

high individual variation of serum insulin concentration (high CVs in the treated and control group at 10 hours, and in the treated group at 30 hours) the differences between the means for the treated and control group of piglets were not statistically significant.

Blood serum IGF-I concentrations. The results for serum IGF-I concentrations are presented in table 4.

Table 4. Blood serum IGF-I concentrations (nmol/L) in the control and treated group of neonatal piglets.

	Blood serum sampling time			
	10 hours postpartum		30 hours postpartum	
	Treated	Control	Treated	Control
	N = 20	N =17	N = 20	N =17
M	18.20**	13.70	17.61**	12.48
SD	2.80	4.90	3.27	2.62
SE	0.63	1.02	0.173	0.64
CV (%)	15.38	35.77	18.57	20.99

** - $p<0.001$

They show that the blood serum IGF-I concentrations in newborn piglets treated with clinoptilolite were highly significantly increased at 10 and 30 hours postpartum (18.2 ± 0.63:13.7 ± 1.02, $p<0.001$; 17.61 ± 0.173:12.48 ± 0.64, $p<0.001$). Variability of the blood serum IGF-I concentrations was lower than that for insulin, which is evident from the lower coefficients of variation (CVs). Our data presented in the table 2. also imply that the individual variability of blood serum IGF-I concentrations is very much reduced at the first sampling time (CVs at 10 hours postpartum, treated vs. control, 15.38:35.77), being almost equal at the second sampling time (18.57:20.99).

DISCUSSION

Ingestion of an adequate amount of colostrum is a vital factor for the survival of newborn piglets. Colostrum contains not only maternal immunoglobulins that are essential for the establishment of natural passive immunity, but it is also the source of nutrients, which are important in the thermoregulation process (Herpin *et al*., 1995), highly digestible proteins (Pluske *et al*., 1995) and numerous growth factors and hormones (Donovan *et al.*, 1994; Burrin *et al*., 1997). Concentrations of insulin, IGF-I and IGF-II in colostrum are relatively high and decrease considerably during lactation (Simmen *et al*., 1988; Donovan *et al.*, 1994). Because of the mitogenic and anabolic nature of these growth factors it is a plausible hypothesis

that increased ingestion of those factors from colostrum could be an important factor for enhanced growth rate in newly born piglets.

In a series of studies aimed to determine the effects of colostrum or milk on the rate of the protein synthesis in newborn pigs, it was concluded that the anabolic skeletal muscle response to colostrum could not be attributed to either circulating insulin or amino-acid concentrations but was associated with increased circulating IGF-I concentrations (Burrin *et al.*, 1992; Fiorotto *et al.*, 1995).

The uptake of macromolecules by the intestinal epithelial cells apparently involves both non-specific and receptor mediated endocytosis. It is well documented in newborn pigs that macromolecules such as immunoglobulins, dextrans, and bovine serum albumins are readily absorbed during the immediate postnatal period (Westrom *et al.*, 1984). Homologous immunoglobulin are preferentially absorbed at a higher rate than foreign proteins and nonprotein macromolecules (Burton *et al.*, 1977). In rats selective absorption of immunoglobulins involves binding of the molecules to the Fc receptors of the microvillous membrane of the epithelial cells in the jejunal region, and non-specific absorption of macromolecules occurs in the ileal region of the small intestine (Weaver *et al.*, 1989). In the jejunum, immunoglobulins bound to the receptors are taken up by endocytosis and then discharged at the basolateral membrane by exocytosis. In the ileum macromolecules are taken up by nonselective, fluid-phase endocytosis and then transferred to a giant supranuclear vacuole in which the macromolecules are degraded (Gonnella *et al.*, 1984).

