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Content and gain of macro minerals in the empty body and body tissues of growing bulls

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ABSTRACT

This research aimed to generate basic data for specifying the macro mineral requirements of beef bulls. Hence, the contents of the macro minerals calcium, phosphorus, sodium, potassium, sulfur, and magnesium in the empty body and body tissue fractions of growing Fleckvieh (German Simmental) bulls with 120–780 kg live weight were determined. Results were used to calculate mineral gain rates in bulls within a wide weight range from 100 to 800 kg live weight. Calcium and phosphorus represented the largest shares in the animals' bodies. Body mineral content changed during animal growth due to progressing bone mineralization and increasing amounts of fat in all body tissues. Peak mineral gain rates were observed for calcium, phosphorus, and magnesium during the 200–400 kg live weight range. The gain rates of sodium, potassium, and sulfur declined steadily during cattle growth. The provided data allow to adjust the existing values of net mineral requirements of growing Fleckvieh bulls within the factorial requirement calculation method.

1. Introduction

An essential part of livestock nutrition is the animals' adequate mineral supply. Minerals can be classified in macro minerals, *e.g.*, calcium, phosphorus, sodium, potassium, sulfur, and magnesium, and trace minerals (micro minerals), *e.g.*, iron, zinc, copper, and manganese. The common classification depends on the amounts of minerals in the body. Macro minerals hold >50 mg per kg body weight, while trace minerals are present in smaller quantities. Adequate mineral supply promotes animal growth, health, and performance. These factors are impaired in animals facing mineral deficiency. Deficient mineral supply may result from feeding low quality feedstuffs, reduced mineral bioavailability, and/or in phases of high performance that entail increased mineral demands, *e.g.*, growth and lactation (Radwińska & Żarczyńska, 2014). Hence, mineral malnutrition can occur when animals are not fed according to their specific requirements and should be avoided to ensure good livestock performance and welfare.

This research examined concentrations of minerals in body tissues and respective mineral gains in growing Fleckvieh (German Simmental) bulls. The Fleckvieh breed is a common dual-purpose cattle breed in southern Germany and provides high milk and meat yields simultaneously. The breed's performance potential has been improved by selective breeding and progress in cattle farming and feeding during the past decades. In practice, this is reflected in increased daily weight gains and final weights of the animals, which may be associated with changes in the animals' energy and nutrient requirements. Basic research into energy and nutrient accretion in growing Fleckvieh bulls was performed almost three decades ago (Kirchgessner, Schwarz, Reimann, Heindl, & Otto, 1994; Schwarz, Heindl, & Kirchgessner, 1995). Recently, Honig et al. (2022) showed that energy and nutrient gain rates in growing Fleckvieh bulls today are different from what was observed in the studies by Kirchgessner et al. (1994) and Schwarz et al. (1995). Hence, the question arises if mineral gain has equally changed during the past decades and if feeding recommendations should be adjusted to the requirements of today's growing bulls.

Besides avoiding malnutrition in growing cattle, feeding the animals according to their nutrient requirements is a decisive factor in reducing environmental impact due to nitrogen or phosphorus oversupply. Wu,

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Satter, Blohowiak, Stauffacher, and Wilson (2001) reported increasing phosphorus excretions of cows fed with rations high in phosphorus. High phosphorus excretions can cause environmental pollution due to excessive phosphate leaching into surface water and hence should be avoided. The German Agricultural Society (DLG, 2014) uses data on phosphorus intake and accretion in growing fattening bulls to calculate the amounts of excreted phosphorus per animal and farm. Those data are a basis to implement European law as the EU Nitrates Directive (Council Directive 91/676/EEC, 1991) into national law, *e.g.*, Fertilizer Application Ordinance (DüV, 2017) and the Ordinance for Substance Flow Analysis (StoffBilV, 2017) in Germany. Data on phosphorus accretion in fattening bulls and other farm animals are scarce what may be a reason for different phosphorus excretion coefficients used in different countries (Šebek, Bikker, Vuuren, & Krimpen, 2014).

This research project aimed to determine the content and gain of the macro minerals calcium, phosphorus, sodium, potassium, sulfur, and magnesium in the body and body tissue fractions of growing Fleckvieh bulls.

2. Material and methods

2.1. Animals and treatments

The research was conducted at the Bavarian State Research Center for Agriculture (LfL) according to European guidelines for animal experiments (Directive 2010/63/EU, 2010) and approved by the LfL-committee for Ethics of Animal Experiments. Material and methods employed during calf rearing and bull fattening were previously described by Honig et al. (2020).

The experiment included 72 male Fleckvieh calves (German Simmental; age: 42 d ± 9, body weight (BW) 80 kg ±6), randomly acquired from cattle farms in Bavaria, Southern Germany. For six weeks, the calves were fed with restricted amounts of milk replacer (120 g/l) with a maximum of 6 l/d and a total mixed ration (TMR) based on concentrates and hay until weaning at an average BW of 121 kg ±10. Subsequently, the animals were fed a weekly adapted TMR based on maize silage and concentrates for *ad libitum* intake. During the rearing phase, the feed intake was recorded daily per pen, and individual milk replacer intake was recorded using automatic calf feeders. Calf BW was determined using a calf scale every second week.

