The impact of rare earth elements on growth, energy-, carbon- and nitrogen-balance of piglets

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Inaugural-Dissertation

zur Erlangung der Doktorwürde der
Vetsuisse-Fakultät Universität Zürich

vorgelegt von

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Zürich 2006

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1. Summary

Forty weaned barrows at the weight period of 9-56 kg were used to investigate the impact of dietary Rare Earth Elements citrate (REE) on their energy (GE), nitrogen (N) and carbon (C) balance. Furthermore, possible influence of REE on selected blood serum parameters and bone mineral content was determined and its properties as a growth promoter were evaluated.

Three different additives were supplemented in a usual diet and fed ad libitum (150 ppm REE (EG1), 300 ppm REE (EG2) or 100 ppm sodium citrate (CO)). In two weight periods (20 kg (P1) and 50kg (P2)) piglets were set in indirect respiration calorimetry chambers for balance analyses, each time for 96 h. Blood samples were taken four times during the fattening period and after slaughtering the bones of the left metatarsus were collected for the assay of ash, calcium (Ca), phosphorus (P) and magnesium (Mg).

Supplementation of REE had no effect on daily weight gain. GE and N digestibility enhanced as well as feed conversion ratio (FCR) decreased by 7% in piglets fed EG1 compared to CO. Furthermore EG1 barrows indicated a tendency to a 14% better nitrogen utilization and a 17% enhanced protein accretion during P2. Nutrient digestibility and FCR were not modified by EG2 diet.

Blood parameters were not affected by REE intake. Metatarsi of piglets fed REE diet contained less Ca and exhibited a decreased Ca/P ratio compared to CO. Mg concentration in ash was reduced by feeding REE citrate compared to CO (13% in EG1 and 24% in EG2; $P<0.001$).

2. Introduction

Rare Earth Elements (REE) are 17 elements within the periodic table of elements, which are Lanthanum (La), Scandium (Sc), Yttrium (Y) and the 14 lanthanides Cerium (Ce), Praseodymium (Pr), Neodymium (Nd) , Promethium (Pm), Samarium (Sm), Europium (Eu), Gadolinium (Gd), Terbium (Tb), Dysprosium (Dy), Holmium (Ho), Erbium (Er), Thulium (Tm), Ytterbium (Yb) and Lutetium (Lu). They are similar in most of their physical and chemical properties. In China they have been successfully used as fertilizer in plant production and as growth promoter in animal production for more than 40 years. In this country there are a lot of studies about the positive performance enhancing effects of REE on almost all kinds of productive livestock (Rambeck et al., 2005 and Redling 2006). So for example Chen (1997), Hu et al. (1999) and Xu et al. (1999) reported that the application of REE to feed of piglets increases daily weight gain up to twenty percent and feed conversion rate up to fifteen percent proportional to not supplemented control. But the Chinese results can not be simply transferred to European agriculture conditions because there are a lot of differences, especially in genetics, housing and feed of animals. Since the ban of antibiotics 1999 in Switzerland and from 2006 in the European Union there is a great demand for alternative growth promoters. Advantages of REE for this application purpose are that they leave only low residues in environment and carcasses after supplementation and that there is a large margin separating the effective dosis from the toxic dosis (Arvela, 1979 and Borger, 2003). Therefore since 1999 studies were conducted to evaluate REE as a growth promoter in European agriculture, especially in pig fattening. The predominant number of studies about REE supplemented piglets in Europe had evinced at least numerically an increased growth promoting effect (Table I.), although to date significant positive effects on daily weight gain and feed conversion rate were only observed in a few of these studies (Borger, 2003; Kessler, 2004 and Knebel, 2004). In contrast to these findings Böhme et al. (2002), Eisele (2003) and Kraatz et al. (2004) noticed negative effects of REE. The additives which were applied varied largely in the composition, the organic compound and the doses of the used REE. Therefore standardized products are required because of a better comparability of the results. Furthermore for a successful and safe use of a growth promoter, knowledge is necessary about the acting mechanisms of these supplements in organism. According to Arvela (1979) generally only a small amount of lanthanids of less than 0.05% of oral admission would be absorbed and Cochran et al. (1950) evaluated a total retention of radio marked Lanthanum chloride of less than 0.3% in rats after four days of oral substitution. So it stands to reason that the acting site of REE additives basically would be the gastrointestinal tract and thus they could influence digestibility of nutrients. Böhme et al. (2002)

observed no affect on digestibility by REE supplemented pigs in opposite to Chinese studies (Hu et al, 1999). Besides Muroma (1958 and 1959) evaluated blocking and stimulating affects of REE on different microorganism populations in vitro, but studies in artificial rumen as well as in gut flora of poultry and piglets (Schuller et al., 2002; Knebel, 2004 and Kraatz et al., 2004) did not sign up any impact on microflora in vivo. Nevertheless lanthanides are in discussion to influence nutrient absorption and/ or enzyme activation in the gastrointestinal tract (Darnall et al., 1970; 1973 and Tanswell et al., 1974). Chemical and physical properties as well as structure of REE ions are on the main lines in analogy with Calcium (Ca) and other alkaline earth metal ions like Magnesium (Mg). This is why REE are frequently able to replace these ions in their specific binding sites. In most cases REE have even stronger chemical reactions and they bond more stable than alkaline earth metal ions do (Evans, 1990). So it is possible, that even a low absorbed amount of REE could have an influence on a lot of different biological moieties and on the intermediary metabolism of mammalians. The definite mode of action, especially their exertion of influence on growth parameters is not totally known, although there are a lot of experimental information about metabolism and acting sites of the lanthanides (Ellis, 1977). Additional knowledge on this might help to verify why the results on the function of REE as a growth promoter are inconsistent. So the aim of the study was to determine if REE citrate has an impact on energy (GE), carbon (C) and nitrogen (N) balance and digestibility of growing piglets, as well as to evaluate the potential of a commercial REE citrate compound (composed with La, Ce, Pr and Nd citrate and starch) as a growth promoter. Furthermore the effect of the chronicle REE supplementation on selected blood serum parameters and on the concentration of the main minerals in bones of metatarsus (Ca, Mg and Phosphorus (P)) were analyzed. For these objects piglets were fattened in three different feeding variants (150 ppm REE citrate, 300 ppm REE citrate and 100 ppm sodium citrate).

