

Growth, Body Composition, and Marginal Efficiency of Methionine Utilization Are Affected by Nonessential Amino Acid Nitrogen Supplementation in Male Broiler Chicken^{1,2}

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ABSTRACT The main objective of this experiment was to study the effect of CP level in the diet on the efficiency of Met utilization. Male Ross broilers were grown in floor pens in groups of 10. Three pens were allocated 1 of 16 experimental diets from d 8 to 21 posthatch. Dietary Met concentration ranged from 1.8 to 7.0 g/kg with 183 (low protein; LP) or 229 (normal protein; NP) g of CP/kg of diet. Inclusion rates of DL-Met and nonessential amino acids were varied to achieve these differences. Prececal net disappearance was additionally studied for the NP diet without Met supplementation in 4 pens of 10 birds each. Net disappearances were 83 and 78% for CP and Met, respectively, and ranged from 66% (cystine) to 96% (Ala) for other amino acids. Birds significantly and nonlinearly responded to increased Met intake in BW gain, which was significantly lower for NP than for LP. Al-

though the concentration of protein in gained BW was unaffected by Met and CP levels, the contents of fat and energy in gained BW were lower with NP than with LP and rose with increasing Met intake until a plateau was attained. The content of Met in accreted whole-body protein rose with increasing Met intake and plateaued at about 2.0 g/16 g of N. With the exception of Lys and Gly, Met intake did not significantly affect the concentration of amino acids in accreted whole-body protein. The marginal efficiency of Met utilization was, at its maximum, 8% lower with NP than with LP. Concentrations of 3.4 and 3.6 g of Met/kg of diet were needed to achieve 95% of y_{max} in protein accretion with LP and NP, respectively. It was concluded that an increase in the dietary Met requirement often found with elevated CP concentrations was the consequence of a reduced capacity to use Met for protein gain.

(Key words: methionine, broiler, marginal efficiency, accretion, amino acid)

2005 Poultry Science 84:1584–1592

INTRODUCTION

Under most dietary conditions for broiler chickens, the first limiting amino acids (AA) are sulfur-containing AA (SAA). The efficiency of AA use is an important factor that codetermines the requirement for total or digestible AA and needs to be known when requirements are calculated with the factorial approach. Modeling of quantitative AA requirements also depends on data for maintenance need, rate of protein gain, and the AA pattern in gained body protein.

Several studies on Met and SAA requirements have been conducted in growing chickens (e.g., Kalinowski et

al., 2003; Rama Rao et al., 2003; Sklan and Noy, 2003), but protein and AA accretions were not measured in most of these studies. Studies specifically aimed at determination of efficiency of AA use are rare in growing chickens. A few studies that have been conducted were restricted to the ascending portion of the growth curve, where growth appears to respond linearly. Studies on Met and SAA with broilers in which protein and AA accretions were measured only covered the suboptimal level of AA supply (Edwards and Baker, 1999). However, studies with a wider range in supply of Lys in broilers (Fatufe et al., 2004) and pigs (Gahl et al., 1994) and with 9 essential AA (EAA) in rainbow trout (Rodehutscord et al., 1997) have shown that nonlinear relationships exist between intake and efficiency of AA use in growing animals. Diminishing response to AA supplementation is also apparent in the range of marginal supply from these studies.

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Received for publication March 31, 2005.

Accepted for publication June 30, 2005.

¹Presented in part at the Annual Conference of the Society of Nutrition Physiology, March 9 to 11, 2004 in Göttingen, Germany (Fatufe, A. A. and Rodehutscord, M. 2004. Marginal efficiency of methionine utilisation in broiler chicks depending on the supply of methionine and nonessential amino acid nitrogen. Proc. Soc. Nutr. Physiol. 13: 38).

²With financial support from the German Academic Exchange Office (DAAD) in form of a doctoral scholarship for A. A. Fatufe.

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Abbreviation Key: AA = amino acid; EAA = essential amino acids; Eff_M = marginal efficiency of utilization; LP = low protein; NEAA = nonessential amino acids; NP = normal protein; SAA = sulfur containing amino acids.

Table 1. Composition of the low protein (LP) and normal protein (NP) diets and analyzed concentrations (g/kg)

Ingredient	Composition		Analyzed concentration ¹	
	LP	NP	LP	NP
Corn	483.5	483.5	DM	898 (893 – 902)
Field beans	130.0	130.0	N × 6.25	183 (178 – 188)
Peas	130.0	130.0	Crude fat	66 (61 – 71)
Wheat gluten	60.0	60.0	Crude ash	54 (50 – 55)
Cornstarch ²	73.4	25.4	Crude fiber	34 (29 – 43)
Soya bean oil	40.0	40.0	Met ⁷	1.7 – 6.9
Vitamin-mineral premix ³	10.0	10.0	Cystine	2.4 (2.2 – 2.5)
CaHPO ₄	26.0	26.0	Lys	12.5 (11.2 – 13.0)
CaCO ₃	8.0	8.0	Thr	8.9 (7.9 – 9.4)
NaCl	3.0	3.0	Val	11.7 (10.6 – 13.1)
Glycine	2.0	2.0	Ile	6.7 (6.2 – 7.1)
EAA mix ⁴	28.1	28.1	Leu	14.6 (14.1 – 15.1)
NEAA mix 1 ⁵	6.0	6.0	Phe	7.7 (7.2 – 8.1)
NEAA mix 2 ⁶	—	48.0	His ⁸	4.0
			Arg	12.5 (12.2 – 12.8)
			Trp ⁸	1.9
				12.7 (11.7 – 13.7)
				1.9

¹Values given are the averages (minimum and maximum in parenthesis) for 8 (low protein; LP) and 9 (normal protein; NP) diets (except Met). The Trp and His were not analyzed.