The fattening period was initiated at an average BW of 225 kg ±29 and age of 154 d ± 15. At this stage, the bulls were randomly allocated to normal energy (NE) and high energy (HE) treatment groups fed 11.6 and 12.4 MJ ME/kg DM for *ad libitum* intake, respectively. Differences in TMRs energy concentrations were achieved by varying the amount of maize silage and concentrates in the rations. The TMRs' compositions were constant during the fattening period and crude protein contents per kg DM remained constant in both diets. The feed mineral content was based on the recommended mineral supply for fattening bulls (GfE, 1995). The feed mineral concentrations were kept constant in relation to feed dry matter. Hence, feed mineral content was not subject to NE and HE groups' experimental treatment. During the fattening period, individual feed intake was recorded daily, and BW was determined using a cattle scale at four-week intervals.

2.2. Slaughter and body tissue sampling

Animals were slaughtered at the LfL Research Abattoir in Grub, Germany, in compliance with Council Regulation (EC) No 1099/2009 (2009). Slaughter and tissue sampling methods were previously described by Honig et al. (2020, 2022). In short, bulls from both feeding groups were slaughtered at final live weights of 120, 200, 400, 600, and 780 kg. During slaughter, the bulls' empty body weights (EBW) were determined as final live weight minus the content of urinary bladder and content of gastrointestinal tract (GIT). Subsequently, the entire empty body was dissected to individual body tissue fractions: hide, blood, organs, empty GIT, body fat, muscle, tendon, and bone. The tissue fractions organs and blood, empty GIT, body fat, muscle, and tendon were ground separately in a meat grinder (FW 114, K + G Wetter GmbH, Germany), and homogenized using an industrial stirrer. The hide was homogenized using a bowl cutter (bowl volume 65 l, Krämer & Grebe, Germany), while bones were crushed using a bone crusher (FX-300, Zhengzhou Fusion Machinery Equipment Co., Ltd., China). Bone material was not defatted prior to grinding. Three samples of each tissue fraction were taken and analyzed for their mineral composition. The experimental design allowed to analyze the empty bodies' and individual body tissues' mineral composition in different stages of the animals' maturity. Feeding varying energy concentrations reflected the range of different growth intensities under practical conditions.

2.3. Mineral analyses of feedstuffs and body tissues

The mineral analyses were conducted at the LfL Department of Quality Assurance and Analytics according to methods of the Association of German Agricultural Analytic and Research Institutes (VDLUFA, 2012). Samples of individual feedstuffs and body tissues were analyzed for their calcium (Ca), phosphorus (P), sodium (Na), potassium (K), sulfur (S), and magnesium (Mg) contents. For this, feedstuff and body tissue samples were homogenized and dissolved in nitric acid and hydrogen peroxide. Minerals were subsequently extracted by pressure digestion, using a microwave pressure digestion system, and analyzed using inductively coupled plasma optical emission spectrometry (Agilent 725 ICP-OES, Agilent Technologies, Santa Clara, United States of America). The mineral contents of feedstuffs used in the TMRs given during calf rearing and the fattening period are presented in Table 1 and were calculated by the TMRs' compositions and the mineral content in the individual feed components. The bulls' empty body mineral contents were calculated by their body tissue composition and the mineral contents in the individual body tissues.

2.4. Data analysis

Statistical analysis of feed and body mineral contents was performed using the Proc Mixed procedure of SAS (Version 9.4, SAS Institut, Cary, NC, USA) and the Kenward-Roger method to provide corrected degrees of freedom. The analysis included a two-way analysis of variance (ANOVA) with interaction (feed energy, weight group, feed energy x weight group). Differences between the groups were tested using the PDIFF option with effects stated as significant when $p < 0.05$. The results are presented as least squares means (LSM) and the standard error of the

Table 1
Mineral contents of feedstuffs fed during calf rearing and the fattening period.

Feedstuff	Minerals					
	Ca g/kg DM	**P** g/kg DM	**Na** g/kg DM	**K** g/kg DM	**S** g/kg DM	**Mg** g/kg DM
Barley	0.5	3.7	0.08	5.0	1.6	1.4
Brewer's yeast	2.4	6.6	0.5	13.8	0	1.6
Calcium Carbonate, cattle salt	381	0	0	0	0	2.0
Calf milk replacer	10.6	5.9	4.4	13.6	3.1	1.3
Feed grade urea, 46.5% N	0	0	0	0	0	0
Hay	3.5	2.7	0.7	20.2	2.2	2.0
Maize grain	0.1	2.4	0.1	3.5	1.3	1.1
Maize silage	2.0	2.0	0.1	9.5	1.2	1.6
Minerals, 26% Ca, 2% P	255	20	78	0.8	0	20
Molasses	1.0	0.1	5.7	38.3	3.1	0.1
Pressed beet pulp	9.0	1.0	1.1	8.3	2.9	2.1
Rapeseed meal	8.2	12.2	0.4	14.5	7.4	6.0
Soybean oil	0	0	0	0	0	0
Wheat	0.6	4.3	0.1	5.4	2.2	1.5

mean (SEM).