Table I. *Survey of European study results of the use of different REE compounds in fattening pigs*[1]

Animals		Additives	DWG*	FCR*	Author
Start weight	**Term**		**% Control**	**% Control**	
7 kg	6 weeks	75 mg La chloride	102	95	Rambeck et al. (1999)
		75 mg REE chloride	100	97	
		150 mg La chloride	102	96	
		150 mg REE chloride	105	93	
18 kg	11 weeks	300 mg diff. REE chloride	105	101	Eisele (2003) Trial I
			100	100	
			98	100	
18 kg	11 weeks	300 mg diff. REE chloride	104	101	Eisele (2003) Trial II
			105	98	
			104	98	
8 kg	to 20 kg	200 mg REE chloride	110	98	Eisele (2003) Trial III
8 kg	16 days	200 mg REE chloride	103	91	Eisele (2002) Trial IV
42 kg	to 90 kg	100 mg REE (diff. org. comp.)	97	103	Böhme et al. (2002)
18 kg	to 50 kg	150 mg La/Ce chloride	119[a]	89[b]	Borger (2003)
50 kg	to 105 kg		112	97	
7 kg	6 weeks	200 mg REE citrate	97	99	Kraatz et al. (2004)
			101	103	
9 kg	6 weeks	50 mg REE citrate	100	98	Knebel (2004)
		100 mg REE citrate	91	94	
		200 mg REE citrate	113[a]	94[b]	
25 kg	to 104 kg	200 mg REE citrate	109[a]	96[b]	Kessler (2004)
8 kg	5 weeks	150 mg REE citrate	96	99	Gebert et al. (2005)
		300 mg REE citrate	96	96	

[1] Mean values with different superscripts in a row are significantly different ($P<0.05$).

3. Material and Methods

3.1. Animals and treatments

Forty weaned barrows at the age of 32±2 days were housed at the stables of the Institute of Animal Sciences at the Swiss Federal Institute of Technology, Zurich. Pedigree races of the crossbreds were in each case share in 25% Swiss Large White and 25% Swiss Landrace combined with either 50% Duroc or 50% Swiss Large White. Their initial weight was 8.6±0.4 kg. At intervals of two weeks four piglets of two litters were randomly assigned pairwise in the three different supplemented feeding groups (Table II.).

Table II. *Variable supplements*

	Sodium citrate (mg/kg)	**Lancer®[1] (mg/kg feed)**
Control (CO)	100	0
Experimental group 1 (EG1)	0	300
Experimental group 2 (EG2)	0	600

[1] 100 mg Lancer® = 50 mg Rare Earth Elements citrate + 50 mg wheat starch**;** Lancer® (Zehentmayer AG, CH - 9305 Berg)

At the outset seven couples were disposed in the control group (CO) and in the 150 ppm REE supplemented group (EG1) while in the 300 ppm REE group (EG2) six pairs of piglets were stabled. The 1.5 m^2 sized pig pens were arranged under habitual husbandry conditions with a nipple watering place. During nine days piglets were gradually adapted from the piglet start up feed restrictively accustomed to the lasting experimental feed which was offered ad libitum. So day nine was the start term of the relevant data collection of the trial. In feed Celite 545 was added as an indicator for the digestibility of nutrients by increasing hydrogen chloride insoluble ash. The three different supplemented diets were pelleted without any other growth promoting essence (Table III.).

Table III. *Composition of the basal diet*

Basal diet

Components	**%**	**Calculated nutrient content (89% dry matter)**	**per kg feed**
Barley	20.0	Ash	48.4 g
Wheat	20.0	Crude Protein	178.3 g
Maize	20.0	Crude fat	57.1 g
Oat	5.0	Crude fiber	28.7 g
Dextrose	1.5	NfE[4]	573.4 g
Oat flakes	10.0	Gross energy	14.1 MJ
Soybean meal 43% CP	7.0		
Potato protein	2.5		
Fish meal 70/ 72 CP	5.0		
L- Lysine- HCl	0.48		
DL- Methionine	0.13		
L- Threonine	0.16		
Calcium carbonate	0.85		
Mono- calcium phosphate	0.74		
Sodium chloride	0.34		
Animal fat	2.5		
Calcium propionate	0.3		
Organic acids[1]	0.8		
Vitamins and minerals[2]	0.6		
Celite 545	1.1		
Variable supplements[3]	1.0		

[1] Organic acids: 50% formiate acid and 50%.lactate acid

[2] Vitamins and minerals supplied per kg feed: Vit.A, 20000 IE; $VitD_3$, 2000mg; Vit E, 50 mg; Vit. K, 1.25 mg; Vit. B1, 2 mg; Vit. B2, 6 mg; Vit. B6, 5 mg; Vit. B12, 0.02 mg; Biotin, 0.2 mg; Ca-pantothenate, 15 mg; Niacin, 30 mg; folic acid, 0.5 mg; Betaine, 165 mg; Fe, 100 mg; Zn, 100 mg; Mn, 40 mg; Cu, 15 mg; I, 1 mg; Se, 0.5 mg

[3] Variable supplements: Table I, each complemented with maize starch to 1%

[4] NfE: Nitrogen free extractives

3.2. Experimental procedure

Two weeks after the start of the trial the piglets were initially set pairwise in steel collection cages for 24 hours (h) for the purpose of acclimatization. After two days of intermission they were placed there again for 96 h. That was the first metabolic measure period (P1). The cages were equipped with a permanent nipple watering place, feeding dispenser and a steel grid floor with an underlying large funnel

to collect faeces and urine separately. During the respiration period, boxes were positioned in one of the both 1.21 m^3 capacious respiration chambers in which gaseous exchange could be recorded according to an open circuit indirect calorimetry system (Hadorn, 1994). A climate control unit adjusted temperature at 20.5±1.1° C, humidity at 50.9±7.0% and air pressure constant at 964±4 hPa. Air flow rate was tuned in at 13.9±0.1 m^3/h and was permanently measured with inline electronic flow meters (Swingwirl DV 630; Flowtec AG, Reinach Switzerland). Carbon dioxide and methane production were recorded with an infrared analyzer (NGA 2000, Fisher-Rosemount, Ohio, USA). In addition the usage of oxygen was detected paramagnetic with an Oxymat (Siemens AG, Karlsruhe, Germany). Every 22.5 h the measurement phases stopped for 1.5 h to take urine and faeces samples, to refill feed, to clean the cages and to calibrate gas analyzers manually with reference gases. Data were subsequently extrapolated to 24 h.