²The NP diet that was used to determine net disappearance contained 5 g/kg TiO₂ at the expense of cornstarch.

³The vitamin-mineral premix was the same as that described in Table 1 of Fafafe et al. (2004).

⁴Essential amino acid (EAA) mix comprised (g): L-Thr, 4.4; L-Trp, 0.7; L-Val, 5.1, L-Ile, 1.7; L-Arg, 4.7; L-Lys·HCl, 8.5; L-His, 0.5; L-Leu, 1.7; L-Phe, 0.8

⁵Comprised (g): L-glutamic acid 4.0, L-aspartic acid 1.0, L-alanine 1.0. In 7 further diets per CP level, this mix was gradually replaced by DL-Met in the following steps (g/kg of diet): 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, and 6.0.

⁶Nonessential amino acid (NEAA) mix comprised (g): L-Glu, 7.5; L-Asp, 7.5, L-Ala, 33.0.

⁷Individual values were (g/kg of diet): 1.7, 2.3, 2.8, 3.2, 4.0, 4.8, 5.7, 6.9 (LP) and 1.8, 2.1, 2.8, 2.9, 3.2, 4.3, 5.2, 7.0 (NP).

⁸Calculated from ingredient values.

It is often presumed that efficiency of AA use is unaffected by dietary CP concentration. Some studies have indicated that the requirement for Met is affected by dietary CP concentration (Mendonca and Jensen, 1989; Jeroch and Pack, 1995; Huyghebaert and Pack, 1996). It has not been examined whether this effect is caused by changes in body composition or efficiency of use of the limiting AA. It is also unclear whether this is only an effect of the level of N supply or whether it was caused by a single AA. Chamruspollert et al. (2002), for example, showed that the growth-depressing effect of excess Arg supply could partly be compensated for by supplementation with Met.

The objectives of this study were to examine the effect of CP concentration on the efficiency of Met use and investigate the degree to which a reduced CP level may affect the Met requirement. The CP concentration was varied only by nonessential AA (NEAA) to keep the EAA supply constant. The prececal net disappearance of AA in the terminal small intestine, often referred to as apparent ileal digestibility, was also studied.

MATERIALS AND METHODS

Diets

Two basal diets were used and differed solely in CP content (Table 1). One was calculated to contain a conventional 230 g of CP/kg (normal protein; NP), and the other contained 180 g of CP/kg of diet (low protein; LP). The

diets were mainly based on maize, peas, field beans, wheat gluten, and free AA. They were calculated to be deficient in SAA content (1.8 g of Met and 2.5 g of Cys/kg of diet). The contents of all other EAA were calculated to exceed the Gesellschaft für Ernährungsphysiologie (1999) recommendations by approximately 10% to avoid any limitation by EAA other than Met. These recommendations are similar to those of NRC (1994) except for Val (about 15% lower by NRC, 1994) and Trp (about 10% higher by NRC, 1994). The variation in CP concentration was achieved by replacing 48 g of cornstarch in the NP diet with the equivalent amount of a NEAA mixture (L-glutamic acid: L-aspartic acid: L-alanine = 1.0:1.0:4.4). The analyzed CP concentrations were 183 (LP) and 229 (NP) g/kg of diet. Calculated contents of AME_n were 3,150 (LP) and 3,110 (NP) kcal/kg of diet, equivalent to 13.2 and 13.0 MJ/kg. In 7 other diets per CP level, the Met concentration was gradually and isonitrogenously increased by inclusion of DL-Met at the expense of equivalent masses of a NEAA mix. The following increments were used (g of DL-Met/kg of diet): 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, and 6.0. Analyzed Met concentrations (g/kg of diet) were 1.7, 2.3, 2.8, 3.2, 4.0, 4.8, 5.7, and 6.9 in LP diets and 1.8, 2.1, 2.8, 2.9, 3.2, 4.3, 5.2, and 7.0 in NP diets. These values confirmed the intended levels. Intended concentrations for the other EAA except Ile were confirmed by analysis, and variations did not exceed the ranges of analytical error (Table 1). L-Lys·HCl, DL-Met, L-Trp, and L-Thr were feed-grade quality, whereas the other supplemented AA were pharmaceutical grade. Approximately 17% (LP)

and 34% of N (NP) in the basal diets were provided by free AA (15% from EAA and 2% from NEAA in LP; 12% from EAA and 22% from NEAA in NP). For determination of net disappearance, we used another diet based on the NP basal diet with replacement of 5 g of cornstarch/kg of diet by TiO₂, which was the indigestible marker (Jagger et al., 1992).

All ingredients except the variable ones were mixed in a single batch to ensure uniformity of the mixes and then were divided into 17 portions. Each portion was then mixed with the variable ingredients (cornstarch, TiO₂, DL-Met, and NEAA) in their respective proportions. Diets were pelleted through a 3-mm screen without using steam.

