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Effects of supplementing a CP-reduced diet with rumen-protected methionine on Fleckvieh bull fattening

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ABSTRACT

The objective of this study was to evaluate the effect of supplementing a CP-reduced diet with rumen-protected methionine on growth performance of Fleckvieh bulls. A total of 69 bulls (367 ± 25 kg BW) were assigned to three feeding groups ($n = 23$ per group). The control (**CON**) diet contained 13.7% CP and 2.11 g methionine/kg diet (both DM basis) and was set as positive control. The diet reduced in CP (nitrogen) (**RED**) diet as negative control and the experimental RED + rumen-protected methionine (**MET**) diet were characterised by deficient CP concentrations (both 9.04% CP). The RED + MET diet differed from the RED diet in methionine concentration (2.54 g/kg DM vs. 1.56 g/kg DM, respectively) due to supplementation of rumen-protected methionine. Rumen-protected lysine was added to both RED and RED + MET at 2.7 g/kg DM to ensure a sufficient lysine supply relative to total and metabolisable protein intake. Metabolisable energy (**ME**) and nutrient composition were similar for CON, RED, and RED + MET. Bulls were fed for 105 days (**d**) on average. Individual feed intake was recorded daily; individual BW was recorded at the beginning of the experiment, once per month, and directly before slaughter. At slaughter, blood samples were collected and carcass traits were assessed. Reduction in dietary CP concentration reduced feed intake, and in combination with lower dietary CP concentration, daily intake of CP for RED and RED + MET was lower compared with CON ($P < 0.01$). Daily ME intake was reduced in RED and RED + MET compared with CON ($P < 0.01$). Consequently growth performance and carcass weights were reduced (both $P < 0.01$) in both RED and RED + MET compared with CON. Supplemental rumen-protected methionine was reflected in increased serum methionine concentration in RED + MET ($P < 0.05$) as compared to RED but it did not affect growth performance, carcass traits and serum amino acid (**AA**) concentrations, except for lysine which was reduced ($P < 0.01$) compared to CON and RED. In conclusion, bulls fed RED or RED + MET diets were exposed to a ruminal CP deficit and subsequently a deficit of prececal digestible protein, but methionine did not appear to be the first-limiting essential AA for growth under the respective experimental conditions.

Implications

Efficiency in meat production (i.e., poultry and swine) is particularly enhanced by supplementing single, limiting amino acids to protein-reduced diets to meet the animals' amino acid requirements more precisely. In a few studies, this concept was also successfully applied to bulls for fattening. In this study, a protein-reduced diet with limited supply of prececal digestible methionine depressed growth performance. Addition of rumen-protected methionine did not resolve depression of performance. Therefore, methionine did not seem to be the first-limiting amino acid under these feeding conditions. Hence, this strategy cannot be generally applied to beef cattle farmers to reduce nitrogen emissions.

Introduction

Dietary amino acid (**AA**) supplementation based on digestible or metabolisable AA requirements is common practice in conventional swine and poultry production (van Milgen and Dourmad, 2015). However, knowledge on limiting AA for growing cattle under different feeding conditions and diet formulations is still

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limited. Ruminal fermentation and microbial protein synthesis impede the quantification of the amount and composition of AA absorbed in the small intestine (Titgemeyer, 2003). Flux of microbial protein towards the duodenum combined with dietary protein escaping ruminal degradation is the main AA source in intestinal digesta (Chalupa, 1975).

Numerous studies have investigated the effects of rumen-protected AA on milk production and health in dairy cows. Broderick et al. (2008) demonstrated that supplementation of rumen-protected Met to cows consuming a CP-reduced diet (16.1% CP vs. 17.3% CP in a standard diet) reduced urinary N excretion, increased N efficiency measured as milk N/N intake, and improved milk fat content and milk yield. Several other studies found ameliorating effects on milk yield, milk composition, and N utilisation with rumen-protected methionine (Met), lysine (Lys) and histidine (His) supplementation (Kudrna et al., 2009; Lee et al., 2012; Arriola Apelo et al., 2014; Lee et al., 2015; Giallongo et al., 2015; Giallongo et al., 2016). Contrary to work in dairy cattle, limited research has been conducted to evaluate the effects of supplemental rumen-protected AA in growing beef cattle.

Foundational work conducted by Richardson and Hatfield (1978) indicated that the sequence of the three first-limiting AAs in growing cattle was Met, Lys, and threonine (Thr). Therefore, Met and Lys appear to be the most promising AA to investigate limitations in growing cattle. Hill et al. (1980) determined the effects of supplementing rumen-protected Met to growing Angus × Hereford steers. Steers (230 kg BW) were fed a diet adequate in Lys and sulphur. Lysine and varying amounts of Met were infused abomasally. Under their experimental conditions, Met did not appear to be the first-limiting AA for growth. In contrast, recent work by Cantalapiedra-Hijar et al. (2020) may indicate improvements in N metabolism of young fattening Charolais bulls (320 kg BW) when Met was supplemented in high-forage diets. In a 2 × 2 factorial design, a protein adequate (13.2% CP) and a high protein (16.2% CP) diet were either balanced with rumen-protected Met (supplemented at 2.6% of metabolisable protein) or unbalanced (rumen-protected Met supplemented at 2.0% of metabolisable protein). Average daily gain (**ADG**) was improved when diets were balanced for Met, with a larger improvement observed in the high CP diet. Teixeira et al. (2019) indicated that supplemental rumen-protected Arg and Lys did not improve performance, but Lys can increase lean meat yield in Angus × Simmental finishing steers.