Urine was disposed in 20 litre containers with 25 ml of thymol isopropanol solution in function as the preserving additive. After filtration an aliquot (1%) of the daily urine volume was taken. Each day in P1 a similar amount of faeces was collected, too. Received urine and faeces samples of current 96 h were admixed and frozen at -20 ° C.

One week after the P1 was finished animals were singled in pig pens. Three weeks later the heavier piglet of the primal couple was single placed in a metabolic cage that was set in one of the both 5.44 m^3 capacious respiration chambers for 96 hours (P2). System and performance were similar to that described in P1. A week shifted the remaining second piglet was put in the same treatment. The piglets were slaughtered at the weight of 55.5±4.1 kg, either four or five days after uncasing of respiration chambers. Left lower legs were drawn off the carcasses. In the following the metatarsal bones were anatomized and manual dissected of soft tissues. After that the bones were accordingly stored at -20 ° C until analysis. Four times during weaning (start of the trial, start day of P1, start day of P2 and the day before slaughtering) blood samples were taken by vena cava cranialis punctuation 12 hours after feed removal. Serum without any ingredient was separated by centrifugation 15 minutes at 3500 rpm and 4 ° C (Varifuge K, Heraeus-Christ GmbH, Osterode, Germany) and placed in freezer at -20 ° C until assay. During the fattening period piglets were individually weighed weekly and feed consumption was recorded pen wise daily.

3.3. Laboratory analyses

Faeces were lyophilized for 48 hours (Beta 116, Christ, Osterode, Germany). Dehuminidified faeces and feed were milled centrifugally through a 1 mm sieve (Retsch ZM 1, Arlesheim, Switzerland). Both were determined for dry matter (DM) and total ash (automatically by TGA-500, Leco Corporation, St. Joseph, Michigan, USA), carbon and nitrogen (C/N Analyzer, Leco-Analysator Typ FP-2000, Leco Instrumente GmbH, Kirchheim, Germany (Nitrogen x 6.25 = crude protein)) and heat of combustion (adiabatic combustion calorimeter C 7000, IKA-Werke GmbH und Co. KG, Staufen, Germany) as well as crude fiber and crude fat concentrations of feed were analyzed according to methods of VDLUFA (Naumann et al., 1997). Concentrations of La, Ce, Pd and Nd in feed were analyzed with the Varian Ultra Mass^{TM} ICP-MS- system (Varian Optical Spectroscopy Instruments, Melbourne, Australia) at methods of Forrer et al. (1998). Urine energy was calculated according to the equation of Hoffmann et al. (1980) by use of determined contents of C and N in urine with C/ N analyzer. Digestibility was calculated using indicator method (Prabucki et al., 1975) and displayed in the ratio of the sum of the digestible nutrients proportional to the intake of nutrients. Displayed balances are based on metabolic body mass incorporating factor 0.75 ($BM^{3/4}$). Balances are generated according to Hadorn (1994). In doing so the equation of Brouwer (1965) was used to investigate heat production (Q), energy balance (calculated as metabolizable energy (ME) divided Q) and energy efficiency (K) indirectly according to ARC (1981). Retained protein (protein = N x 6.25) and retained fat were expected to contain 23.8 kJ/g and 39.7 kJ/g, respectively, to estimate protein and fat accretion.

Bones of the left metatarsus were set for 96 hours at 105 ° C to record dry matter first and thereafter they were set there at 600 ° C for 96 h to determine ashes. After weighing 160 ml of an 8% hydrochloric acid was added to each of the ash samples, then they were filtered and mineral contents in solutions were analyzed by calorimetry (Cobas Mira®, Roche-autoanalyzer, Hoffmann-La Roche, Basel, Switzerland) using methods of methylthymol blue for Ca, phosphomolybdate without precipitation of proteins for P and calmagite for Mg concentration determination. Serum parameters of total protein, albumin, urea, creatinine, alkaline phosphatase (AP), glucose, triglycerides, and cholesterol were analyzed photometric by employing commercial kits (Cobas Mira®, Hoffmann-La Roche, Basel, Switzerland). Ca and P concentrations in serum were determined in bone ash.

3.4. Statistical analyses

The mean values of a couple of piglets constituted the experimental units for the determination of feed consumption, feed conversion ratio and of all assigned results of P1. Parameters of P2 and body gain were recorded and calculated for individual animals. All data were interpreted statistically by disposal of the program SYSTAT 7.0® for Windows®. It was accomplished with a multi-attribute analysis of variance (ANOVA). When significant dependent variable effects were determined the Bonferroni multiple range test was operated to detect which of the variants were different. P-Values <0.05 were accounted for significant.

4. Results

4.1. Diets

Nutrient and energy concentrations did not prominently vary between feeding variants (Table IV.). The REE La, Ce, Nd and Pr were contained in all of the three different feed samples. The concentrations of the analyzed REE increased CO<EG1<EG2 as intended by formulation.

4.2. Growth performance

Three of the 40 barrows were excluded before the trial ended, because one animal of CO died shocked after first blood sampling and one couple of piglets in EG1 was slaughtered ahead of schedule because in provided time of P1 they suffered from pulmonary infections and diarrhoea attended with high fever.

Results of growth performance are demonstrated separately in two phases (before and after singling) and over the whole period, because feed consumption is recorded initially per couple and later on individually (Table V).

Barrows had an initial weight of 8.6±0.4 kg by housing. After nine days of adaptation the mean weight of all piglets was 10.2±0.9 kg. 31 days further they were singled and weighed 29.4±3.9 kg. The animals achieved their end weight of 55.5±4.1 kg after an average time of 67±3 days. Daily weight gain (DWG) was 0.62±0.11 kg/day in time of pair stabling (Period A) and 1.02±0.08 kg in time of the beginning of single keeping up to slaughtering (Period B). DWG of total time (Period AB) was 0.80±0.07 kg. During any time of fattening mean body mass (BM) and DWG did not present any relevant differences between

treatments. Mean daily feed intake (DFI) in Period AB was 1.40±0.13 kg feed/day and was reduced in EG1 by 7% and in EG2 by 3% compared to CO (not significant (n.s.)). Feed conversion ratio (FCR) in Period AB was in a mean of 1.75±0.08. In Period B and Period AB the FCR was significantly lower in EG1 than in CO.