Birds and Experimental Procedures

The experiment was approved by the animal welfare authorities in accordance with the German Animal Welfare Act. Day-old male chicks from a commercial broiler hybrid (Ross) were obtained from a local hatchery.⁴ Birds were fed a commercial broiler starter diet⁵ until d 8. On the basis of uniform BW distribution, birds were divided into groups of 10 on d 8 and kept on straw bedding in floor pens. At the same time, 3 groups of 20 representative birds were taken to determine initial body compositions. Average BW on d 8 was 107 g. A lighting regimen was maintained at 20 h of light and 4 h of darkness, and room temperature incrementally decreased from 34 to 25°C as follows: d 1 to 2: 34°C, d 3 to 4: 32°C, d 5 to 7: 30°C, d 8 to 14: 28°C, and d 15 to 21: 25°C.

In the dose-response trial, 3 pens of 10 birds were randomly allocated to each of the 16 diets. The trial lasted for 2 wk beginning on d 8. Feed was offered ad libitum from one trough per pen. Free access was given to water through a nipple drinker with an attached cup. Body weight was measured at the beginning and end of the trial. Feed intake was determined pen-wise. Dead birds were weighed, and feed consumption up to the day of a bird's removal was recorded. On d 21, all birds were slaughtered and used for whole-body chemical analysis. Details of the slaughtering procedure, further handling of treatment and baseline birds, preparation for chemical analyses, and determination of protein, AA, fat, and energy accretions are described in detail by Fatufe et al. (2004).

For determination of CP and AA net disappearance, 4 groups of 10 birds were fed ad libitum the NP basal diet containing TiO₂ for 8 d beginning on d 13. Birds were then killed through CO₂ asphyxiation, and samples from the small intestine posterior to Meckel's diverticulum were obtained as described by Rodehutscord et al. (2004).

The gut content was pooled within the 10 birds of 1 pen, immediately frozen at -18°C, and freeze-dried for later chemical analyses.

Calculations, Chemical, and Statistical Analyses

Diets were analyzed for DM, ash, CP, crude fat, crude fiber, AA, and TiO₂ in the case of the net disappearance diet. Freeze-dried ileal digesta samples were analyzed for CP, AA, and TiO₂. Body homogenates were analyzed for DM, CP, fat, energy, and AA. Crude nutrients were determined according to the official methods in Germany (Naumann and Bassler, 1976). Amino acid analysis also followed standard procedures, and details are given by Timmler and Rodehutscord (2003). After an oxidation step, samples were hydrolyzed in 6 N HCl. Norleucine was used as the external standard. Tryptophan, His, and Tyr were not determined. Separation of AA was done with an AA analyzer⁶ using different buffer solutions and ninhydrin. The TiO₂ content was determined by the method described by Brandt and Allam (1987). Energy was determined with a bomb calorimeter.⁷

Net disappearance (ND) was calculated using the following standard equation:

$$\text{ND (\%)} = 100 - \frac{[(\text{TiO}_2\text{Diet} \times \text{AA}_{\text{Digesta}})}{(\text{TiO}_2\text{Digesta} \times \text{AA}_{\text{Diet}})} \times 100 \quad [1]$$

where TiO₂_{Diet}, TiO₂_{Digesta}, AA_{Diet}, and AA_{Digesta} are the concentrations of the marker and the respective AA (or CP) in diet and digesta samples (g/kg).

Results were subjected to routine ANOVA from general linear model procedures using the software package SPSS for Windows.⁸ The following 4-parameter logistic equation (Gahl et al., 1994) was fitted to the data to describe the responses of broilers depending on Met concentration or Met intake.

$$y = \frac{y_{\max} + [b \times (1 + c) - y_{\max}] e^{-kx}}{1 + c \times e^{-kx}} \quad [2]$$

where x = dietary Met concentration (g/kg of diet) or Met intake (g/bird), y = response criterion, y_{max} = asymptotic maximum response at infinite intake, b = y-intercept or response to zero intake, c = parameter related to the inflection point or shape, k = parameter related to the scale of the data, and r² and s_{y,x} are presented as parameters for the goodness of fit. The s_{y,x} values are the standard deviations of the residuals, which are the distances between the individual points from the calculated line.

The cumulative efficiency of Met use was calculated as grams of accreted Met per gram of Met intake, and accreted Met was based on the predicted response curves. The marginal efficiency (Eff_M) of use ($\Delta y / \Delta x$) was calculated to describe the proportion of each increment in Met intake that was recovered in the body protein. This is described by the first derivative of equation 2 when Met

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⁵Mischfutter und Landhandel GmbH, Edderitz, Germany.

⁶Eppendorf LC 3000, Hamburg, Germany.

⁷IKA-Calorimeter C7000 isoperibolic, Janke&Kunkel IKA Analysentechnik, Staufen, Germany.

⁸Version 11.0, SPSS Inc., Chicago, IL.

Table 2. Net disappearance (%) of CP and amino acids determined for the normal protein basal diet¹

	Mean	SE
CP	83	1.9
Ala	96	0.4
Arg	92	0.6
Asp	85	1.2
Cystine	66	2.6
Glu	91	0.7
Gly	78	2.1
Ile	84	1.4
Leu	85	1.4
Lys	89	1.2
Met	79	2.9
Phe	85	1.4
Pro	82	2.2
Ser	78	1.9
Thr	81	1.7
Val	85	1.2

¹n = 4 pens of 10 birds.

accretion is plotted against quantitative Met intake (Gahl et al., 1996):

$$\frac{\Delta y}{\Delta x} = \frac{k e^{-kx}}{(1 + c e^{-kx})^2} \times (y_{max} - b) \times (1 + c) \quad [3]$$

Nonlinear regressions were calculated using GraphPad Prism 4.02.⁹

RESULTS

Survival rate was over 99%. Five birds died when on Met levels of 1.7 (LP), 2.8, 3.3, 4.8, and 7.8 g Met/kg of diet (NP) on d 8, 5, 11, 5, and 13, respectively.