The experiments mentioned above applied sophisticated experimental procedures to evaluate the limiting potential of Met and Lys, such as abomasal infusions via cannulas and unusual dietary compositions. Research investigating applications of Met and Lys under practical conditions, such as the supplementation of rumen-protected AA to common rations, is still lacking. Therefore, the objective of our study was to evaluate the relevance of rumen-protected Met as a putative first-limiting AA in dual-purpose Fleckvieh bulls for fattening under conditions of sufficient Lys supply under practical feeding and housing conditions.

Material and methods

Animals

A total of 69 growing-fattening Fleckvieh bulls (238 ± 11 days (**d**); 367 ± 25 kg initial BW) were evenly assigned to three dietary treatment groups: control diet (**CON**), diet reduced in CP (nitrogen) (**RED**) or CP-reduced diet with supplemental rumen-protected Met (RED + **MET**) (n = 23/treatment). The treatment groups were balanced for age, initial BW and feed intake, measured during two weeks prior to the start of the experiment. The bulls were kept in two pens per treatment equipped with fully slatted floors. Each of the two pens assigned to one treatment housed 11 and 12 bulls, respectively. Bulls in the same pen had access to all feeding troughs of their pen (six feeding troughs/pen) all the time. The bulls' access to the feeding trough was monitored during the whole experiment (LfL Institute for Agricultural Engineering and Animal Husbandry, Grub, Germany; Wendl et al., 2001). All diets were offered as total mixed ration. The total mixed rations were prepared and delivered once per d (0800) for *ad libitum* intake. Approximately 10% of feed refusals were targeted; refusals were removed daily before refilling the feeding trough. Refusals were not analysed for feed sorting.

Feeding groups, feed analyses, and feed evaluation

Experimental diets (Table 1) contained maize silage and ground premixed concentrate as major diet components. The CON diet contained 13.7% CP, 15.7% utilisable CP (**UCP**), and 12.3 MJ metabolisable energy (**ME**) per kg DM (Table 2) and met the recommendations for nutrient and energy supply to growing Fleckvieh bulls in the BW range of 350–600 kg (German Society for Nutrition Physiology, 1995). The second diet (RED) induced a deficit in CP (9.04% CP) by removing rapeseed meal and urea from the CON diet; proportion of dried beet pulp was increased to replace rapeseed meal and urea (Table 1). Rumen-protected Lys (fat coated; LysiGEM, Kemin Industries, USA) was added to achieve the same dietary Lys concentration as CON. LysiGEM contains 70% Lys–HCl with reported 85% rumen stability and 95% intestinal availability of bypass Lys. The third diet (RED + MET) was the same as RED, except for the addition of 1.6 g rumen-protected Met (Smartamine M®, Adisseo, France) per kg DM. Smartamine M ® consists of small beads that are physically protected by a pH-sensitive coating (Graulet et al., 2005). The product contains a minimum of 75% DL-Met with 80% rumen stability and 99% intestinal availability of rumen stable Met. The RED diet contained 9.04% CP and the RED + MET diet contained 9.08% CP. The experimental diets were kept constant throughout the entire study.

The DM content of the total mixed ration (Table 2) was analysed twice per week, and DM content of the concentrate feed was analysed once for every batch. Dry matter analysis was conducted in accordance with the Association of German Agricultural Analytic and Research Institutes (VDLUFA 2012, method 3.1). Nutrient composition of the maize silage, total mixed ration, and concentrate feed were analysed by wet chemistry analyses according to VDLUFA (2012; method 8.1 for crude ash, method 4.1.2 for CP, method 7.1.1 for sugar, method 6.5.1 for NDF after amylase treat-

Table 1
Ingredient composition of the control (CON), CP-deficient (RED) and RED supplemented with rumen-protected Methionine (RED + MET) diet fed to Fleckvieh bulls for fattening.

Item	Diet	
	CON	RED and RED + MET[1]
Ingredient (% DM)		
Maize silage	38.07	37.89
Corn, dry-rolled	25.35	25.01
Barley, dry-rolled	12.69	12.63
Rapeseed meal, extracted	8.88	–
Dried beet pulp	8.88	18.94
Barley straw	3.81	3.79
Urea	0.80	–
Mineral and Vitamin premix	0.72	0.72
Calcium carbonate	0.63	0.63
Salt	0.13	0.13
LysiGEM™	–	0.27
Smartamine M®[1]	–	0/0.16

[1] RED and RED + MET were equal in their ingredient composition except for supplemental rumen-protected methionine (0.16%, DM basis).

Table 2
Chemical and amino acid composition of the control (CON), CP-deficient (RED) and RED supplemented with rumen-protected Methionine (RED + MET) diet fed to Fleckvieh bulls for fattening.

Item	Diet		
	CON	RED	RED + MET
DM (g/kg)	539	537	531
Analysed Composition (% of DM, if not indicated differently)			
Ash	5.3	5.0	4.9
$aNDF_{om}$	28	29	29
Starch and Sugar	40	42	42
Crude Fat	3.0	2.9	2.9
Nitrogen (g/kg DM)	22	15	15
Amino Acids (g/kg DM)			
Lys	4.8	4.9	4.8
Met	2.1	1.6	2.5
Cys	2.1	1.4	1.2
Thr	4.7	3.6	3.4
Trp	1.1	0.8	0.7
Ile	3.9	3.5	3.1
Leu	9.6	8.3	7.8
Val	5.4	4.8	4.4
Ala	7.0	6.0	5.7
Arg	5.0	3.4	3.1
Asx	8.5	6.7	6.
Glx	20	16	15
Gly	5.4	4.1	3.9
His	3.2	2.4	2.2
Phe	4.5	3.90	3.6
Pro	8.2	6.8	6.3
Ser	5.5	4.0	3.7
Tyr	3.3	2.8	2.6
Calculated parameters (g/kg DM, if not indicated differently)			
CP[1], total	137	90	90
from urea	23	–	–
Utilisable CP[2]	157	149	149
Prececal Digestible Protein	103	97	99
Prececal Digestible Met	2.3	2.2	3.2
Prececal Digestible Lys	6.3	7.8	7.8
Prececal Digestible Thr	4.7	4.6	4.6
Metabolisable Energy[3] (MJ/kg DM)	12.3	12.2	12.2