Table IV. *Analyzed nutrients and REE concentrations in feed[1] and in the supplement Lancer®[2]*

	Treatment[1]			**Lancer®[2]**
	CO	**EG1**	**EG2**	
Supplemented REE-citrate (mg/kg)	**0**	**150**	**300**	**500.000**
Nutrient content/kg feed				
Dry matter (%)	88.7	89.1	88.8	
Gross energy (MJ)	17.1	17.2	17.3	
Crude ash (g)	56	54	57	
Crude protein (g)	185	184	185	
Crude fat (g)	52	53	52	
Crude fiber (g)	34	31	32	
Carbon (g)	411	414	414	
Element				
Lanthanum (mg/kg)	0.15	7.61	16.92	28300
Cerium (mg/kg)	0.25	22.7	49.00	87600
Praseodymium (mg/kg)	0.04	3.87	8.00	13300
Neodymium (mg/kg)	0.12	0.70	1.27	1800

[1] Feed analyzed with ICP-MS by Rhea Forrer at the Institute of Physiology, Vetsuisse-Faculty, University of Zurich, Switzerland

[2] Lancer® analyzed with ICP-MS by Sächsische Landesanstalt für Landwirtschaft, Leipzig, Germany

Table V. *Treatment effects on growth performance of growing piglets*[1]

			Treatment							
	Period[2]	**n**	**CO**	***% CO***	**EG1**	***% CO***	**EG2**	***% CO***	**SEM**	**P-Value**
Body mass (kg)	Start A	12	10.32	**100**	10.28	**99.6**	10.12	**98.1**	0.254	0.845
	Start B	12	29.79	**100**	28.98	**97.3**	29.29	**98.3**	1.154	0.879
	End B	12	56.05	**100**	55.51	**99.0**	54.94	**98.0**	1.216	0.807
Daily weight gain (g)	A	12	628	**100**	603	**96.0**	618	**98.4**	0.031	0.848
	B	12	1013	**100**	1038	**102.5**	1004	**99.1**	0.233	0.569
	AB	12	805	**100**	800	**99.4**	794	**98.6**	0.019	0.929
Daily feed intake (g)	A	6	990	**100**	928	**93.7**	960	**97.0**	0.048	0.621
	B	12	2016	**100**	1853	**91.9**	1950	**96.7**	0.051	0.087
	AB	6	1455	**100**	1345	**92.4**	1408	**96.8**	0.054	0.260
Feed conversion ratio (gain/feed)	A	6	1.56	**100**	1.54	**98.7**	1.54	**98.7**	0.039	0.954
	B	12	1.99^{a}	**100**	1.79^{b}	**89.9**	1.94^{a}	**97.5**	0.041	0.002
	AB	6	1.81^{a}	**100**	1.68^{b}	**92.8**	1.77^{ab}	**97.8**	0.024	0.003

[1] Results are presented as least square means and standard error of mean (SEM). Mean values with different superscripts in a row are significantly different ($P<0.05$).

[2] Period A: from begin of trial to singling; Period B: from singling to slaughtering; Period AB: Period A + Period B

4.3. Balance analyses

In P1 six units with a couple of piglets and a unit further with a single piglet in CO (n=6) were measured in each treatment. During P2 one piglet of CO had an accident and on this account it could not be evaluated for P2. So in all of the feeding variants data of twelve single piglets were analyzed (n=12). Furthermore, it is to note that two animals of EG2 and one piglet of EG1 had diarrhoea during P2. All

results in Table VI. and Table VII. are represented in metabolic life mass ($BM^{3/4}$) per day. In P1 the average weight of the barrows was 20.1 ± 3.1 kg and in P2 their BM were 49.8±4.2 kg.

4.3.1. Digestibility of nutrients

During P1 and P2 the amount of intake and digestible GE, N and C was not significantly affected, although numerically differences could be measured (Table VI.). In P1 GE and C digestibility of EG1 was significantly higher compared to CO and there was a tendency to higher nitrogen digestibility ($P<0.1$). During P2, nitrogen digestibility was higher in EG1 compared to EG2. It was reduced by 6% ($P<0.05$). But if data of the mentioned diarrheic piglets were excluded, differences of N digestibility were below significance. Results changed as follows if data of the mentioned diarrheic piglets were excluded (data not shown): In EG1 (n=11) intake of GE was 1934 kJ/kg $BM^{3/4}$ and intake of CP was 18.40 g/kg $BM^{3/4}$ as soon as digestible GE was 1682 kJ/kg $BM^{3/4}$ and digestible CP was 15.68 g/kg $BM^{3/4}$(digestibility of GE 87% and digestibility of CP 85%). In EG2 (n=10) intake of GE was 1932 kJ/kg $BM^{3/4}$ and intake of CP was 18.34 g/kg $BM^{3/4}$ as soon as digestible GE was 1651 kJ/kg $BM^{3/4}$ and digestible CP was 15.15 g/kg $BM^{3/4}$(digestibility of GE 85% and digestibility of CP 83%).

4.3.2. Gross energy-, carbon- and nitrogen balance

In P1 and P2 there was no significant difference in energy, carbon or nitrogen balance of the growing piglets (Table VII.). DFI in P1 was enhanced in EG1 (7%) and EG2 (9%) compared to CO. Furthermore energy balance (EB), nitrogen balance (NB) and carbon balance (CB) increased CO<EG1<EG2 (n.s.). In P2 there was a tendency ($P<0.1$) to a 14% increased nitrogen utilization (k (N)) in EG1 compared to CO. Furthermore during the second balance period (P2) EB, NB and CB of EG2 decreased compared to CO and EG1. But after eliminating values of diarrheic animals, calculated value of EB was 843 k J, of NB was 1.38 g and of CB was 13.46 g. Therefore values were increased CO<EG2<EG1 (n.s.).