Net disappearance of CP was 83%, and net disappearance for AA ranged between 66 and 96% (Table 2). Methionine net disappearance (79%) was lower than net disappearance of the other investigated EAA. Cystine and Ala were the AA with the lowest and highest net disappearance, respectively.

The chickens responded significantly and nonlinearly to increasing Met supply in feed intake, BW gain, and gain/feed ratio (Table 3). A significant interaction between the dietary concentrations of Met and CP was not detected in any response criterion. During the 14-d experimental period, an estimated y_{max} for BW gains of 550 g (LP) and 510 g (NP) was attained (Table 4), and the effect of dietary CP level on BW gain was significant (Table 3). The estimated y_{max} for gain/feed ratio was 0.75 (LP) and 0.77 g/g (NP) without a significant CP effect.

Body weight gain of broilers contained significantly less fat and energy when the NP diets were fed, as compared with the LP diets (Table 3). Both fat and energy contents rose with increasing dietary Met concentrations. On the contrary, the content of protein in gained BW was not affected by Met or CP concentration. However,

quantitative protein accretion nonlinearly responded to increasing Met supply and approached y_{max} values of 87 (LP) and 82 g/bird (NP; Figure 1, Table 4). The CP effect on protein accretion was significant ($P = 0.003$). The dietary Met concentrations that were needed to achieve 95% of y_{max} depended upon the response criterion and varied between 3.0 to 3.4 (LP) and 3.2 to 3.6 g Met/kg of diet (NP) (Table 4). Among the responses studied, protein accretion led to the highest need for Met in the diet.

As with protein accretion, the estimated y_{max} in Met accretion was lower for NP as compared with LP (Figure 2). The Eff_M of Met use was maximal at a level of intake, which was approximately half of that needed for high Met accretion (Figure 2). Maximal Eff_M of 103% (LP) and 95% (NP) were calculated for intakes of 1.1 (LP) and 0.9 g of Met/bird (NP). Maxima in cumulative efficiency of utilization also occurred at suboptimal Met intakes and were 85% (LP) and 76% (NP) (Figure 3).

In gained whole-body protein, the concentrations of Lys and Met increased ($P < 0.001$), and those of Gly ($P = 0.004$) decreased with increasing dietary Met (Table 5). The dietary CP level significantly affected the concentrations of Asp, Glu, Ile, Met, and Thr in gained whole-body protein. There was no significant interaction between CP and Met concentrations on AA pattern of gained whole-body protein.

DISCUSSION

Effects on Growth and Implication for Requirement Estimates

There was a difference between the 2 CP levels in the response of broilers to supplemental Met. The magnitude in response was consistently higher with LP compared with NP, which cannot be attributed to differences in feed intake (Table 3). In previous studies that focused on the effect of CP on SAA requirement for broiler chicks, diets were formulated by varying the intact CP sources to achieve the intended CP levels (Mendonca and Jensen, 1989; Jeroch and Pack, 1995) or by diluting diets (Huyghebaert and Pack, 1996), thus also varying the concentrations of EAA, even though similar AA profiles were often assumed. In contrast to most previous studies, we varied dietary CP concentration with a mixture of NEAA only. The supply of EAA remained unchanged and slightly exceeded the recommended levels (except Met). This finding explains why the LP level was not inferior to the NP level in growth performance (Tables 3 and 4). The Met concentrations required to achieve a plateau in the response criteria were higher for NP than for LP (Table 4). This latter observation is consistent with previous findings (Mendonca and Jensen, 1989; Jeroch and Pack, 1995; Huyghebaert and Pack, 1996). Huyghebaert and Pack (1996) were unable to quantify the magnitude to which dietary CP (197 to 259 g/kg of CP) may elevate the requirement for dietary SAA, because a plateau was not achieved in the range of SAA fed. Upon reexamination of their diets, one will notice a basal Met level of 3.1

⁹GraphPad Software, Inc. San Diego, CA.

Table 3. Feed intake, BW gain and composition of BW gain of chickens fed different Met levels in diets with 183 g (low protein; LP) or 229 g (normal protein; NP) of CP/kg of diet during d 8 to 21 of age^{1,2}