Abbreviation: MJ = Megajoule.
[1] CP = N × 6.25.
[2] Calculation of utilisable CP according to German Society of Nutrition Physiology (1995) excluding CP from urea.
[3] Estimation of metabolisable energy concentration according to German Society of Nutrition Physiology (2008) and DLG (2011).

ment and ashing ($aNDF_{om}$)) in samples pooled over four weeks. Crude fat (method 152-H) and starch (method 152-L) were determined according to the methods of Commission Regulation (EC) No. 152/2009. Feed AA, except tryptophane (Trp), was analysed according to methods of Commission Regulation ((EG) 152/2009 App. III, F). Tryptophane analysis was conducted according to the method of Commission Regulation (EG) 152/2009 App. III, G. Utilisable CP concentration was calculated as follows: $(11.93 - (6.82 \times (RBP/CP_{(without\ urea)}))) * ME + 1.03 \times RBP$ (German Society of Nutrition Physiology, 2001) with RBP presenting rumen bypass protein (**RBP**) according to German Agricultural Society (DLG, 1997). In this formula, we used dietary CP that originated from feed components except urea. This component of dietary CP was considered to provide 30% of CP as RBP. Utilisable CP of microbial origin (**MUCP**) was calculated by subtracting RBP from total UCP. Prececal digestible (**pcD**) protein was calculated as the sum of absorbed protein from both MUCP and RBP. Microbial contribution to pcD protein was calculated as follows: 80% of MUCP was considered to be true protein according to National Research Council (NRC, 2001), which was assumed to be 80% intestinal digestible according to the 'PDI system' of the Institute National de la Recherche Agronomique (Sauvant and Nozière, 2016; Institut National de la Recherche Agronomique, 2018). Contribution of RPB to pcD protein was calculated by RBP × 0.7, reflecting the conversion factor of the 'PDI system' of the Institut National de la Recherche Agronomique (2018). Accordingly, pcDMet, pcDLys and pcDThr concentrations comprise the respective sum of pcDMet, pcDLys and pcDThr from both the MUCP and RBP. Respectively, contributions from MUCP were calculated as follows: $(0.028 \times 0.8 \times MUCP \times 0.8)$ for Met with 2.8% of Met in the true protein proportion of MUCP, $(0.079 \times 0.8 \times MUCP \times 0.8)$ for Lys with 7.9% of Lys in the true protein proportion of MUCP and $(0.058 \times 0.8 \times MUCP \times 0.8)$ for Thr with 5.8% of Thr in the true protein proportion of MUCP. Average AA concentrations in the true protein proportion of MUCP were obtained from the National Research Council (NRC, 2001; 2016) that uses data from Clark et al. (1992). Intestinal digestibility was set to 0.8 as indicated above (Institut National de la Recherche Agronomique, 2018). Accordingly, contributions of RBP to pcDMet, pcDLys and pcDThr were calculcated as respective dietary concentrations of Met, Lys, and Thr multiplied 0.3 and 0.7 with the latter factors representing the proportion of RBP to dietary CP and the conversion factor from RBP to pcD protein (Institut National de la Recherche Agronomique, 2018). In case of added dietary rumen-protected Met or rumen-protected Lys, their contributions to pcDMet and pcDLys were calculated by multiplying with 0.8 and 0.85 for rumen escape and 0.99 and 0.95, for digestibility, respectively, as stated above in the product description.

Slaughter, blood sampling and analyses

The experiment was divided into three periods. Period one lasted from d 1 until d 28, period two from d 29 until d 57. Period three lasted from d 58 until slaughter. Bulls were slaughtered across eight d and bulls from all three treatments were slaughtered on each date. The average duration of the experiment was 105 d.

On d of slaughter, bulls were transported from the stable to the research abattoir at 0600 h. Duration of transport was not longer than five minutes. Bulls were always weighed immediately before leaving the stable and refilling the feeding troughs and when entering the slaughterhouse; bulls were not fasted before slaughter. Carcasses were classified (EUROP with E = excellent, U = very good, R = good, O = Fair and P = poor) and carcass quality was determined following European Standards (Council Regulation No. 1249/2008).

Blood samples were collected using vacuette tubes (VACUETTE TUBE 4 ml CAT Serum Clot Activator, Greiner Bio-One International GmbH, Kremsmünster, Austria) during exsanguination. Tubes were inverted, centrifuged (2 000*g* × 10 min at room temperature) and then stored at −20 °C for further analysis.

The AAs in the blood serum were analysed at the Bavarian Centre for Biomolecular Mass Spectrometry (BayBioMS, Freising, Germany) using stable isotope dilution analysis and LC-ESI-MS/MS (MRM) measurement after the extraction of AA from the serum with a mixture of methanol/water 70/30 (v/v). Fifteen isotope-labelled standards were used to quantify 19 proteinogenic AA. Method parameters were applied according to Hillmann and Hofmann (2016).

Urea concentrations in the blood serum were analysed using a BioChrom30 Amino Acid Analyser following the standardised recommended procedure of analysing urea and AA in physiological liquids (Biochrom Ltd., Cambridge, UK). Cystine and cysteine (Cys) concentrations were below detection limit and therefore omitted from statistical analyses.

BW measurements and calculations

The BW of the bulls was recorded using an electronic scale directly before beginning of the study (BW at start), at the end of

period one and period two, and immediately prior to transportation to the slaughterhouse (BW at slaughter).