Table VI. *Treatment effects on gross energy (GE), nitrogen (N) and carbon (C) digestibility* [1]

		Treatment							
per kg BM$^{3/4}$/ day^2		**CO**	***% CO***	**EG1**	***% CO***	**EG2**	***% CO***	**SEM**	**P-Value**
P1		*n=7*		*n=6*		*n=6*			
GE	intake	1916	**100**	2013	**105**	2069	**108**	88.22	0.447
(kJ)	digestible	1637	**100**	1785	**109**	1780	**109**	78.98	0.369
N	intake	3.29	**100**	3.43	**104**	3.60	**109**	0.15	0.362
(g)	digestible	2.75	**100**	2.94	**107**	2.99	**109**	0.13	0.336
C	intake	46.20	**100**	48.38	**105**	49.75	**108**	2.12	0.476
(g)	digestible	39.75	**100**	42.45	**107**	43.04	**108**	1.91	0.415
Digestibility[3]									
GE		0.85^a	**100**	0.87^b	**102**	0.86^{ab}	**101**	0.01	0.025
N		0.83	**100**	0.86	**103**	0.84	**101**	0.01	0.070
C		0.86^a	**100**	0.88^b	**102**	0.86^{ab}	**100**	0.01	0.034
P2		*n=12*		*n=12*		*n=12*			
GE	intake	1912	**100**	1972	**103**	1908	**100**	60.66	0.706
(kJ)	digestible	1641	**100**	1710	**104**	1604	**98**	57.24	0.427
N	intake	2.92	**100**	2.99	**102**	2.91	**100**	0.09	0.756
(g)	digestible	2.44	**100**	2.54	**104**	2.34	**96**	0.09	0.288
C	intake	40.82	**100**	42.18	**103**	40.67	**100**	1.29	0.664
(g)	digestible	34.64	**100**	36.13	**104**	33.75	**97**	1.19	0.371
Digestibility[3]									
GE		0.86	**100**	0.87	**101**	0.84	**97**	0.01	0.121
N		0.84^{ab}	**100**	0.85^a	**101**	0.80^b	**95**	0.01	0.043
C		0.85	**100**	0.86	**101**	0.83	**97**	0.02	0.099

[1] Results are presented as least square means and standard error of mean (SEM). Mean values with different superscripts in a row are significantly different ($P<0.05$)

[2] $BM^{3/4}$: metabolic body mass

[3] Digestibility: digestible nutrients proportional to intake of nutrients

Table VII. *Treatment effects on gross energy, nitrogen and carbon metabolism*[1]

		Treatment							
per kg BM$^{3/4}$/day^{2}		**CO**	***% CO***	**EG1**	***% CO***	**EG2**	***% CO***	**SEM**	**P-Value**
P1		*n=7*		*n=6*		*n=6*			
Energy									
ME[2]	(kJ)	1602	**100**	1712	**107**	1742	**109**	78.222	0.397
m(E)[3]	(kJ)	0.84	**100**	0.85	**102**	0.84	**101**	0.005	0.147
Q[4]	(kJ)	985	**100**	1032	**105**	1018	**103**	29.750	0.511
EB[5]	(kJ)	616	**100**	680	**110**	724	**118**	53.160	0.346
K[6]		0.38	**100**	0.40	**103**	0.41	**107**	0.015	0.459
Nitrogen									
NB[7]	(g)	2.36	**100**	2.37	**100**	2.59	**109**	0.139	0.446
k(N)[8]		0.72	**100**	0.69	**96**	0.72	**101**	0.017	0.376
Carbon									
CB[9]	(g)	14.35	**100**	15.33	**107**	16.56	**115**	1.182	0.411
Accretion									
k_g[10]		0.54	**100**	0.54	**100**	0.56	**104**	0.014	0.443
protein	(g)	14.78	**100**	14.80	**100**	16.16	**109**	0.871	0.446
fat	(g)	12.05	**100**	12.85	**107**	14.54	**121**	1.322	0.398
P2	**n = 12**	*n=12*		*n=12*		*n=12*			
Energy									
ME	(kJ)	1591	**100**	1664	**105**	1555	**98**	56.092	0.392
m(E)	(kJ)	0.83	**100**	0.84	**101**	0.81	**97**	0.009	0.066
Q	(kJ)	780	**100**	799	**102**	792	**101**	14.555	0.678
EB	(kJ)	811	**100**	865	**107**	764	**94**	48.406	0.348
K	(kJ)	0.51	**100**	0.52	**102**	0.48	**94**	0.016	0.251
Nitrogen									
NB	(g)	1.31	**100**	1.53	**117**	1.29	**98**	0.085	0.104
k(N)		0.45	**100**	0.51	**114**	0.44	**98**	0.021	0.050
Carbon									
CB	(g)	13.11	**100**	14.17	**108**	12.05	**92**	0.856	0.231
Accretion									
k_g		0.72	**100**	0.72	**100**	0.68	**96**	0.015	0.222
protein	(g)	8.22	**100**	9.58	**117**	8.07	**98**	0.531	0.104
fat	(g)	15.49	**100**	16.05	**104**	14.40	**93**	1.014	0.514

[1] Results are presented as least square means and standard error of mean (SEM).

[2] ME: metabolizable energy; [3] m (E): energy metabolisability; [4] Q: Energy for heat production; [5] EB: energy balance; [6] K: total efficiency of utilization of ME; [7] NB: nitrogen balance; [8] k (N): nitrogen utilization; 9 CB: carbon balance

[10] k_g: partially efficiency of utilization for ME for growth

4.4. Bone mineralization

Dry matter (DM) of left metatarsal bones was 64.8±3.0% on an average and the mean ash content of natural bones was 23.7±2.0% (data not shown). Ash concentration relating to natural and dried bones was not different among feeding variants (Table VIII). The fraction of P was in an amount of 15.19± 0.60% of bone ash balanced among variants, too. Ca in ash was reduced by supplementing REE citrate (CO>EG1>EG2). Ca/P ratio decreased by 3% in EG1 and by 5% in EG2 compared to CO (n.s.). Mg concentration in bone ash and DM decreased with rising dietary REE. Mg in ash was 13% lower in EG1 and 24% lower in EG2 compared to CO ($P<0.001$). Mg in DM was also reduced (14% EG1 to CO and 24% EG2 to CO ($P<0.05$)). Furthermore Ca/Mg ratio was significantly higher in EG2 than in CO.

4.5. Serum parameters

Ca and P concentration and their ratio in serum were not significant affected by dietary treatments. But P and Ca/P ratio in sera of all piglets varied among the values before the beginning of the trial (B1) with regard to the mean values of the three samples per piglet taken during the trial (B2). The concentration of P was 2.11±0.29 in B1 and 2.70±0.15 in B2 ($P<0.0001$). Total protein (TP) was decreased in both REE citrate groups compared to CO in B1 as well as in B2 and Albumin (Alb) concentration was reduced in equal measure in B1 and B2. However, in the variation of both samples there was not any significant difference of analyzed TP and Alb in sera. Creatinine of B1 and B2 as well as urea of B2 was significantly decreased in CO compared to EG2. The variations of B2–B1 were not influenced relevantly. Further blood parameters like triglyceride, cholesterol, glucose, and AP were not significantly affected by treatments.