Since there is significant absorption of intact macromolecules during the perinatal period (Lecce, 1973) there is a reasonable probability that ingested polypeptide growth factors can be absorbed intact from the intestinal lumen into the blood and induce an increase in the circulating concentration. However, many studies in neonatal pigs, calves and rodents have demonstrated that oral administration of IGF-I, even in pharmacological doses, does not affect circulating IGF-I concentrations (Baumrucker *et al.*, 1994; Houle *et al.*, 1997). Further evidence, based on direct measurements of [^{125}I]IGF-I administered orally to formula-fed neonatal pigs, also suggested that intestinal absorption of IGF-I is probably not responsible for the increased circulating IGF-I concentration observed in neonatal pigs fed colostrum. It seems that circulating IGF-I largely originates from hepatic secretion. In neonatal animals nutrient intake could have a major influence on the hepatic expression and circulating concentration of IGF-I (Breier *et al.*, 1989). An increase in circulating IGF-I concentration in response to feeding occurs gradually: typically a significant increase above the fasting baseline requires at least 6 hours (Davis *et al.*, 1996). Our previous results concerning serum IGF-I levels in newborn calves indicated that it is dependant on the amount of ingested colostrum (Kirovski *et al*., 2002). Our present study indicates that clinoptilolite treatment in newborn piglets effectively increases serum IGF-I concentration. This increase is consistent and less variable than the apparent increase in serum insulin concentration. Assuming that the amount of ingested colostrum is uniform in the treated and control group of piglets, the difference in the blood serum IGF-I concentrations could not be attributed to this factor, as was the case in our previous experiment in calves (Kirovski *et al.*, 2002). However, we cannot exclude the possi-

bility of greater endogenous synthesis of IGF-I in various tissues of the neonatal piglets. Nevertheless, the regular increase in the serum IGF-I concentration of newborn piglets might indicate that, contrary to the conclusions of some authors (Baumrucker *et al.*, 1994; Houle *et al.*, 1997), there is effective absorption of intact porcine IGF-I molecules from the intestine of newborn piglets during the first 30 hours of life and that clinoptilolite treatment is an effective stimulator of such absorption in that critical period of life.

The exact place and mechanism of clinoptilolite action has not been completely elucidated, but several possible modes of action have been proposed. It is well known that it can efficiently bind aflatoxins B1 and G1 (Tomašević-Čanović *et al.*, 1994). This led to a hypothesis that clinoptilolite could bind some degradation products of colostral proteins in the intestine, thus preventing possible negative effects on the intestinal epithelial cells (Stojić *et al.*, 1995; Stojić *et al.*, 1998). Another possible mechanism of clinoptilolite action on the intestine epithelial cells could be direct effects on specific cell receptors. Namely, it has been documented that clinoptilolite treatment of mice and dogs suffering from various types of tumor led to improvement of the overall health status, prolonged life span, and decrease of tumor size (Pavelić *et al.*, 2001). *In vitro* tissue culture studies showed that finely ground clinoptilolite inhibits protein kinase B (c-Akit), induces the expression of $p21^{WAF1/CIP1}$ and $p27^{KIP1}$ tumor suppressor proteins, and blocks cell growth in several cancer cell lines. We could speculate that clinoptilolite treatment reduces the turn-over exchange rate of intestine epithelial cells, prolonging their lifespan and activity. This could influence the rate of colostral IGF-I absorption.

Insulin was the first peptide shown to be absorbed from the neonatal GI tract in a biologically active form. Oral administration of pharmacological levels of insulin to the suckling piglet (20 U/100 g BW) resulted in hypoglycemia (Asplund *et al.*, 1962), which indicates that insulin is absorbed intact and retains its ability to stimulate glucose uptake. Insulin receptors on jejunal and ileal brushborder membranes may allow for direct action upon the enterocyte and/or receptor –mediated uptake. Recent studies have both supported and contradicted these earlier observations on insulin absorption. In studies in which neonatal piglets and calves were fed colostrum or mature milk, serum insulin levels were two- and fourfold higher, respectively, in colostrum-fed neonates than in those fed mature milk (Burrin *et al*., 1992; Schams *et al.*, 1991). These results supported the concept of insulin absorption from the neonatal intestine. Neither study determined whether this discrepancy was due to absorption of colostral insulin or to enhanced endogenous secretion, although endogenous insulin secretion is thought to be suppressed for at last 12-48 hours postpartum (Grütter *et al.*, 1991). In contrast, two studies in which insulin was added to a formula (85 U/L) (Shulman, 1990) or administered orally (50 mg/100 g BW) (Grütter *et al.*, 1991) immediately prior to feeding colostrum did not result in a rise in serum insulin or a decline in blood glucose.