Calculations were conducted for the three distinct periods (periods one, two, three) as well as for the entire time on feed. Average daily gain (g/d) total was calulcated as (BW at slaughter (kg) − BW at start (kg))/total (d) × 1 000. Accordingly, ADG during periods one, two and three was calculated as the ratio of BW gain (kg) during the period and the time being the number of d in the period (i.e., between two measurements). Daily total DM, nutrient and ME intake were calculated as the ratio of total DM, nutrient, and ME intake and total d on feed and were conducted accordingly for periods one, two and three. Feed conversion ratio of each bull was calculated as the ratio of total DM intake/total weight gain and calculated accordingly for periods one, two, three, and total.

Supply of pcD protein, pcDMet, pcDLys, and pcDThr was calculated by dietary contents multiplied with DM intake in the respective periods of time. Requirements of pcD protein of animals were calculated by estimating the net requirements of (ideal) protein and assuming a metabolic utilisation of pcD protein of 0.7 according to German Society of Nutrition Physiology (1995). Net requirements comprised estimates of maintenance requirements (i.e., urinary, faecal, and surface losses derived by DM intake and mean BW according to German Society of Nutrition Physiology (1995)) and net protein requirements for growth that were assumed to account for 20% of ADG (Honig et al., 2020). Supply of pcD protein, pcDMet, pcDLys, and pcDThr was expressed as percentage of respective requirements.

Statistical analyses

Statistical analysis was performed using SAS (SAS 9.4, SAS Institute, Cary, NC, USA). Zootechnical data of total time on feed and of each period (1, 2, 3) were analysed with a general linear mixed model (**GLMM**) with dietary treatment as fixed effect and pen × treatment as random effect. Residual variance was determined to be pen × treatment. Dietary treatment group means were tested using a Student-Newman-Keuls posthoc test included in the GLMM. In the case of EUROP carcass classification and fat grade, a non-parametric posthoc test was applied (Kruskal-Wallis H Test).

The $P\text{-value}_{GLMM}$ represents the statistical significance of the GLMM model in total. Linear contrasts, except for EUROP carcass classification and fat grade, were calculated to detect differences in response variables due to CP reduction (*P*-value CON vs. RED and RED + MET) and subsequently, due to the supplementation of rumen-protected Met (RED vs. RED + MET). The standard error of the mean over the whole GLMM is indicated as SEM.

Significant differences were declared at $P \leq 0.05$. Differences at $0.05 \leq P \leq 0.10$ were considered a trend.

Results

DM, nutrient, and metabolisable energy intake

Table 3 presents the average DM, ME, nutrient, and pcDAA intake during experimental periods one to three and over the entire feeding period, referred to as 'total'. The reduction in CP decreased DM intake ($P < 0.01$) in both RED and RED + MET (8.49 and 8.27 kg/d, respectively) compared to CON (9.43 kg/d) during the whole time on feed. Consequently, ME and nutrient intake were also lower ($P < 0.01$) in RED and RED + MET compared to CON. In total, pcD protein intake of CON bulls accounted for 968 g/d, which represented 134% of their daily requirement. In comparison, RED and RED + MET bulls had lesser ($P < 0.01$) supply of pcD protein (both 822 g/d) meeting their requirements (calculated retrospectively on the base of actual performance) at 133 and 138%, respectively. Intake of pcDLys accounted for 59.7, 66.0 and 64.2 g/d in CON, RED and RED + MET, respective intake of pcDThr accounted for 44.1, 38.9 and 37.8 g/d. In CON and RED bulls, intake of pcDMet (21.2 and 18.8 g/d, respectively) matched their requirements at 98 and 101%, respectively. RED + MET bulls had a greater ($P < 0.01$) daily pcDMet intake (26.2 g) relative to CON and RED, which accounted for 146% of their daily requirement.

Serum amino acids and urea concentrations

The reduction in dietary CP concentration depressed serum concentrations (Table 4) of almost all essential AA, with Lys, Met, Thr, and the sum EAA being statistically different (linear contrasts of both RED and RED + MET vs. CON; $P < 0.05$). Furthermore, Ala and Asx were reduced ($P < 0.01$), while serum levels of Tyr increased ($P < 0.05$) relative to RED and RED + MET vs CON. Serum urea decreased from 2 081 to ∼702 mol/L on average in CP deficient bulls ($P < 0.01$). Within CP deficient treatments, addition of rumen-protected Met (group RED + MET) increased serum Met ($P < 0.01$) and reduced Lys (linear contrast between RED and RED + MET; $P = 0.02$).

Growth performance

The initial BW was 366 kg in CON and RED + MET bulls and 368 kg in RED bulls (Table 5). BW at slaughter was lower ($P < 0.01$) in both RED and RED + MET groups (499 and 498 kg) compared to CON bulls (532 kg). Additionally, higher dietary CP concentration increased ($P < 0.01$) ADG in CON (1 579 g/d) as compared to RED (1 256 g/d). On average, RED + MET bulls gained 1 199 g/d. Due to reduction in dietary CP concentration, total feed conversion ratio (**FCR**) of RED and RED + MET bulls was impaired (7.07 and 7.15, respectively) compared to FCR of CON bulls (6.07; $P < 0.01$).

Carcass traits

Reduced dietary CP concentration (RED and RED + MET) decreased both carcass weight (Table 6, 298 kg for CON vs. 274 kg for average of RED and RED + MET, $P < 0.01$) and dressing percentage (56.9% for CON vs. 55.6% for average of RED and RED + MET, $P < 0.01$) relative to CON; the addition of rumen-protected Met had no effect on either parameter (RED vs. RED + MET, $P \geq 0.33$). Carcass quality was not affected by dietary CP reduction or Met supplementation ($P = 0.2$). Fat grade of CON bulls was found to be 2.34. Compared to CON, RED + MET bulls had a lower fat grade (2.34 vs. 2.04) while that of RED bulls was intermediate (2.13; $P = 0.3$). There were no differences between treatments for pH after 1 and 24 h or in the kidney fat proportion.