To calculate the bulls' mineral composition and mineral gain, third-order polynomial regression equations and their derivatives were used according to methods described by Honig (2022) and Honig et al. (2022). The use of polynomial regressions instead of power functions commonly used to describe allometric growth was preferred because power functions can describe the contents of body fractions at a given live weight, but their derivatives lack the ability to reproduce growth rate minima or maxima, which are important elements of allometric growth processes in different body parts and tissues (Honig, 2022). In terms of estimating the animals' mineral composition and mineral gain, third-order polynomial regressions and their derivatives can describe the body mineral contents as well as the mineral gain rates at a given live weight and they adequately reflect changes in mineral accretion rates during allometric animal growth. Compared to power functions, the use of third-order polynomial regressions better reflects the mineral content and accretion at the models' boundaries, represented by the lowest and highest weight groups.

The regression analyses were calculated using the Proc NLIN procedure in SAS and based on the following model:

$$y_i = aLW_i + bLW_i^2 + cLW_i^3 + e_i$$

Where LW is the live weight and e is the residual error.

Mineral accretion for a given body weight was calculated using the first derivative of the body composition function. The residuals of the fitted models for the NE and HE bulls were calculated to estimate significant differences between the feed intake groups and to evaluate the goodness of fit of the regression equations. A two-way ANOVA (interaction feed energy x weight group) showed no significant differences in the residuals of both feed intake groups. Hence, combined regression equations were calculated for both groups and presented in the results. The model predictive performance was determined by calculating the coefficient of determination (R^2) for each equation as $R^2 = 1 - SSE/CSS$, where SSE is the sum of squares error and CSS is the corrected sum of squares.

3. Results and discussion

3.1. Fattening performance and mineral intake

The results on feed intake, fattening performance, and efficiency of growing Fleckvieh beef bulls were published by Honig et al. (2020, 2022). In brief, bulls in the NE and HE treatment groups exhibited daily weight gains of 1699 and 1792 g/d from 200 to 780 kg live weight, respectively ($p < 0.1$). High-energy feeding increased daily weight gains among certain stages of the fattening period ($p < 0.05$) and thus shortened the fattening period in 780 kg HE-fed bulls by 21 days ($p < 0.05$). Increasing the amount of concentrate in the HE-ration led to greater daily dry matter (Honig et al., 2020) and mineral intake of HE bulls ($p < 0.0001$), except for daily potassium intake which was increased in NE bulls due to increased maize silage intake (Table 2).

3.2. Empty body mineral composition

Average empty body weights (EBW), body fraction weights and the empty bodies' and body tissue fractions' mineral contents in bulls in different weight groups are presented in Table 3. The combined results for both animal groups are shown since dietary energy concentration had no significant effect ($p > 0.05$) on the body mineral content of bulls in the NE and HE treatment groups. The amount of GIT content decreased in growing bulls from 14 to 6%. Hence, the ratio of empty body weight to live weight increased from 86 to 94% in bulls with 120–780 kg live weight.

The bulls' empty body mineral contents showed decreasing amounts of P, Na, K, and S with increasing live weight of the animals. Empty body Ca and Mg concentration did not change during growth. These results are in line with research by Schulz, Oslage, and Daenicke (1974) and Schwarz et al. (1995). However, recalculating the empty body mineral concentration in growing Fleckvieh bulls from these studies revealed lower mineral concentration in former bulls at defined live weights. *Ad libitum* fed Fleckvieh bulls in studies by Schwarz et al. (1995) featured P, Na, and K concentrations of 6.4–5.9, 1.0–0.9, and 2.1–1.9 g/kg EBW in 200–650 kg bulls, respectively. Amounts of Ca and Mg in the bulls' empty bodies averaged at 10.4 and 0.3 g/kg EBW, respectively (Schwarz et al., 1995). Schulz et al. (1974) reported P, Na, and K amounts decreasing from 7.2 to 6.6, 1.4–1.1, and 2.1–1.8 g/kg EBW, respectively, in *ad libitum* fed growing Schwarzbunte (an ancient dual-purpose breed) bulls with 152–581 kg live weight, while Ca and Mg concentration remained constant at an average of 13.4 and 0.4 g/kg EBW, respectively. Comparing the Fleckvieh bulls' empty body mineral concentration to those of Nellore x Red Angus bulls in research by Chizzotti et al. (2009) reveals higher Ca, P, and Na concentration in the crossbred animals, while K and Mg concentration is comparable between cattle breeds. Hence, current Fleckvieh bulls feature higher mineral concentration at defined final weights compared to former Fleckvieh and Schwarzbunte bulls, but lower Ca, P, and Na concentrations compared to Nellore x Red Angus bulls. The empty body Mg concentration was comparable in all cattle breeds. Sulfur concentration was not determined in research by Schulz et al. (1974), Schwarz et al. (1995), and Chizzotti et al. (2009) and thus cannot be compared with previous studies.

Body mineral concentration depends mainly on bone mineral concentration, since bone tissue features the highest amounts of total ash, which contains the highest amounts of minerals, as demonstrated by Honig et al. (2022). In bone tissue, amounts of Ca, P, Na, and Mg increased during cattle growth, while K and S proportions decreased with increasing live weight of the animals. These observations agree with recalculated bone mineral concentration in research by Schulz et al. (1974), which determined bone mineral concentration to range between 85.1 and 123.5, 38.0–54.1, 3.2–3.6, and 2.4–2.6 g/kg natural bone tissue for Ca, P, Na, and Mg, in growing Schwarzbunte bulls with 152–581 kg live weight, respectively. Recalculated bone mineral concentration in research on former Fleckvieh bulls published by Schwarz et al. (1995) showed bone Ca, P, Na, and Mg concentration to range

Table 2
Mineral intake of bulls in normal and high energy treatment groups in different weight ranges.