Table VIII. *Treatment effects on mineral concentrations in bones of metatarsus*[1]

		Treatment				
in DM*[2] *of bones		**CO**	**EG1**	**EG2**	**SEM**	**P-Value**
in DM of bones						
Ash	%	36.80	36.25	36.58	0.612	0.806
Ca[3]	%	15.68	14.83	14.81	0.434	0.266
P[4]	%	5.62	5.45	5.58	0.112	0.553
Mg[5]	%	0.22[a]	0.19[ab]	0.17[b]	0.010	0.001
in ash of bones						
Ca	%	42.68	40.94	40.40	1.071	0.286
P	%	15.27	15.04	15.26	0.175	0.589
Mg	%	0.60[a]	0.52[b]	0.46[b]	0.023	<0.001
Ca/ P ratio		2.79	2.72	2.65	0.062	0.275
Ca/ Mg ratio		72.75[a]	80.48[ab]	89.31[b]	4.010	0.019

[1]Results are presented as least square means and standard error of mean (SEM). Mean values with different superscripts in a row are significantly different ($P<0.05$) n=12
[2] DM: dry matter
[3]Ca: Calcium
[4]P: Phosphorus
[5]Mg: Magnesium

Table IX. *Treatment effects on selected blood serum parameters of growing piglets*[1]

		Blood 1 (B1)[2]				**Mean value blood 2; 3; 4 (B2)**[3]				**Difference B2– B1**			
		Treatment				***Treatment***				***Treatment***			
Parameter		CO	EG1	EG2	p- Value	CO	EG1	EG2	p-Value	CO	EG1	EG2	p-Value
Calcium	mmol/ l	2.74	2.75	2.71	0.833	2.92	2.87	2.82	0.110	0.18	0.12	0.11	0.325
Phosphorus	mmol/ l	2.10	2.15	2.08	0.873	2.74	2.64	2.72	0.238	0.64	0.50	0.63	0.444
Ca/ P	ratio	1.32	1.31	1.32	0.993	1.07	1.09	1.04	0.140	-0.26	-0.23	-0.28	0.778
Protein	g/ l	53.95	50.44	50.88	0.095	59.02^{a}	55.92^{b}	56.05^{b}	0.034	5.07	5.48	5.18	0.960
Albumin	g/ l	36.71^{a}	30.53^{b}	31.23^{b}	0.000	39.61^{a}	32.84^{b}	33.48^{b}	0.000	-2.90	-2.30	-2.25	0.877
Triglyceride	mmol/ l	0.43	0.46	0.37	0.483	0.43	0.43	0.40	0.658	0.00	-0.03	0.03	0.598
Cholesterol	mmol/ l	2.27	2.02	2.00	0.566	2.48	2.39	2.55	0.301	0.21	0.37	0.55	0.427
Glucose	mmol/ l	5.89	6.01	5.94	0.934	5.53	5.39	5.45	0.886	-0.36	-0.61	-0.49	0.749
Urea	mmol/ l	2.70	2.23	2.14	0.401	3.04^{a}	2.50^{b}	2.65^{ab}	0.020	0.34	0.26	0.51	0.837
Creatinine	µmol/ l	104.38^{a}	90.17^{ab}	76.58^{b}	0.011	99.92^{a}	91.64^{ab}	76.49^{b}	0.014	-4.46	1.47	-0.08	0.772
Alcaline Phosphatase	U/ l	249	291	275	0.690	273	282	269	0.933	24.87	-9.03	-6.03	0.464

[1] Results are presented as least square means. Mean values with different superscripts in a row are significantly different (P< 0.05);

[2] Blood serum 1 is taken before starting trial diets

[3] Results of mean values and P-Value which are presented, are of an average of three blood samples during trial per individual piglet

5. Discussion

5.1. Diets

The calculated diet preparation was in compliance with the feed references for fattening weaned piglets according to NRC (1998). The analyzed nutrient and energy contents in feed corresponded approximately to the calculated values and they were balanced between variants. Na citrate was added to the feed of CO as to adjust the supplemented citrate in the REE compound of experimental diets.

The REE La, Ce, Nd and Pd were contained in low dose in feed of CO, although REE were not intentionally supplemented in diet of CO. But REE are natural constituents in soils and thus are found ubiquitous (Krafka, 1999). Yet concentrations and compounds of REE vary high between areas. Plants assimilate and incorporate these elements in all parts including the seed (Aidid, 1994; Wyttenbach et al., 1998 and Ding et al., 2005). So traces of REE can be found in almost all kinds of herbal diets. In experimental feed of EG1 and EG2 concentrations of the four supplemented lanthanides (La, Ce, Pr, and Nd) increased close to the amount as expected.

5.2. Growth performance

Daily weight gain (DWG) was neither positively nor negatively affected to a relevant degree by supplementation of REE citrate. This is in line with results presented by Eisele (2003), Kraatz et al. (2004) and Gebert et al. (2005). Gebert et al. (2005) fattened 157 piglets with an initial weight of 8.5 kg for 35 days. They used the same feed and additives as applied during the present study. The aim was to detect the influence of REE citrate on growth performance and on digestibility parameters under field conditions. In opposite to these findings a lot of Chinese studies (Shen et al., 1991; Yuan, 1994; He et al., 1998 and Hu et al., 1999) determined positive enhancing effects of REE on DWG of growing piglets. Under European conditions Borger (2003), Knebel (2004) and Kessler (2004) ascertained significantly increasing effects on DWG of up to 23%. Significantly negative effects on DWG were not noticed in European reports up to now. The studies performed so far in Europe were very dissimilar in design. Concentration, organic compound and composition of rare earth elements differed within the diets, in addition animals were of different age and the term of the trials also varied (Table I). Furthermore in some studies growth promoting effects were only significantly evident within a limited period of fattening (Borger, 2003; Knebel, 2004). It is known that growth promoters generally exert only

minor or no effects on growth (LAS, 2001). In the trial of Knebel (2004) a large number of piglets suffered from diarrhoea during fattening. Under these suboptimal growth conditions DWG was increased up to 23% in REE citrate supplemented animals compared to control animals. This might also be a reason for the enormous growth promoting effects evinced by supplementing REE in Chinese studies. Feeding and housing conditions of growing piglets are usually inferior in China compared to that in Western Europe and mean DWG is lower.