Discussion

Studies evaluating metabolic AA requirements of ruminants must consider microbial degradation of dietary protein and de novo microbial protein synthesis in the rumen, as they alter the AA composition of protein reaching the small intestine (Titgemeyer, 2003). The dominant proportion of the duodenal AA flow is of microbial origin (Clark et al., 1992). Apart from that, ruminal bypass protein and endogenous secretions add to the total duodenal flow of AA (Richardson and Hatfield, 1978). The relevant protein supply to the animal, however, is the AA absorbed from duodenal digesta into the bloodstream. These are assessed differently by various protein evaluation systems - such as 'metabolisa-

Table 3
Daily nutrient intake of the bulls fed the control (CON), CP-deficient (RED) and RED supplemented with rumen-protected Methionine (RED + MET) diet.

	Diet				*P*-value		
	CON	RED	RED + MET	SEM	GLMM[1] (CON vs. RED vs. RED + MET)	Lin. Contrast I[2] (CON vs. RED/RED + MET)	Lin. Contrast II[2] (RED vs. RED + MET)
Period 1							
DM (kg)	8.27	7.56	7.01	0.47	0.19	<0.01	0.03
Metabolisable Energy (MJ)	102	92	86	5.77	0.17	<0.01	0.02
CP (g)	1 133[a]	683[b]	637[b]	49.0	0.02	<0.01	0.03
Prececal Digestible Protein (g)	850	732	696	46.3	0.09	<0.01	0.03
% requirement	*137*	*136*	*156*				
Prececal Digestible Met (g)	18.6[ab]	16.7[a]	22.2[b]	1.2	0.04	0.13	<0.01
% requirement	*100*	*103*	*166*				
Prececal Digestible Lys (g)	52.4	58.7	54.4	3.5	0.32	<0.01	0.02
% requirement	*106*	*136*	*153*				
Prececal Digestible Thr (g)	38.7	34.6	32.0	2.17	0.15	<0.01	0.02
% requirement	*120*	*124*	*139*				
Period 2							
DM (kg)	9.14	8.6	8.31	0.54	0.21	0.01	0.29
Metabolisable Energy (MJ)	113	105	101	6.66	0.17	<0.01	0.28
CP (g)	1 251[a]	777[b]	754[b]	55.8	<0.01	<0.01	0.41
Prececal Digestible Protein (g)	938	833	825	53.4	0.07	<0.01	0.29
% requirement	*128*	*129*	*131*				
Prececal Digestible Met (g)	20.6[a]	19.0[a]	26.3[b]	1.39	<0.01	<0.01	<0.01
% requirement	*94*	*98*	*139*				
Prececal Digestible Lys (g)	57.8	66.8	64.5	4.07	0.08	<0.01	0.25
% requirement	*99*	*130*	*128*				
Prececal Digestible Thr (g)	42.8	39.4	38.0	2.50	0.13	<0.01	0.26
% requirement	*112*	*118*	*116*				
Period 3							
DM (kg)	10.24	9.1	9.22	0.60	0.08	<0.01	0.7
Metabolisable Energy (MJ)	126	111	113	7.37	0.06	<0.01	0.72
CP (g)	1 402[a]	823[b]	837[b]	65.0	<0.01	<0.01	0.66
Prececal Digestible Protein (g)	1 051[a]	881[b]	916[b]	59.5	0.03	<0.01	0.7
% requirement	*130*	*132*	*133*				
Prececal Digestible Met (g)	23.0[a]	20.1[b]	29.2[c]	1.54	<0.01	0.02	<0.01
% requirement	*95*	*100*	*141*				
Prececal Digestible Lys (g)	64.8	70.7	71.5	4.42	0.11	<0.01	0.71
% requirement	*100*	*132*	*130*				
Prececal Digestible Thr (g)	47.9	41.7	42.1	2.78	0.05	<0.01	0.75
% requirement	*114*	*120*	*118*				
Total duration of the experiment							
DM (kg)	9.43	8.49	8.27	0.52	0.07	<0.01	0.43
Metabolisable Energy (MJ)	116	104	101	6.42	0.06	<0.01	0.42
CP (g)	1 292[a]	767[b]	751[b]	54	<0.01	<0.01	0.58
Prececal Digestible Protein (g)	968[a]	822[b]	822[b]	0.02	0.03	<0.01	0.43
% requirement	*134*	*133*	*138*				
Prececal Digestible Met (g)	21.2[a]	18.8[b]	26.2[c]	1.35	<0.01	0.04	<0.01
% requirement	*98*	*101*	*146*				
Prececal Digestible Lys (g)	59.7	66.0	64.2	3.91	0.13	<0.01	0.39
% requirement	*103*	*134*	*134*				
Prececal Digestible Thr (g)	44.1	38.9	37.8	2.41	0.05	<0.01	0.39
% requirement	*117*	*121*	*122*				

Abbreviation: GLMM = general linear mixed model, SNK = Student-Newman-Keuls posthoc test.
[1] *P*-values of the SNK in the GLMM.
[2] *P*-values of the linear contrasts of CON vs. RED and RED + MET or RED vs. RED + MET.
[a,b,c] Values within a row with different superscripts differ significantly in the SNK at $P < 0.05$.

ble protein' according to the National Research Council (2001) and the Cornell Net Carbohydrate System (Lapierre et al., 2018), as 'intestinal digestible protein' (DVE) according to the Dutch 'DVE/OEB system' (Tamminga et al., 1994; 2007; van Duinkerken et al., 2011), as 'protein digestible in the intestine' (PDI) according to the French 'PDI system' (Institut National de la Recherche Agronomique, 2018), or as 'AA absorbed in the small intestine' (AAT_n) according to the Scandinavian 'NorFor system' (2011). In our study, we refer to pcD according to the system used in monogastrics (German Society of Nutrition Physiology, 2008).