Mineral intake	Weight range								SEM	p-value		
	80-120 kg	120-200 kg	200 – 400 kg		400-600 kg		600-780 kg			feed	weight	feed x weight
			NE	HE	NE	HE	NE	HE				
	n = 72	n = 64	n = 27	n = 27	n = 18	n = 18	n = 9	n = 9				
Calcium (g/d)	19	46	60^A	73^B	82^A	97^B	94^A	106^B	0.79	<0.0001	<0.0001	<0.0001
Phosphorus (g/d)	9	20	27^A	33^B	36^A	44^B	40^A	48^B	0.36	<0.0001	<0.0001	<0.0001
Sodium (g/d)	10	9	10^A	14^B	14^A	19^B	16^A	20^B	0.18	<0.0001	<0.0001	<0.0001
Potassium (g/d)	28	46	71^A	64^B	93^A	83^B	104^A	92^B	0.84	<0.0001	<0.0001	<0.0001
Sulfur (g/d)	5	11	15^A	19^B	20^A	27^B	23^A	29^B	0.20	<0.0001	<0.0001	<0.0001
Magnesium (g/d)	4	12	17^A	19^B	24^A	28^B	27^A	29^B	0.22	<0.0001	<0.0001	<0.0001

Means within a weight range sharing the same superscript are not significantly different.

Table 3
Empty body and body tissues' mineral contents in bulls in different weight groups.