	Met supplementation (g/kg)								Pooled SEM	P (ANOVA)		
	0.0	0.5	1.0	1.5	2.0	3.0	4.0	6.0		Met	CP	Met×CP
BW gain ³ (g/bird)												
LP	151	218	342	532	555	566	573	492	33.5	<0.001	<0.001	0.767
NP	100	174	294	490	532	533	487	476	34.0			
Feed intake (g/bird)												
LP	319	417	485	730	723	754	774	663	35.9			
NP	289	343	530	683	680	759	613	619	37.6	<0.001	0.106	0.676
BW gain/feed (g/g)												
LP	0.47	0.54	0.71	0.73	0.77	0.75	0.74	0.74	0.04			
NP	0.36	0.51	0.65	0.72	0.78	0.71	0.79	0.77	0.04	<0.001	0.485	0.898
Protein in BW gain (g/kg)												
LP	153	153	161	155	150	162	157	160	1.44			
NP	167	150	152	151	162	159	160	165	1.97	0.332	0.365	0.201
Fat in BW gain (g/kg)												
LP	84	107	113	129	128	126	128	134	3.70			
NP	53	77	92	108	107	96	96	87	3.89	<0.001	<0.001	0.410
Energy in BW gain (MJ/kg)												
LP	7.1	7.9	8.5	9.1	8.9	9.1	8.9	9.1	0.19			
NP	6.4	7.1	7.3	7.9	8.4	7.9	7.8	7.8	0.15	<0.001	<0.001	0.931

¹n = 3 pens of 10 birds per treatment.²Initial whole-body concentrations determined in baseline birds on d 8 were (per kg BW): 146 g of protein, 80 g of fat, and 6.45 MJ of energy.³Initial body weight on d 8 was 107 g.

g/kg and an upper level of 4.7 g Met/kg of diet. In the present study, 95% of the y_{max} values in responses were already achieved with 3.0 to 3.6 g of Met/kg of diet. This difference may be due in part to the range of supplementation, because we used a basal Met level of 1.7 g/kg. The range in supplementation influences the shape of the curves and the estimated plateau (Rodehutscord and Pack, 1999). Also, the chosen response criteria and used mathematical model often influence the derived requirement (Baker, 1986). Although we implemented a 4-param-

eter logistic model to describe the response, the study by Huyghebaert and Pack (1996) implied fitting an exponential model. Differences between these 2 models are relevant for requirement estimates (Rodehutscord and Pack, 1999). Fitting an exponential model rather than equation [2] to the protein accretion data for the LP level of the present study resulted in an estimate for the Met requirement of 4.5 instead of 3.4 g/kg, respectively. This result demonstrates how sensitive the outcome of dose-response studies is, depending on study details such as

Table 4. Results of the parameter estimate when responses of broilers to increasing Met concentration in diets with 183 (low protein; LP) and 229 (normal protein; NP) g of CP/kg of diet were described with equation 2

	Estimated parameter					$s_{y,x}$	Met for 95% of y_{max} (g/kg of diet)
	y_{max}	b	c	k	r^2		
BW gain (g/bird in 14 d)							
LP	550.1	162.3	282,102	4.643	0.94	42.6	3.2
NP	510.2	115.3	579,915	4.721	0.95	38.9	3.4
BW gain/feed (g/g)							
LP	0.753	0.4471	2,244	3.327	0.61	0.09	3.0
NP	0.767	0.2254	215.5	2.388	0.64	0.12	3.3
Protein accretion (g/bird in 14 d)							
LP	86.49	23.15	55,360	4.087	0.94	6.57	3.4
NP	82.06	18.35	361,676	4.474	0.94	7.18	3.6
Met accretion (g/bird in 14 d)							
LP	1.770	0.3363	9,741	3.549	0.93	0.16	3.1
NP	1.609	0.3155	471,453	4.588	0.95	0.14	3.2
Fat accretion (g/bird in 14 d)							
LP	70.89	16.49	2.4×10^6	5.336	0.96	5.23	3.2
NP	50.19	8.972	7.8×10^9	8.069	0.85	7.98	3.2
Energy accretion (MJ/bird in 14 d)							
LP	4.965	1.2680	1.1×10^6	5.116	0.94	0.43	3.2
NP	4.066	0.8203	2.9×10^6	5.229	0.90	0.50	3.3

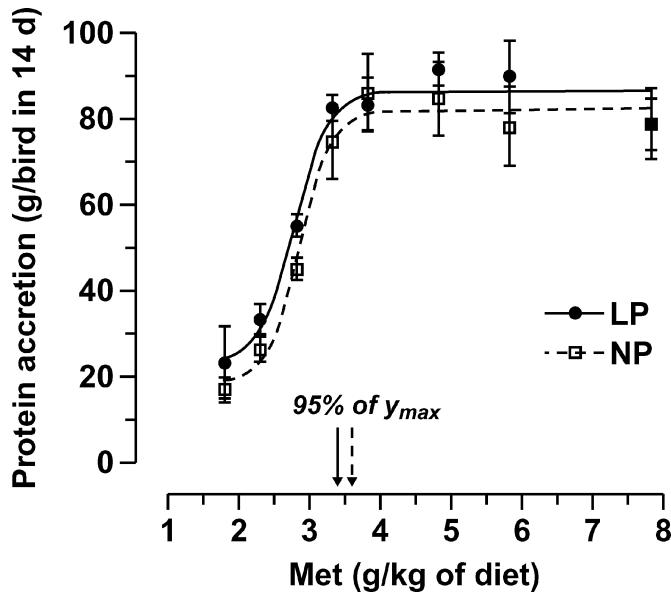


Figure 1. Effect of dietary Met concentration on protein accretion of broiler chickens fed diets with 183 (LP) or 229 g CP/kg (NP) between d 8 and 21 of age (means and SD; $n = 3$ pens of 10 birds per treatment). Equation 2 was fitted to the data, and results of parameter estimate are given in Table 4. The arrows indicate the Met concentration necessary to achieve 95% of the estimated y_{max} .