Our previous results concerning the effects of a clinoptilolite based mineral adsorbent on colostral immunoglobulin (Ig) absorption in newborn calves and piglets showed that it significantly increases Ig absorption (60% increase in newborn piglets 24h postpartum) (Stojić *et al.*, 1995; Stojić *et al.*, 1998). Our present results indicate that mineral adsorbent treatment in the newborn piglets could

also result in increased serum insulin and IGF-I concentrations despite their much shorter half-lives in the peripheral circulation (Table 3 and 4). Allthough mean insulin concentration increased by almost 20% compared to the control group of piglets, the high individual variation prevented statistical significance of the difference. Serum insulin concentrations change rapidly in relation to nutrient intake. The high individual variation was probably due to different amounts and rates of colostrum ingestion, as well as differences in the intestinal absorption capacity for biologically active substances from colostrum, and individual variation in the rate of gut closure, which occurs between 8 and 36 hours pospartum (Burrin *et al.*, 1997). Nowak (1990) reported that, in newborn piglets, serum glucose concentration before suckling is highly differentiated, and intramuscular loading of nursing sows with insulin (80 IU per animal) caused an increase in the concentration of insulin in colostrum from 1.365 to 3.449 nmol/L. At the same time mean insulin level (0.313 ± 0.04 nM/L) in the piglet blood plasma ($n = 42$) increased significantly to 1.234 ± 0.07 nmol/L ($p < 0.001$) after suckling by sows loaded with exogenous insulin. It is interesting that the glycaemic response of the piglets was very different, being poor in two litters, but showing a statistically significant increase at the same time, while in the other three litters the glucose concentrations in blood plasma samples did not change after sucking. The author excluded the hypothesis that the high level of insulin in colostrum could be the cause of hypoglycaemia in healthy piglets after sucking, but at the same time these results showed that there was definite increase of blood serum insulin concentration after intake of colostrum containing insulin. Previous results from the same author (Nowak, 1989) indicated that insulin administered orally to newborn piglets is very effective in decreasing blood serum glucose concentration (from an initial level of 4.7 nmol/L to 2.55 nmol/L), and that "gut closure" occurs in piglets between 30-40 hours after birth. Our finding of a slight increase in blood serum insulin concentration after clinoptilolite treatment could be at least partly due to an increase in the rate of absorption of intact insulin molecules from colostrum.

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KONCENTRACIJA INSULINA I IGF-I U KRVNOM SERUMU NOVOROĐENE PRASADI TRETIRANE KLINOPTILOLITOM

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SADRŽAJ

Cilj rada je bio da se ispita uticaj peroralnog davanja praparata Min-a-Zel S, koji sadrži aktivnu komponentu klinoptilolit, na nivo insulina i insulinu-sličnog faktora rasta-I (IGF-I) u krvnom serumu novorođene prasadi u prvih 30. časova postnatalnog života. U ogled je bilo uključeno 40 prasadi koji su poticali iz 4 legla. U svakom leglu polovina prasadi je dobijala po 10 ml 15% suspenzije Min-a-Zela S, i to odmah posle rođenja, 12. i 24. časa po rođenju. Druga polovina prasadi je služila kao kontrolna grupa. Krv za analizu nivoa ovih biološki aktivnih jedinjenja je uzimana 10. i 30. časa neonatalnog perioda.

Koncentracija insulina kod ogledne grupe prasadi u ispitivanim vremenskim periodima bila je za 20% veća u odnosu na kontrolnu grupu, ali zbog velikih individualnih varijacija razlika nije bila statistički značajna.

Na drugoj strani, koncentracija IGF-I u krvnom serumu ogledne grupe prasadi u oba ispitivana perioda je bila statistički vrlo značajno viša u odnosu na kontrolnu grupu.

Ovi rezultati ukazuju da peroralno davanje klinoptilolita u vreme napajanja prasadi sa kolostrumom može uticati na značajno povišenje koncentracije ovih biološki aktivnih jedinjenja u krvnom serumu. U radu su razmatrani i mogući mehanizmi delovanja klinoptilolita na stepen resorpcije i/ili na povećanje endogene sinteze ovih jedinjenja u organizmu novorođene prasadi.