Body fractions	Fraction weight, mineral composition	Weight group 120 kg n = 8	200 kg n = 10	400 kg n = 18	600 kg n = 18	780 kg n = 18	SEM	p-value weight
Empty body	Weight (kg)	104.0^{A}	175.7^{B}	370.0^{C}	553.1^{D}	734.2^{E}	3.64	<0.0001
	Calcium (g/kg)	15.39	14.39	13.72	14.23	13.62	0.50	0.1718
	Phosphorus (g/kg)	8.91^{A}	8.36^{AB}	8.07^{B}	8.12^{B}	7.73^{B}	0.24	0.0337
	Sodium (g/kg)	1.56^{A}	1.42^{B}	1.33^{C}	1.27^{D}	1.19^{E}	0.02	<0.0001
	Potassium (g/kg)	2.46^{A}	2.29^{B}	2.21^{B}	2.12^{C}	1.99^{D}	0.03	<0.0001
	Sulfur (g/kg)	1.72^{A}	1.62^{B}	1.60^{B}	1.52^{C}	1.47^{D}	0.02	<0.0001
	Magnesium (g/kg)	0.43^{AB}	0.45^{A}	0.45^{A}	0.44^{A}	0.41^{B}	0.01	0.0191
Hide	Weight (kg)	9.4^{A}	18.7^{B}	43.1^{C}	60.9^{D}	75.9^{E}	0.99	<0.0001
	Calcium (g/kg)	0.19	0.18	0.18	0.17	0.17	0.01	0.5634
	Phosphorus (g/kg)	0.61^{A}	0.54^{B}	0.42^{C}	0.36^{D}	0.32^{E}	0.01	<0.0001
	Sodium (g/kg)	1.95^{A}	1.89^{A}	1.88^{A}	1.76^{B}	1.69^{B}	0.03	<0.0001
	Potassium (g/kg)	1.28^{A}	1.04^{B}	0.82^{C}	0.73^{D}	0.70^{D}	0.02	<0.0001
	Sulfur (g/kg)	2.89^{A}	2.33^{B}	2.10^{BC}	2.00^{C}	2.10^{BC}	0.09	<0.0001
	Magnesium (g/kg)	0.08^{A}	0.07^{AB}	0.06^{B}	0.05^{C}	0.05^{C}	0.003	<0.0001
Blood & Organs	Weight (kg)	13.4^{A}	21.6^{B}	41.5^{C}	58.0^{D}	69.9^{E}	0.80	<0.0001
	Calcium (g/kg)	0.12^{AB}	0.12^{A}	0.12^{AC}	0.09^{B}	0.10^{BC}	0.01	0.0221
	Phosphorus (g/kg)	1.63^{A}	1.61^{A}	1. 57^{A}	1.35^{B}	1.34^{B}	0.03	<0.0001
	Sodium (g/kg)	1.70	1.61	1.65	1.68	1.66	0.04	0.7397
	Potassium (g/kg)	1.94^{A}	1.88^{A}	1.71^{B}	1.70^{B}	1.65^{B}	0.05	0.0002
	Sulfur (g/kg)	1.43^{A}	1.46^{AB}	1.50^{AC}	1.53^{BC}	1.55^{C}	0.02	0.0132
	Magnesium (g/kg)	0.11^{A}	0.11^{A}	0.10^{B}	0.09^{C}	0.09^{C}	0.002	<0.0001
Gastrointestinal tract	Weight (kg)	7.6^{A}	12.0^{B}	20.6^{C}	23.9^{D}	28.5^{E}	0.79	<0.0001
	Calcium (g/kg)	1.31^{A}	1.40^{AB}	1.95^{BC}	1.79^{AB}	2.39^{C}	0.20	0.0021
	Phosphorus (g/kg)	2.13^{A}	1.96^{AB}	1.82^{B}	1.75^{B}	1.77^{B}	0.08	0.0104
	Sodium (g/kg)	1.07^{A}	1.08^{A}	0.95^{B}	0.95^{B}	0.95^{B}	0.02	<0.0001
	Potassium (g/kg)	2.24^{A}	2.17^{A}	1.70^{B}	1.67^{BC}	1.60^{C}	0.04	<0.0001
	Sulfur (g/kg)	1.20^{AB}	1.22^{A}	1.11^{C}	1.13^{BC}	1.11^{C}	0.03	0.0041
	Magnesium (g/kg)	0.22^{A}	0.19^{AB}	0.17^{B}	0.17^{B}	0.19^{B}	0.01	0.0120
Fat	Weight (kg)	3.8^{A}	11.8^{B}	36.7^{C}	77.1^{D}	134.3^{E}	3.54	<0.0001
	Calcium (g/kg)	0.21^{A}	0.16^{B}	0.11^{C}	0.10^{C}	0.09^{C}	0.01	<0.0001
	Phosphorus (g/kg)	0.87^{A}	0.56^{B}	0.42^{C}	0.32^{D}	0.27^{E}	0.02	<0.0001
	Sodium (g/kg)	1.13^{A}	0.79^{B}	0.58^{C}	0.51^{D}	0.49^{D}	0.02	<0.0001
	Potassium (g/kg)	1.17^{A}	0.75^{B}	0.53^{C}	0.45^{D}	0.37^{E}	0.02	<0.0001
	Sulfur (g/kg)	0.71^{A}	0.49^{B}	0.38^{C}	0.32^{D}	0.29^{D}	0.02	<0.0001
	Magnesium (g/kg)	0.07^{A}	0.04^{B}	0.03^{C}	0.02^{D}	0.02^{E}	0.001	<0.0001
Muscle	Weight (kg)	44.2^{A}	73.5^{B}	157.0^{C}	235.0^{D}	305.1^{E}	3.33	<0.0001
	Calcium (g/kg)	0.10^{A}	0.09^{B}	0.07^{C}	0.06^{CD}	0.06^{D}	0.004	<0.0001
	Phosphorus (g/kg)	2.15^{A}	2.05^{B}	2.05^{B}	1.99^{C}	1.96^{C}	0.02	<0.0001
	Sodium (g/kg)	0.68^{A}	0.64^{B}	0.56^{C}	0.59^{D}	0.57^{CD}	0.01	<0.0001
	Potassium (g/kg)	3.84^{A}	3.72^{BC}	3.77^{AB}	3.72^{B}	3.62^{C}	0.03	0.0002
	Sulfur (g/kg)	1.96^{A}	1.95^{A}	2.00^{AB}	2.02^{B}	2.02^{B}	0.02	0.0405
	Magnesium (g/kg)	0.23	0.22	0.22	0.22	0.22	0.002	0.6539
Tendon	Weight (kg)	4.3^{A}	7.1^{B}	15.8^{C}	22.8^{D}	29.6^{E}	0.49	<0.0001
	Calcium (g/kg)	0.33^{A}	0.31^{A}	0.22^{B}	0.23^{B}	0.23^{B}	0.02	<0.0001
	Phosphorus (g/kg)	0.91^{A}	0.86^{A}	0.76^{B}	0.78^{B}	0.72^{B}	0.03	<0.0001
	Sodium (g/kg)	1.67^{A}	1.53^{B}	1.43^{C}	1.35^{D}	1.27^{D}	0.03	<0.0001
	Potassium (g/kg)	1.30	1.28	1.24	1.28	1.21	0.04	0.4365
	Sulfur (g/kg)	1.18^{A}	1.15^{AB}	1.09^{BC}	1.09^{BC}	1.05^{C}	0.03	0.0128
	Magnesium (g/kg)	0.08	0.08	0.07	0.08	0.07	0.003	0.0549
Bone	Weight (kg)	19.4^{A}	27.8^{B}	49.1^{C}	67.8^{D}	79.9^{E}	0.85	<0.0001
	Calcium (g/kg)	81.04^{A}	89.96^{A}	101.83^{B}	114.50^{C}	123.63^{D}	3.40	<0.0001
	Phosphorus (g/kg)	40.04^{A}	44.58^{A}	51.24^{B}	56.48^{C}	60.74^{D}	1.57	<0.0001
	Sodium (g/kg)	3.67^{A}	3.56^{A}	3.92^{B}	3.94^{BC}	4.10^{C}	0.07	<0.0001
	Potassium (g/kg)	1.08^{A}	0.94^{B}	0.94^{B}	0.73^{C}	0.66^{C}	0.04	<0.0001
	Sulfur (g/kg)	1.49^{A}	1.38^{AB}	1.42^{A}	1.22^{B}	1.22^{B}	0.06	0.0046
	Magnesium (g/kg)	1.55^{A}	2.00^{B}	2.41^{C}	2.60^{D}	2.64^{D}	0.08	<0.0001

Means within a row sharing the same superscript are not significantly different.