model choice. Huyghebaert et al. (1994) also observed that in growing chickens no plateau was achieved by feeding dietary Met ranging from 3.1 to 5.5 (low CP) and 3.9 to 5.9 (high CP) in low (205 g/kg) and high (242 g/kg) CP diets. They concluded that the SAA requirement could be higher than 8.6 g/kg SAA at low dietary CP and 9.5 g/kg SAA at high dietary CP. Poorer growth rate at similar Met intake for high CP treatment compared with low CP treatment and the absence of any advantage of low CP over high CP in gain/feed in their study is consistent with observations in the present study. Mendonca and Jensen (1989) observed that the requirement for SAA of growing male broilers as a percentage of the diet rose with increased CP concentration. By supplementing DL-Met to diets ranging from 164 to 287 g/kg CP, the SAA requirement for BW gain and gain/feed increased from 7.1 and 7.7 to 10.8 and 10.8 g/kg, respectively. This present study tends to support the proposition that Met requirement may be affected by CP concentration. Measurements of Eff_M show that the reason for this can be observed in a reduced capacity to use limited Met with increased CP supply.

Our results do not correspond with those of Corzo et al. (2005), who found lower growth at d 21 with broilers that were fed an EAA balanced diet with reduced CP concentration (17 vs. 21%). However, with supplementation with Gly to the reduced CP diet, growth was similar to the normal CP diet. Diets in our study were also supplemented with Gly. It can be concluded that under consideration of Gly supplementation, the requirement for N from NEAA was met in the LP diets. Arginine has also been described to interact with Met metabolism (Chamruspol-

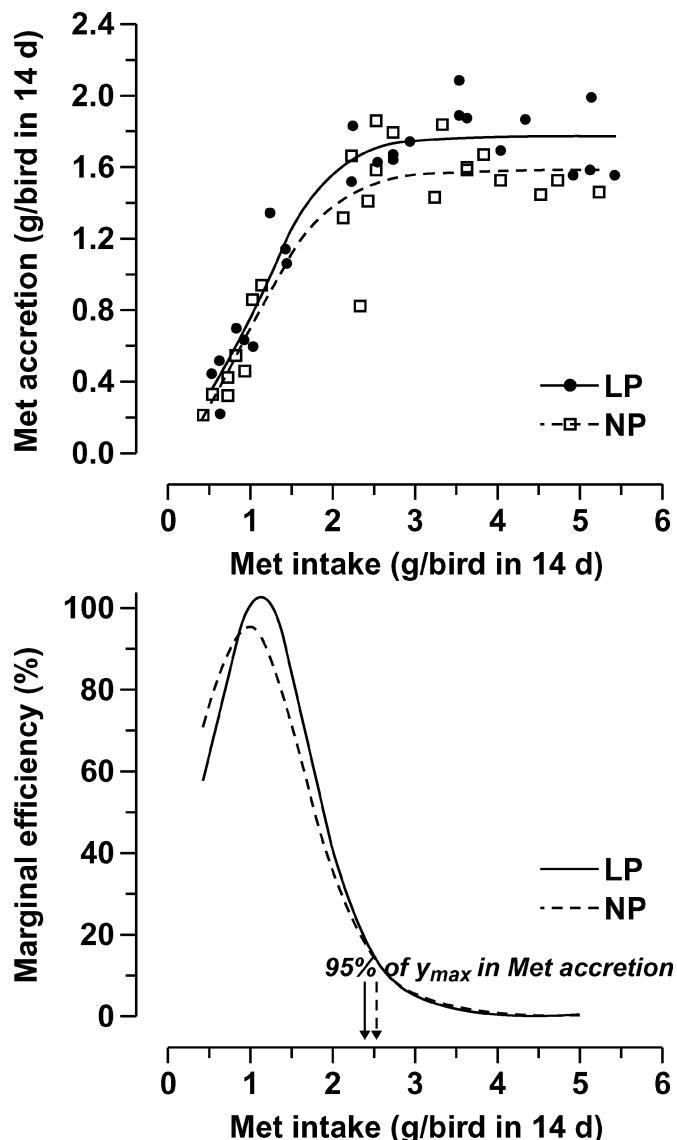


Figure 2. Effect of dietary Met intake on Met accretion (upper panel) and marginal efficiency of Met utilization (Δ accretion/ Δ intake $\times 100$) of broiler chickens fed diets with 183 (LP) or 229 g CP/kg (NP) between d 8 and 21 of age. Equation 2 was fitted to the data in the upper panel, and the following parameters were estimated: $y_{max} = 1.767$, $b = 0.1196$, $c = 12.30$, $k = 2.308$, $r^2 = 0.92$, and $s_{y,x} = 0.18$ (for LP), and $y_{max} = 1.579$, $b = -0.03669$, $c = 6.852$, $k = 2.056$, $r^2 = 0.87$, and $s_{y,x} = 0.21$ (for NP). Marginal efficiency was calculated with these parameters according to equation 3. Arrows indicate the Met intake necessary to achieve 95% of y_{max} in Met accretion.

lert et al., 2002), but the Arg concentration was kept constant in our study.