DFI was not significantly affected by feeding REE. However, DFI was decreased numerically in dietary REE compared to CO (CO>EG2>EG1). Gebert et al. (2005) reported a numerically lowered DFI in REE supplemented experimental groups, too. But in most European reports DFI increased by REE supplementation in diets. Aroma and flavour of the diets could have an influence on DFI (Fontanillas et al., 2002). But REE are tasteless and all of the other components in diet of the three variants were identical in the present study thus this aspect is not verisimilar. The reduced DFI could have been an individual variance or an impact of REE on the repletion within the intermediary or gastrointestinal sector.

FCR in Period AB was positively affected by the supplementation of low-dosed REE compared to CO. The positive effect on FCR in Period AB was because of the decreased FCR of EG1 compared to CO in Period B. In this term the FCR of EG1 compared to EG2 was significantly improved, too. So in Period AB low-dosed REE had the best feed utilization among all of the three variants. In opposite to that Gebert et al. (2005) did not ascertain any significant affect on FCR using the same feed in a field trial. But during their study piglets were fattened from 8.5 to 22 kg. This weight period was nearly according to Period A of the present study. The FCR in Period A did neither vary between feeding variants. In most of the published European studies FCR was at least numerically increased in REE supplemented groups in comparison to control (Table I and Redling, 2006). Significantly negative effects of REE on FCR were not found in European studies up to now. There are only some studies that presented results after supplementation of REE (Böhme et al., 2002 and Kraatz et al., 2004). Reasons for the improvements of FCR after REE supplementation could be an enhancement of digestibility of nutrients and/or an intermediary impact due to superior utilization of convertible energy. REE might improve or inhibit these mechanisms dose dependently. Thus lanthanides could exert their influence on the gastrointestinal tract as well as on the intermediary metabolism (Evans, 1983 and Rambeck et al., 2005).

5.3. Balance analyses

5.3.1. Digestibility of nutrients

In P1 the amount of digestible GE, C and N were elevated by 7 up to 9% in both of the REE variants compared to CO. It was not significant because of the high variations between the individuals. However, there was a significant 2% demanding of GE and C digestibility ($P<0.05$) and a tendency of 3% to a better N digestibility ($P<0.1$) in EG1 compared to CO whereas digestibility of EG2 was not affected relevantly. In P2 there was a significantly reduced N digestibility in EG2 compared to EG1. Diarrhoea has usually a relevant effect on digestibility, so that data of diarrheic animals should be evaluated separately (Dünser, 2004). After the exclusion of results from diarrheic animals (two of variant EG2 and one of EG1) the differences of N digestibility were not significant anymore, whereas C digestibility EG2 compared to EG1 was decreased ($P<0.05$). The GE, N and C digestibility of CO and EG2 were indeed very similar after excluding the data of diarrheic piglets, while nutrient digestibility of EG1 compared to CO and EG2 increased.

These results are largely in analogy with the results of Gebert et al (2005) in field trial: Analyzed GE digestibility in groups of feeding variant of 150 ppm REE citrate was significantly positively affected compared to CO and dietary 300 ppm REE groups. N digestibility was not significantly affected, but there was a numeric difference REE 150>control>REE 300. Furthermore many Chinese studies reported a positive impact of REE on digestibility and disposability of nutrients (Li et al., 1992; Cheng et al., 1994; Lu et al., 1996 and Xu et al., 1998). This is in opposite to results of Böhme et al. (2002), who ascertained the digestibility of nutrients of a total of 20 barrows growing from 35 to 60 kg which were supplemented with either 100 mg of Lanthanum-chloride, REE-citrate, REE-nitrate or REE-ascorbate compared to those receiving a control diet (n=4). They did not determine any significant affect on digestibility of crude nutriments.

Digestibility could be influenced by an impact on the gut flora of the gastrointestinal tract. Muroma (1958), Evans (1990) and Ruming et al. (2002) reported dose dependent effects of REE on microorganism in vitro. Low doses of REE could enhance the population of microorganisms, whereas at high doses REE caused a growth inhibition of different bacteria, fungal and virus. In contrast to these findings Schuller et al. (2002), Böhme et al. (2002), Knebel (2004) and Kraatz et al. (2004) did not ascertain any significant effect in vivo on gut flora of piglets and poultry or microorganisms in artificial rumen. Furthermore REE are able to influence several enzyme activities (Ellis, 1977 and Evans, 1990).

For example the conversion of trypsinogen to the active form trypsin is accelerated and also protected from auto digestion by Ca (Buck et al., 1962). As mentioned lanthanides are able to substitute Ca and in that case REE expedite the autocatalytic activation at 100 fold lower concentrations and more efficiently as Ca does (Darnall et al., 1970 and Evans, 1990). The individual lanthanides vary in intensity of reaction because of their differences in the ionic radius. Higher concentrations of the REE could operate in opposite and inhibit trypsin (Gomez et al., 1974). REE can similarly impact many further enzymes which have interactions with Ca or Mg like α-amylase, enolase and phospholipase (Smolka et al., 1971; Hershberg et al., 1976; Brewer et al., 1981 and Evans, 1990). So a better digestibility of the low dose REE variant (EG1) compared to CO in P1 and an increased C digestibility EG1 compared to EG2 in P2 could be based on the opposite properties of REE depending on doses and ion dimensions. Additionally it is possible that lanthanides could impact nutrient intestinal motility and/or nutrient absorption. REE are able to influence nervous tissues interfering with transmembrane Ca fluxes (Kalix, 1971 and Van Breemen et al., 1990). So lanthanides could influence the gastrointestinal motility, absorption and secretion, via vegetative nervous system yet their transport to the nervous systems is very low (Evans, 1990).

5.3.2. Gross energy-, carbon- and nitrogen balance

There were not any significant differences on GE-, C- and N balance among the growing piglets of REE supplemented feeding groups and CO in P1 and P2. During P1 nutrient balances EG2 compared to CO were distinct numerically increased, but in a wide variance. So there was a 9% increased protein accretion and a 21% enhanced fatness EG2 compared to CO (n.s.). These findings could be probably caused by the 8% heightened feed intake EG2 compared to CO, because protein and lipid accretion in body are affected by energy intake (Kyriazakis et al., 1992 and Bikker et al., 1995). The increased DFI between these diet groups was only temporary. Furthermore, during Period AB the DFI of EG2 was on an average less than CO. In P2 a significant affect of REE was detected neither before nor after excluding data of diarrheic piglets. In P2 there was a tendency to an increased nitrogen utilization and protein accretion. These results could be caused by the 2% increased N intake of EG1 compared to EG2 and CO and the increased N digestibility of EG1 (Kyriazakis et al., 1992; Tome et al., 2000). The maintenance requirements were not affected by supplementing REE. Although presented data of indirect calorimetry did not establish any significant impact of REE on nutrient metabolism of growing piglets, it

can not excluded, that REE citrates are able to participate in processes of metabolism in organism. According to Ellis, (1977), Arvela, (1979), Evans (1990), and Siwang et al. (2004) there are a lot of biochemical properties of REE because of their high reactivity although they are only absorbed to a very low degree.