between 69.8 and 98.7, 35.1–46.0, 2.8–3.2, and 1.7–1.8 g/kg in growing bulls with 200–650 kg live weight. Hence, current Fleckvieh bulls exhibit higher bone mineral concentration compared to Fleckvieh bulls in previous decades. Comparing the bulls' bone mineral concentrations between breeds reveals comparable Ca, P, Na, and Mg concentration in 581 kg Schwarzbunte and current 600 kg Fleckvieh bulls. Bone mineral concentration had a constant Ca:P ratio of 2:1, which is in line with previous studies on Fleckvieh bulls (Schwarz et al., 1995). However, Schulz et al. (1974) and Ebeledike, Nwokedi, Ndu, Okoye, and Ochiogu (2010) reported the bone Ca:P ratio in cattle to be 2.3:1 and 2.6:1, respectively, exhibiting higher Ca:P ratio in bone tissue of other cattle breeds. Differences in bone mineral concentration may occur due to different bone sampling and processing methods. This study analyzed non-defatted homogenized bone samples, including all bones from the right side of the body. The samples included bone marrow which shows high ether extract content and thus decreased the samples' overall mineral content. The bone tissues' crude fat concentration in bulls with 120–780 kg live weight ranged between 12.1 and 14.3%, while bone ash concentration increased during growth from 21.0 to 33.1% (Honig et al., 2022).

Contrary to bone tissue, fat tissue showed the lowest total ash concentration of all body tissues (Honig et al., 2022). Proportions of individual minerals in fat tissue decreased with increasing live weight of the animals, which is in agreement with research by Schulz et al. (1974) and Schwarz et al. (1995). Decreasing mineral concentration in fat tissue can be attributed to increasing amounts of ether extract, as demonstrated by Honig et al. (2022). Current Fleckvieh bulls featured lower fat Ca, P, Na, K, and Mg concentrations than Fleckvieh bulls in previous studies by Schwarz et al. (1995). Differences in fat mineral concentration may depend on the analyzed fat tissue. Combining carcass and visceral fat tissues for mineral analysis induces higher ether extract and lower mineral concentration compared to the sole analysis of carcass fat tissue in studies by Schwarz et al. (1995).

Muscle mineral concentration in growing bulls was characterized by decreasing Ca, P, Na, and K concentration, whereas S proportion increased during growth and Mg remained constant. These results agree with changes in the muscle mineral concentration in former Fleckvieh bulls, described by Schwarz et al. (1995). Furthermore, muscle tissue in former Fleckvieh bulls featured lower Ca, Na, and K, but comparable P and Mg concentrations (Schwarz et al., 1995). Previous research on the muscle mineral concentration in cattle showed lower Ca and P contents in other cattle breeds (Ebeledike et al., 2010; Holló et al., 2007; Mateescu et al., 2013; Somogyi, Holló, Csapó, Anton, & Holló, 2015). Lower muscle mineral concentration may depend on muscle sampling methods as well as cattle age and breed. Somogyi et al. (2015) demonstrated that muscle mineral concentration differs between breeds and individual muscles in cattle. Analyzing the mineral concentration of individual muscles (*e.g., longissimus dorsi*) excludes connective tissue and intermuscular fat, which influence the samples' mineral composition. Moreover, intramuscular fat concentration increases in growing animals and differs between cattle breeds (Honig et al., 2020). Hence, higher intramuscular fat content may be a decisive factor for lower muscle mineral concentration.

It can be concluded that empty body mineral concentration in growing cattle is mainly influenced by increasing mineralization of bone tissue and increasing ether extract proportions in all body tissues.