Within protein supply, the AA Met and Lys are likely to be most limiting to growing animals (Nimrick et al., 1970; Williams and Smith 1974; Richardson and Hatfield, 1978, Storm and Ørskov 1984; Wilkerson et al., 1993, Klemesrud et al., 2000; van Milgen and Dourmad, 2015). The first condition to prove the limitation of an EAA is a deficient protein supply while all other nutrients and ME are supplied in sufficient amounts. Secondly, the AA in question must be added to the protein-deficient diet and must relieve protein deficit (e.g., stimulation of growth). In the case of ruminants, the AA must be supplied postruminally, either via abomasal or duodenal infusion, or by a rumen-protected AA added to the diet. However, the positive effect of this AA stops once the next critical AA starts to become limiting (Storm and Ørskov, 1984; Titgemeyer, 2003).

This study was conducted to determine whether Met limits growth performance of fattening Fleckvieh bulls. Dietary concentrations of ME and other nutrients were adequate in all treatments. Regarding ME, bulls with approx. 450 kg BW have a daily ME

Table 4
Concentrations of amino acids and urea in blood serum from bulls fed the control (CON), CP-deficient (RED) and RED supplemented with rumen-protected Methionine (RED + MET) diet.

	Diet				*P*-value		
	CON	RED	RED + MET	SEM	GLMM[1] (CON vs. RED vs. RED + MET)	Lin. Contrast I[2] (CON vs. RED/RED + MET)	Lin. Contrast II[2] (RED vs. RED + MET)
Amino Acid [μmol/L]							
Lys	90.8[a]	69.4[b]	45.6[c]	4.1	<0.01	<0.01	0.02
Meth	33.5[a]	35.0[a]	43.4[b]	1.6	<0.01	<0.01	<0.01
Cys[3]	n.q.	n.q.	n.q.				
Thr	120[a]	100[b]	97.1[b]	5.15	0.12	<0.05	0.70
Trp	61.3[a]	54.8[ab]	53.1[b]	2.55	0.15	0.08	0.46
Ile + Leu	251	220	227	11.7	0.25	0.1	0.81
Val	260	226	236	10.8	0.25	0.12	0.49
His	95.6	83.9	82.9	4.75	0.276	0.11	0.83
Phe	65.4	69.6	61.6	3.65	0.42	0.67	0.20
Essential Amino Acids	978[a]	859[b]	847[b]	36.9	0.08	0.03	0.80
Ala	221[a]	181[b]	168[b]	13.3	<0.01	<0.01	0.93
Arg	187	179	174	8.80	0.80	0.52	0.91
Asx	124[a]	105[b]	97.1[b]	6.75	<0.01	<0.01	0.09
Glx	481	487	479	24.3	0.99	0.93	0.93
Pro	87.0[a]	90.8[a]	77.2[b]	3.70	0.18	0.60	0.09
Ser	88.3[a]	103.5[b]	95.5[ab]	4.45	0.23	0.11	0.45
Tyr	122	139	141	7.80	0.11	0.04	0.73
Non-Essential Amino Acids	1 309	1 284	1 231	270	0.66	0.40	0.79
Sum of Amino Acids	2 188	2 037	1 970	57.5	0.37	0.17	0.86
Urea	2 081[a]	658[b]	747[b]	94.9	<0.001	<0.001	0.39

Abbreviation: GLMM = general linear mixed model, SNK = Student-Newman-Keuls posthoc test.
[1] *P*-values of the SNK in the GLMM.
[2] *P*-values of the linear contrasts of CON vs. RED and RED + MET or RED vs. RED + MET.
[3] Cys could not be analysed because quantities were below the detection limit.
[a,b,c] Values within a row with different superscripts differ significantly in the SNK at $P < 0.05$.

Table 5
Growth performances of the bulls fed the control (CON), CP-deficient (RED) and RED supplemented with rumen-protected Methionine (RED + MET) diet.

	Diet				*P*-value		
	CON	RED	RED + MET	SEM	GLMM[1] (CON vs. RED vs. RED + MET)	Lin. Contrast I[2] (CON vs. RED/RED + MET)	Lin. Contrast II[2] (RED vs. RED + MET)
Period 1							
BW at start[3] (kg)	366	368	366	14.8	0.65	0.87	0.8
Mean BW (kg)	385	383	377	14.1	0.18	0.46	0.43
Average daily gain (g)	1 290	1 023	805	233	0.12	<0.01	0.07
Feed Conversion Ratio	7.53	8.98	9.62	2.8	0.05	0.15	0.67
Period 2							
Mean BW (kg)	425	416	406	14.7	0.08	0.03	0.21
Average daily gain (g)	1 606	1 339	1 316	228	0.24	<0.01	0.92
Feed Conversion Ratio	6.21	7.13	6.68	1.2	0.76	0.21	0.39
Period 3							
Mean BW (kg)	490	468	462	21.0	0.06	0.01	0.6
Average daily gain (g)	1 791[A]	1 379[B]	1 446[B]	192	<0.01	<0.01	0.51
Feed Conversion Ratio	5.85	6.99	6.7	1.0	0.21	0.02	0.59
Total duration of the experiment							
Mean BW (kg)	449	434	432	19.5	0.15	0.07	0.86
BW at slaughter[4] (kg)	532	499	498	34.1	0.17	0.03	0.93
Average daily gain (g)	1 580[a]	1 256[b]	1 199[b]	0.15	0.01	<0.01	0.46
Feed Conversion Ratio	6.07	7.07	7.15	0.8	0.18	<0.01	0.85