5.4. Bone mineralization

Ash content in bones of left metatarsi was not affected relevantly by feeding REE citrate. So there was no hint for a demineralization of bones. REE are deposited in bones (Ellis, 1977; Arvela, 1979 and Evans, 1990). Behets et al. (2005) analyzed the localization of La in bone of chronic renal failure rats after oral dosing with 2g La per kg per day for 12 weeks. They reported that La was mainly located at the surfaces in bone. They concluded that the possible mechanisms for the deposition of La comprise a binding to the organic matrix, heteroionic exchange with calcium and precipitation of insoluble amorphous lanthanum phosphate. According to Albaaj et al. (2005) and Behets et al. (2004a) La has not any toxic effect on osteoblasts and no correlations were found between La concentration and bone histology or parathyroid hormone levels. In contrast to these findings bone defects were detected in healthy and chronic renal failure rats after oral supplementation of 1g La carbonate per kg/day for 12 weeks (Behets et al., 2004b). It could be caused by high phosphate binding capacity of La carbonate in the gut (>97%) and thus occurring bone phosphate depletion (Behets et al., 2004). Furthermore Zhang et al. (2003) investigated the effects of rare earth ions on bone resorbing function of osteoclasts by culturing rabbit osteoclasts on bone slices. He estimated an inhibition of the osteoclastic activities by low doses of La, Sm, Er, Nd, Gd and Dy as well as an enhancing effect of osteoclastic bone resorption rate by higher doses of La, Sm and Er.

During this study there was no effect in Phosphorus concentration of ash. This was in analogy to the determined P concentrations in bones of first coccygeal vertebrae of piglets which were in a weight of 27 kg by Knebel (2004). She analyzed P and Ca concentrations in bone of piglets which had been additionally fed with 0, 50, 100 or 200 mg REE citrate /kg feed for six weeks.

Ca/ P ratio was lowered by 3% in 150 ppm REE cit. (EG1) and by 5% in 300 ppm REE cit. (EG2) compared to CO (n.s.) because Ca was increased by 4% in EG1 and 5% in EG2. More distinct, significant variations were estimated in Mg concentration of bone ash. It decreased by 13% in EG1 and 24% in EG2 compared to CO. Ca/P ratio was relatively high in an average value of 2.72 compared to

that generally reported in literature (Stockland at al., 1973; Lepine et al., 1985 and Prikoszovitis et al., 1995). However, the basal diet already had a Ca content of 7.4 g/kg and P content of 5.8 g / kg which led to a Ca/ P ratio of 1.44 and so it was in a conventional recommended amount (NRC, 1998). Furthermore using different parts of skeleton, conditioning methods and analyzing modes might lead to variable results (Priskoszovitis et al., 1995). Liesegang et al. (2005) ascertained in a similar large Ca/ P ratio of an average of 2.46 by exercising similar conditioning methods as presented.

To Knebel (2004) Ca content and consequently Ca/P ratio varied significantly by supplementation of REE: Low and medium doses of REE cit (50 ppm and 100 ppm) indicated decreased Ca and Ca/P ratio compared to control (0 ppm) as well to high dose (200 ppm). Isomorphic substitution of Ca by REE ions could be a reason for lower Ca concentrations in bone. Decreasing Mg levels might be interpreted similarly. In plants La is able to replace Mg in the centre of chlorophyll (Tao et al., 2001). Mg is able to influence matrix and mineral metabolism in bone by a combination of effects such as hormonal effects and direct influence on bone (Wallach, 1990 and Rude et al., 1998). So it is possible, that REE could enhance or inhibit these mechanisms dose dependently.

5.5. Serum parameters

The serum value of P in B1 was decreased proportional to the reference values in literature (Kraft et al., 1999 and Kixmöller, 2004). According to Seutter (1995) P in serum is influenced by feed and housing conditions. Piglets in all of the three feeding variants had hypophosphatemia before entering the trial. During the trial P values were normalized.

Ca, P and Ca/P ratio in blood serum was not affected relevantly by REE citrate supplementation. In compliance Borger (2003) also reported no difference in both electrolytes of piglets additionally fed with 150 ppm La chloride. Accordingly, Stewart et al. (2002) did not detect any significant effect on serum levels of Ca, P, Sodium (Na) or parathyroid hormone after the administration of 9 g/day La carbonate to healthy men. In opposite to these findings La carbonate (Fosrenol®) significantly decreased P in serum without causing hypercalcaemia when applied at 750 mg up to 6g /day to human beings for depression of hyperphosphatemia in chronic kidney disease (Behets et al., 2004a; Swanson et al., 2004 and Finn, 2005). As to Stewart et al. (2002) La carbonate binds phosphate effectively in the gut and so it reduces its uptake into the systemic circulation. In healthy man and animals REE normally do not affect P and Ca in serum because of well working electrolyte homeostasis. It needs to be investigated in further

studies whether REE citrate also binds phosphate in the gut of piglets. That would be an undesirable effect on fattening piglets.

Concentrations of albumin (Alb) and total protein (TP) in serum of B1 and B2 were significantly increased in EG1 and EG2 compared to CO. But the changes of concentrations B1 to B2 of Alb and TP were not affected by dietary REE. This is why significant differences are probably caused by individual variations of the animals and it did not vary due to supplementing REE citrate. This assumption is further supported by He et al. (2003) who investigated the effect of dietary supplementation of La chloride and a REE mixture compound on blood serum parameters of rats and Borger (2003) who analyzed blood serum of REE supplemented piglets. Both of them did neither detect any effect on Alb or TP in serum.

Significant differences of creatinine and urea among variants were presumably generated in individual variance of animals, too. Furthermore decreased creatinine and urea values as detected in EG1 and EG2 compared to CO are in opposite to the findings of He et al.(2003) who found increased creatinine and urea concentrations after REE supplementation. Triglyceride, cholesterol, alkaline phosphatase and glucose were not affected by REE.

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