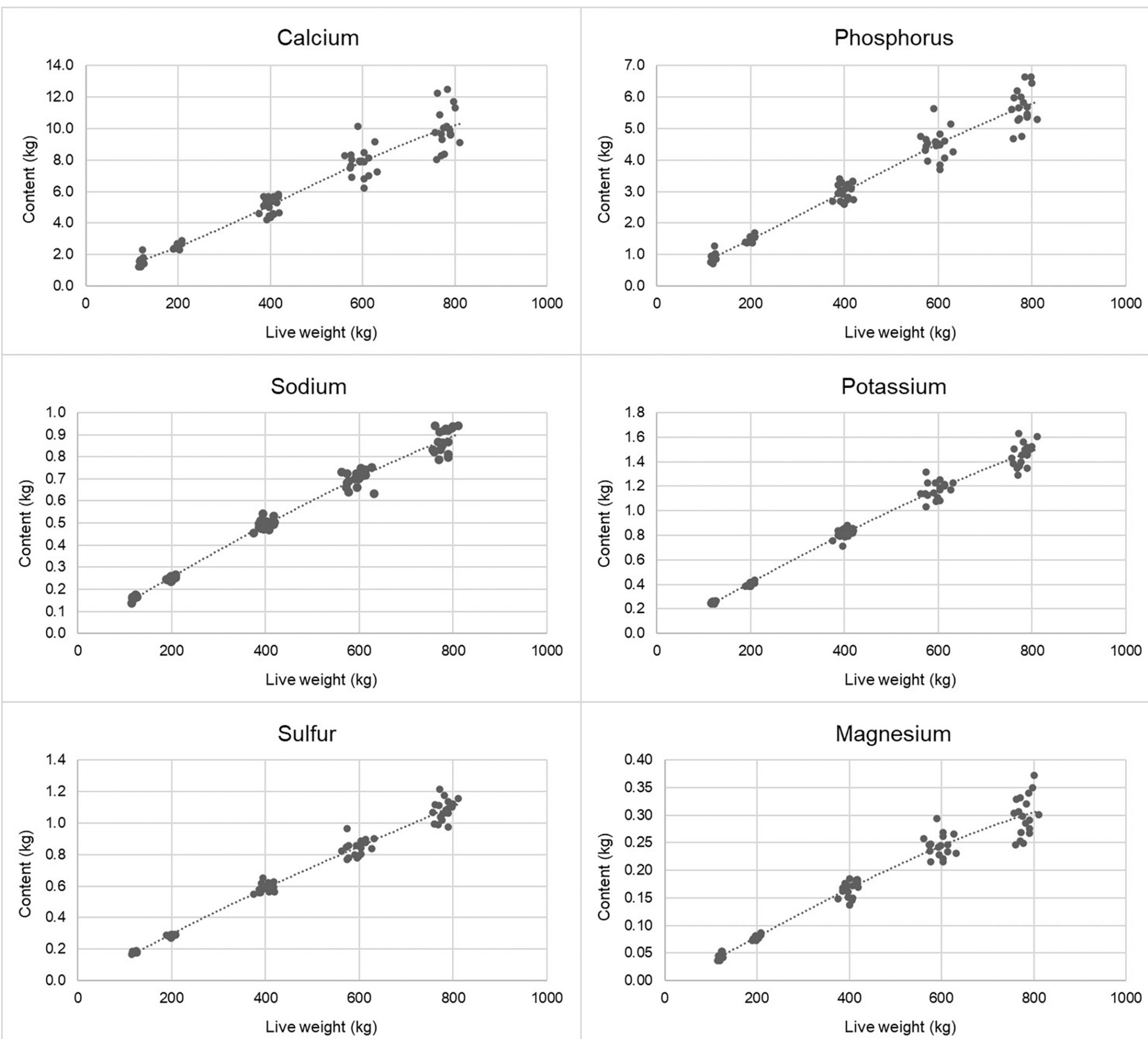

Fig. 1. Empty body mineral content in bulls with different live weights.

Table 4
Parameters for regression equations on empty body mineral content.

y	Regression equation: $y = aLW_i + bLW_i^2 + cLW_i^3 + e_i$							
	Estimated parameter			p-value			SE	R^2
	a	b	c	a	b	c		
Calcium (g)	11.7834 ± 2.1140	0.00474 ± 0.00765	−4.35E-06 ± 6.593E-06	<0.0001	0.5378	0.5116	837.5	0.9291
Phosphorus (g)	7.1222 ± 0.9861	0.00196 ± 0.00357	−2.26E-06 ± 3.076E-06	<0.0001	0.5837	0.4647	390.7	0.9502
Sodium (g)	1.3251 ± 0.0834	−0.00020 ± 0.000302	−7.08E-08 ± 2.601E-07	<0.0001	0.5010	0.7861	33.0	0.9834
Potassium (g)	2.0827 ± 0.1499	0.000033 ± 0.000542	−3.87E-07 ± 4.675E-07	<0.0001	0.9522	0.4103	59.4	0.9813
Sulfur (g)	1.4915 ± 0.1098	−0.00004 ± 0.000397	−1.29E-07 ± 3.423E-07	<0.0001	0.9287	0.7077	43.5	0.9820
Magnesium (g)	0.3569 ± 0.0545	0.000258 ± 0.000197	−2.86E-07 ± 1.701E-07	<0.0001	0.1944	0.0966	21.6	0.9466

LW: live weight; e: residual error.

Table 5
Calculated average mineral contents per kg empty body weight gain in bulls with different live weights.

Empty body mineral gain	Live weight							
	100 kg	200 kg	300 kg	400 kg	500 kg	600 kg	700 kg	800 kg
Calcium (g/kg EBWG)	12.60	13.16	13.45	13.49	13.26	12.77	12.02	11.02
Phosphorus (g/kg EBWG)	7.45	7.64	7.69	7.61	7.39	7.03	6.54	5.92
Sodium (g/kg EBWG)	1.28	1.24	1.19	1.13	1.07	1.01	0.94	0.87
Potassium (g/kg EBWG)	2.08	2.05	2.00	1.92	1.83	1.70	1.56	1.39
Sulfur (g/kg EBWG)	1.48	1.46	1.43	1.40	1.35	1.30	1.25	1.18
Magnesium (g/kg EBWG)	0.40	0.43	0.43	0.43	0.40	0.36	0.30	0.22

Differing mineral concentrations in studies considering varying cattle breeds can be attributed to differing rates of cattle growth and to differences in body tissue sampling, processing, and analyzing methods.

3.3. Empty body mineral gain

Third-order polynomial regressions were calculated to determine the empty body mineral content in growing bulls, as illustrated in Fig. 1. A two-way ANOVA showed no significant differences in the residuals of both feed intake groups. Hence, the combined regression equations were calculated for both groups together and are displayed in Table 4. The coefficient of determination reveals a very good fit in all models throughout the described weight range. The boundaries, represented by the lowest and highest weight groups, were well described by the regressions.