Abbreviation: GLMM = general linear mixed model, SNK = Student-Newman-Keuls posthoc test.
[1] *P*-values of the SNK in the GLMM.
[2] *P*-values of the linear contrasts of CON vs. RED and RED + MET or RED vs. RED + MET.
[3] BW at start indicates BW immediately before start of the experiment.
[4] BW at slaughter indicates BW immediately before transportation to the slaughterhouse.
[A,B] Values within a row with different superscripts differ significantly in the SNK at $P < 0.01$.
[a,b] Values within a row with different superscripts differ significantly in the SNK at $P < 0.05$.

requirement of 96 MJ for ADG of 1 600 g (German Society of Nutrition Physiology, 1995). Mean energy intake was 116, 104, and 101 MJ ME per d in CON, RED, and RED + MET, respectively. In the CON diet, pcDMet, pcDLys, and pcDThr concentrations per 100 g pcDP (2.25 g, 6.33 g, and 4.68 g, respectively; Table 2) were below the recommendations given by Wilkerson et al. (1993) (per 100 g metabolisable protein: 3.0 g, 8.0 g, 5.2 g, respectively). Despite the low dietary AA concentrations, due to high DM intake, daily intake of this pcDAA was near or above the requirement (Table 3).

Table 6
Carcass traits of the bulls fed the control (CON), CP-deficient (RED) and RED supplemented with rumen-protected Methionine (RED + MET) diet.

Items	Diet			SEM	*P*-value		
	CON	RED	RED + MET		GLMM[1] (CON vs. RED vs. RED + MET)	Lin. Contrast I[2] (CON vs. RED/RED + MET)	Lin. Contrast II[2] (RED vs. RED + MET)
Carcass weight[4] (kg)	298	275	272	20.6	0.09	<0.01	0.72
Dressing percentage (%)	56.7	55.7	55.3	0.72	0.11	<0.01	0.33
Kidney fat/carcass weight (%)	2.66	2.68	2.59	0.39	0.94	0.79	0.67
pH1[5]	6.91	6.86	6.89	0.05	0.22	0.10	0.21
pH24[5]	5.47	5.46	5.46	0.04	0.13	0.81	0.83
EUROP[6]	2.65	2.96	2.87		0.20[3]	0.09[3]	0.64[3]
Fat grade[7]	2.34	2.13	2.04		0.02[3]	0.08[3]	0.30[3]

Abbreviation: GLMM = general linear mixed model, SNK = Student-Newman-Keuls posthoc test.
[1] *P*-values of the SNK in the GLMM.
[2] *P*-values of the linear contrasts of CON vs. RED and RED + MET or RED vs. RED + MET.
[3] *P*-values of the Kruskal-Wallis H Test between diet group means.
[4] Weight (kg) of both warm carcass-halves.
[5] Measured 1 and 24 h after slaughter.
[6] EUROP carcass classification (E = 1 = best. U = 2. R = 3. O = 4. P = 5 = poorest). https://www.agriculture.gov.ie/farmingsectors/beef/eubeefcarcaseclassificationscheme/.
[7] Degree of fat is denoted by the numbers 1. 2. 3. 4. and 5 in order of increasing fatness.

RED and RED + MET groups were exposed to a CP deficit in order to reduce microbial protein synthesis and limit protein supply. Thus, DM intake was depressed compared to the CON group as a consequence of low dietary CP concentrations, which reduced pcDP supply and hence limited growth. However, for lower realised growth rates of RED and RED + MET, their true pcDP intake was above the actual requirement – from a retrospective point of view (i.e., RED and RED + MET bulls met the pdDP requirement for their actual rates of growth). In fact, their pcDP intake (822 g/d of both) should have been adequate even for CON growth rates, as 100% pcDP requirement of CON intake accounted for 722 g pcDP/d. Retrospectively, pcDP, pcDLys, and pcDThr intake rates exceeded calculated requirements, while pcDMet supply matched the retrospective requirement in RED bulls (101% of requirement, Table 3). In RED + MET bulls, pcDMet supply was improved compared to RED (142% of requirement; $P < 0.01$, Table 3). Therefore, addition of rumen-protected Met to the RED + MET bulls should stimulate growth, provided Met was the first-limiting AA in our study.

Calculations on protein requirement and supply, however, entail major uncertainties. For example, ideal AA composition of metabolisable protein derived by Wilkerson et al. (1993) was based on data of British type Hereford × Angus crossbred steers with initial BW of 253 kg and ADG of 0.49 kg/d. Hence, these bulls were in an earlier life stage and had considerably lesser ADG. The first uncertainty in the calculation of protein supply is the amount of true protein in MUCP. It is generally assumed to contain 80% true protein (NRC, 2001). Sok et al. (2017), however, proposed 82.4% due to differing AA composition of particle-associated bacteria, fluid-associated bacteria, and protozoa. Secondly, estimates on the AA composition of MUCP true protein differ between protein evaluation systems due to varying methodological approaches. Clark et al. (1992), which is widely used by NRC (2001), took into account data from 18 studies, from which seven were performed in sheep. The Institut National de la Recherche Agronomique (Rulquin et al., 1998) uses the AA composition of fluid-associated bacteria (Le Hènaff, 1991) representing average values of 66 studies in both sheep and cattle. Thirdly, estimates for digestibility of true protein from both sources, the MUCP and the RBP, are different. The Institut National de la Recherche Agronomique (2018) uses 80 and 70%, respectively, whereas German Society of Nutrition Physiology (1995) indicates 85% for both. The fourth uncertainty is AA utilisation efficiency in metabolism. Growing ruminants primarily utilise absorbed AA for muscle tissue accretion, but additional losses due to oxidative processes also take place to a significant extent. Methionine utilisation efficiency, for instance, can range from 14 to 66% (Titgemeyer, 2003).