Effects on Chemical Composition of Body Weight Gain

Supplementation of NEAA caused a significant reduction in the concentrations of fat and energy but not protein in gained BW (Table 3). Similar results are already described in the literature. Nieß et al. (2003) reduced the CP concentration in broiler diets but maintained EAA supply constant by inclusion of free AA and found an

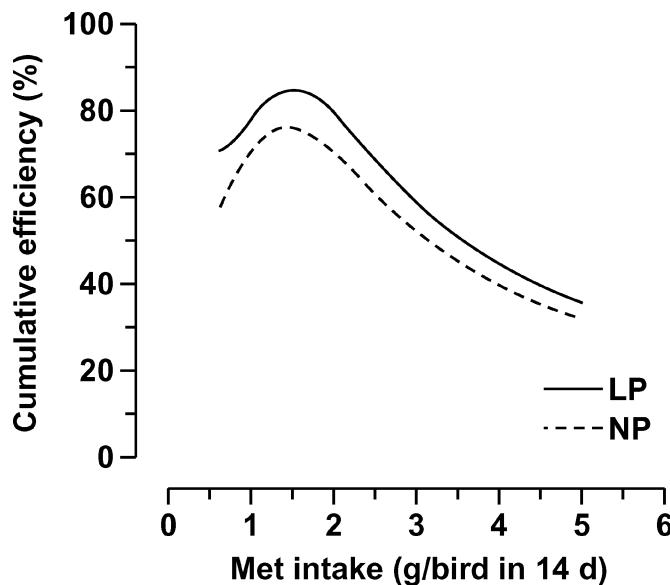


Figure 3. Effect of dietary Met intake on cumulative efficiency (Eff_C) of Met utilization of broiler chickens fed diets with 183 (LP) or 229 g CP/kg (NP) between d 8 and 21 of age. The Eff_C was calculated as grams of accreted Met per gram of Met intake, with accreted Met described with equation 2 as outlined in Figure 2.

increase in fat accretion by 30% without effects on growth or protein accretion. Reevaluating CP by supplementation with NEAA brought fat accretion back to the control level. These authors discussed other relevant papers with similar results. The fact that protein accretion in our study was approximately 5% lower in NP than in LP (Table 4) may, therefore, be a consequence mainly of the lower growth rate. Less energy available for tissue growth due to elevated uric acid synthesis in NP could be the reason for this difference in growth.

The increase in fat and energy concentration of gained BW due to a single supplemented AA could be regarded typical for such dose-response studies with ad libitum feeding, because feed intake increases with increments in limiting AA intake before a plateau is reached (Rosa et al., 2001; Fatufe et al., 2004, 2005). The AA composition of gained whole-body protein was not constant at suboptimal Met supply, and changes were similar at both CP levels. The concentrations of Lys and Met increased significantly, whereas those of Gly significantly declined. These changes occurred in the suboptimal range of Met intake. As discussed by Fatufe et al. (2004), they could have been a consequence of the changing proportions of different protein fractions to whole-body protein. The 4 highest dietary Met concentrations caused a Met concentration in gained whole-body protein of, on average, 2.0 g/16 g of N, which is identical to the value determined for broilers of the same age by Fatufe et al. (2004). Kirchgessner et al. (1987) determined 1.8 or 1.6 g/100 g AA in whole-body protein accreted until wk 2 or in wk 4 to 5, respectively. Dietary energy, but not CP concentration, significantly affected the Met concentration of accreted whole-body protein in their study.

Effect of NEAA Supplementation on the Efficiency of Met Use

The nonlinear response to increasing Met intake showed that the efficiency for protein accretion of incremental Met was not constant even at marginal level of supply. Even though the dosage levels of Met were similar at both levels of CP, Met tended to be less efficiently used in NP diets than in LP diets (Figure 2). The maxima in Eff_M , which demonstrate the potential for use, differed by around 8% between the 2 CP alternatives. The maxima occurred at a suboptimal level of Met intake, meaning that the organism was still in a state that should have forced it to best use a limiting AA. Inevitable catabolism of Met might have prevented a higher Eff_M in the NP treatments. Perhaps the oxidation of the extra NEAA caused a higher inevitable catabolism of free Met as well, thus leaving less available for tissue protein synthesis. Moughan (2003) attributed inevitable catabolism to the presence of active catabolic enzymes in the cell. The hypothesis that lack of specificity in such enzymes for different AA caused the differences in Eff_M for Met needs further clarification.

Maximum values for Eff_M of Met use were achieved at Met intakes that allowed for only 49 and 38% of y_{\max} in Met accretion for LP and NP, respectively (Table 6). This result shows that diminishing returns in retention already occurred at a Met intake of <50% of that required for high Met retention. This finding principally corresponds with the finding of Edwards and Baker (1999), but diminishing return occurred at a lower level of Met intake in the present study. Edwards and Baker (1999) observed a diminishing return of supplemental Met at an intake level above 70% of that required for maximum growth rate. Calculation of efficiency within this range (up to 70% required for maximum growth rate) using a linear model yielded an efficiency of Met use for accretion of 68% in their study. As an approximation, linear regressions for the present data can be calculated for Met accretion (y), depending on Met intake (x) up to ~90% of that required for high protein accretion as follows: $y_{LP} = 0.095 (\pm 0.13) + 0.68 (\pm 0.09) \times (r^2 = 0.86, s_{y,x} = 0.21)$, and $y_{NP} = 0.091 (\pm 0.14) + 0.54 (\pm 0.09) \times (r^2 = 0.77, s_{y,x} = 0.24)$. The efficiency of 68% estimated for LP corresponds with the results of Edwards and Baker (1999). The maximum in cumulative efficiency of Met retention depending on Met intake was 85% for LP and 76% for NP. These values also corroborate the observation about Eff_M with regard to diminishing return to incremental Met intake.