Mineral accretion rates in g/kg empty body weight gain (EBWG) were calculated using the first derivative of each regression equation, a method previously applied by Honig et al. (2022). Using regression equations and their derivatives to calculate mineral gain provides the opportunity to calculate detailed gain rates within growing animals. The mineral accretion per kg EBWG can be converted into mineral accretion per kg body weight gain (BWG) by assuming a mean relation of EBWG to BWG of 0.95 as derived from the present study and also assumed by GfE (1995). Results can be used to adjust feeding recommendations within the factorial requirement calculation method to fit growing Fleckvieh bulls' mineral requirements.

The accretion rates of individual minerals in growing bulls are presented in Table 5. Gain rates of Na, K and S decrease during growth, while Ca, P, and Mg show peak gain rates during the 200–400 kg live weight range and decline afterwards. Previous research on mineral gain determined the peak Ca and P gain to be 14.0 and 7.6 g/kg EBWG in *ad libitum* fed Fleckvieh bulls within the 350–500 kg weight range, respectively (Schwarz et al., 1995). Gain rates of Na and Mg were comparable in present and former Fleckvieh bulls, while former bulls showed increasing rates of K gain during growth (Schwarz et al., 1995). Schulz et al. (1974) demonstrated peak Ca, P, Mg, and K gain in Schwarzbunte bulls within the 267–370 kg live weight range and rapidly decreasing mineral gain afterwards. Decreasing rates of mineral gain were also indicated by Chizzotti et al. (2009), who described the Ca, P, K, and Mg requirements in 250 and 450 kg Nellore x Red Angus cattle to be lower than the respective mineral gain in growing Fleckvieh bulls. Likewise, Gionbelli, Marcondes, Valadares Filho, and Prados (2010) reported lower Ca, P, and Mg, but higher K requirements for weight gain in Zebu beef cattle. However, the total amount of mineral gain depends on the animals' daily weight gain, which was not considered in Table 5.

The ratio of Ca:P gain remained constant at 1.8 g/kg EBWG within the considered weight range, while the ratio of Ca:Mg increased during growth from 1:31 to 1:50 g/kg EBWG. These observations support research conducted by Schwarz et al. (1995) and demonstrate a faster reduction of Mg gain compared to Ca gain. Decreasing mineral accretion rates in growing bulls are associated with decreasing mineral requirements. The Fleckvieh bulls' mineral requirements for performance and maintenance were specified by GfE (1995) and based on mineral gain determined by Schwarz et al. (1995). Calculating the animals' mineral requirements according to GfE (1995) methods reveals higher Ca, P, Na, and Mg requirements in current Fleckvieh bulls. Requirements differentiate especially in the midterm of the fattening period in the 300–500 kg live weight range, when current Fleckvieh bulls required amounts of Ca, P, Na, and Mg to be up to 7, 16, 16, and 20% higher than in former bulls, respectively.

Required minerals are usually supplemented by concentrate feeding. Particularly P supply is an important issue in livestock nutrition because P excretions must be minimized for environmental protection. Comparing calculated daily P requirements and daily P intake (Table 2) reveals a P surplus in high weight groups, especially in HE fed bulls which showed higher P intake due to high concentrate feeding. Likewise, a mineral intake surplus could be observed for Ca, Na, and Mg in NE and HE fed bulls, because mineral intake increased during growth, but mineral accretion declined. Hence, phase feeding should be used to feed growing cattle according to their mineral requirements and reduce phosphorus excretion and the resulting environmental impacts.

4. Conclusion

The minerals Ca and P represent the largest part of the empty body mineral content in cattle. Growing Fleckvieh bulls' bodies exhibited constant Ca and Mg contents, but decreasing P, Na, K, and S proportions. Changes in empty body mineral content in growing animals are mainly influenced by bone mineralization and increasing amounts of ether extract in all body tissues.

Feeding high energy rations did not alter the body mineral composition or mineral gain per kg EBWG. However, total mineral gain depends on the animals' daily weight gain, which was increased by high energy feeding during certain stages of the fattening period.

Peak gain rates were observed for Ca, P, and Mg during the 200–400 kg live weight range, while gain of Na, K, and S declined steadily during cattle growth. The provided data on mineral gain can be used to adjust the feeding recommendations to the mineral requirements of growing Fleckvieh bulls. Furthermore, data on P accretion in growing bulls can be used to adjust the P balance and excretion calculations to the current practical conditions in German fattening bull farms.

Combining adjusted feeding recommendations with phase feeding concepts offers an opportunity to provide feed that closely matches the animals' mineral requirements and thus reduces phosphorus excretion, arising from mineral oversupply. In summary, it can be stated that adequate mineral supply has potential to reduce environmental impact and supports cattle growth and health.

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CRediT authorship contribution statement

Aniela C. Honig: Investigation, Data curation, Formal analysis, Writing – original draft. **Vivienne Inhuber:** Investigation, Writing – review & editing. **Hubert Spiekers:** Resources, Supervision, Writing – review & editing. **Wilhelm Windisch:** Conceptualization, Writing – review & editing. **Kay-Uwe Götz:** Funding acquisition, Writing – review & editing. **Gerhard Strauß:** Formal analysis, Validation, Data curation. **Thomas Ettle:** Conceptualization, Methodology, Supervision, Project administration, Writing – review & editing.

Declaration of Competing Interest

None.

Data availability

Data will be made available on request.

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