In conclusion, the calculation of AA supply through the diets and of the AA requirements of the bulls as done in our study suggests only rough estimates of effective AA supply among treatment groups.

Different Met supplies of the CON, RED, and RED + MET diets were evident in respective blood serum Met concentrations. In particular, RED + MET showed greater serum Met than RED bulls, which proves the efficacy of the added Met source to deliver absorbable Met. Hence, our experimental setup matched in principle the preconditions to test the hypothesis that Met might be the first-limiting AA for the growth performance of Fleckvieh bulls. The fact that serum Met concentration was not reduced in RED compared to CON despite the lower Met intake may have resulted from lower ADG in the RED group which, retrospectively, entailed a lower requirement of pcDMet. Interestingly, blood Lys concentration was significantly lower in RED + MET compared to RED. This may suggest that Lys could have been the first-limiting AA for growth, but not Met. However, theoretical calculations of pcDLys supply show only a mere difference between RED and RED + MET. Hence, it can be assumed that circulating Lys was apparently more utilised for protein synthesis in the RED + MET group compared to RED, but lack of response in growth suggests that it was not translated into pronounced muscle tissue accretion in favour of other body proteins (i.e., organs, functional proteins, etc.). This hypothesis is supported by the fact that supply of pcDLys was sufficiently high and hence did not limit muscle tissue accretion of bulls in our study. Another point that may be considered is the efficacy of the rumen-protection of the Lys product. We did not determine rumen stability of the product in this study, but this has been done by Francia et al. (2020). They indicate ruminal degradation of 23.2% and intestinal digestibility of 87.3% for the Lys product.

Several studies have identified Met as one of the three first-limiting AAs (Richardson and Hatfield, 1978; Greenwood and Titgemeyer, 2000; Froidmont et al., 2001; Cantalapiedra-Hijar et al., 2020). In contrast, Hill et al. (1980) did not find Met as a first-limiting AA. In addition, Ettle et al. (1999) did not consistently elicit the growth-enhancing effects of rumen-protected Met to Fleckvieh bulls for fattening in the live weight range of 100–200 kg. These findings contradict most other studies that applied artificial experimental conditions, but they are in accordance with our results. As the diets in our study and that of Hill et al. (1980) were primarily based on maize silage, this particular feedstuff might have additionally affected experimental outcomes because the AA profile of maize is characterised by comparably high levels of Met. Another point to be considered is the stage of the develop-

ment of the growing bulls. In comparison with the abovementioned studies with beef cattle, our bulls grew to greater live weights, suggesting that the dietary deficit of pcDP was less pronounced. This might have additionally reduced the potential of rumen-protected AA added to the diet to be first-limiting to growth.

In our study, a reduction in dietary CP in RED and RED + MET reduced the fat cover compared to CON. This finding seems to contradict numerous studies with growing monogastric livestock that showed increased body fat accretion when the protein supply fell below requirements (e.g. Kerr et al., 1995). Indeed, quantitative limitations in EAAs force the metabolism to redirect the utilisation of the relative excess of non-limiting AA from protein synthesis towards fat accretion, provided that overall consumption of DM and the corresponding dietary energy remains unchanged. However, in our study, the intake of DM and ME was significantly reduced in RED and RED + MET bulls, which seemed to prevent the expected rise in body fat accretion due to an insufficient supply of dietary AA.

When comparing RED with RED + MET bulls, the addition of rumen-protected Met did not affect fat deposition. This finding additionally supports that Met was not the first-limiting AA for growth, because the relief of such a limitation should have stimulated protein synthesis at the expense of fat deposition as has been widely shown in other studies with monogastric animals (e.g., Kerr et al., 1995; Ettle et al., 2003).

In summary, our data suggest the presence of pcDP deficiency in RED and RED + MET groups due to dietary CP restriction. Furthermore, we succeeded in supplementing RED + MET bulls with rumen-protected Met, which is strongly supported by serum Met concentrations. However, the growth and slaughter performance results did not elicit a significant effect of supplemental Met. Therefore, we assume that under our experimental conditions, Met was not the first growth-limiting AA for Fleckvieh bulls of 350–500 kg BW fed a maize silage-based ration. It may also be possible that another EAA became first-limiting closely behind Met. This could not have been Lys and probably also not Thr, provided that the estimates of pcDLys and pcDThr supply status reflected actual experimental conditions and that rumen-protection of rumen-protected Lys was sufficient. For consecutive studies under practical feeding conditions, we suggest the use of younger bulls, which grow more intensively and have relatively higher rates of protein deposition and, consequently, greater AA requirements.

Ethics approval

The study was conducted at the Bavarian State Research Centre for Agriculture in Grub (LfL, Germany). The experimental procedures followed the guidelines of the German law for animal protection of the German State and Directive 2010/63/EU of the European Parliament and of the Council of September 22nd 2010 on the protection of animals used for scientific purposes. The bulls were slaughtered at the LfL research abattoir (Grub, Germany) according to the German law of animal protection of the German State and Council Regulation (EC) No. 1099/2009 of September 24th 2009 on the protection of animals at the time of killing.

Data and model availability

Data analysis was performed with SAS (SAS 9.4, SAS Institute, Cary, NC, USA). None of the data were deposited in an official repository. The data that support the study findings are available upon reasonable request.

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Declaration of interest

None.

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