We concluded that the efficiency of Met use was affected by the NEAA N concentration in the diet. The efficiency of Met use was not constant at suboptimal supply, and this nonlinearity was unaffected by dietary CP supply. Future AA requirement models should consider this nonlinear relationship between intake and efficiency of use. A reduction in N content of the diets allowed for greater use of limiting EAA, causing lower dietary requirements.

Table 5. Amino acid content of body protein that was accreted by broiler chickens between d 8 and 21 when fed diets with 183 (low protein; LP) or 229 (normal protein; NP) g of CP/kg of diet and different Met supplements (g/16 g of N)^{1,2}

	Met supplementation (g/kg)								Pooled SEM	P (ANOVA)		
	0.0	0.5	1.0	1.5	2.0	3.0	4.0	6.0		Met	CP	Met × CP
Ala												
LP	6.3	6.3	6.5	6.3	6.4	6.5	6.4	6.3	0.08			
NP	6.2	6.5	6.4	6.2	6.4	6.0	6.1	6.1	0.08	0.987	0.346	0.949
Arg												
LP	6.7	6.7	6.9	6.6	6.7	6.8	6.4	6.4	0.16			
NP	5.8	6.6	6.6	6.5	6.7	6.3	6.5	5.9	0.16	0.911	0.232	0.984
Asp												
LP	8.6	8.5	9.0	8.8	8.8	9.0	8.8	8.6	0.06			
NP	8.0	8.4	8.4	8.5	8.7	8.5	8.7	8.3	0.07	0.068	0.001	0.776
Cystine												
LP	1.5	1.4	1.4	1.4	1.3	1.3	1.4	1.4	0.03			
NP	1.5	1.5	1.4	1.4	1.4	1.3	1.4	1.4	0.05	0.905	0.807	0.999
Glu												
LP	14.3	13.8	14.1	14.2	14.6	14.4	14.5	14.2	0.10			
NP	13.4	13.9	13.7	13.8	14.2	13.6	14.0	13.7	0.12	0.729	0.010	0.885
Gly												
LP	9.0	8.7	8.7	7.9	8.3	8.2	8.1	8.1	0.12			
NP	8.9	8.8	8.8	8.1	8.3	7.8	7.9	7.9	0.12	0.004	0.641	0.961
Ile												
LP	4.0	3.7	3.9	3.9	4.0	4.2	3.8	3.8	0.08			
NP	3.2	3.6	3.5	3.7	3.7	3.6	3.7	3.6	0.05	0.878	0.001	0.584
Leu												
LP	7.3	7.2	7.5	7.2	7.4	7.6	7.4	7.5	0.06			
NP	7.1	7.2	7.0	7.3	7.4	7.5	7.5	7.6	0.09	0.549	0.666	0.881
Lys												
LP	5.6	6.2	6.7	6.7	6.9	7.1	6.7	6.6	0.13			
NP	5.5	5.9	5.9	6.5	6.6	6.8	6.9	6.4	0.14	<0.001	0.144	0.835
Met												
LP	1.7	1.9	2.1	2.0	2.0	2.1	2.1	2.0	0.04			
NP	1.7	1.8	1.9	2.0	2.0	2.0	2.0	1.9	0.03	<0.001	0.027	0.779
Phe												
LP	3.8	3.8	3.8	3.6	3.9	4.0	3.9	3.9	0.08			
NP	3.9	3.8	3.8	3.8	3.8	3.8	3.8	3.7	0.05	0.987	0.722	0.995
Pro												
LP	6.9	6.5	6.5	5.8	5.5	5.4	5.5	5.1	0.21			
NP	6.5	5.7	5.6	5.2	5.3	5.6	5.5	5.9	0.28	0.660	0.514	0.971
Ser												
LP	4.7	4.5	4.5	4.4	4.5	4.5	4.4	4.4	0.03			
NP	4.7	4.5	4.4	4.4	4.4	4.3	4.3	4.2	0.05	0.203	0.136	0.876
Thr												
LP	4.1	4.2	4.3	4.2	4.3	4.3	4.2	4.2	0.03			
NP	4.1	4.2	4.1	4.1	4.2	4.0	4.1	4.0	0.03	0.773	0.011	0.781
Val												
LP	4.6	4.6	4.6	4.7	4.7	4.6	4.9	4.8	0.08			
NP	4.4	4.6	4.4	4.5	4.7	4.4	4.5	4.6	0.05	0.959	0.150	0.993

¹n = 3 pens of 10 birds per treatment.

²Amino acid concentrations in body protein at the start of the experiment were (in g/16 g of N): Ala, 6.6; Arg, 6.8; Asp, 8.9; cystine, 1.5; Glu, 13.6; Gly, 9.2; Ile, 3.9; Leu, 7.0; Lys, 5.7; Met, 1.9; Phe, 3.5; Pro, 7.3; Ser, 4.8; Thr, 4.1; Val, 5.2.

Table 6. Relationship between marginal efficiency of Met utilization and Met intake needed for high Met accretion in male broiler chicken when fed diets with 183 (LP) or 229 g CP/kg of diet (NP) between d 8 and 21

	Met intake		Marginal efficiency (%) ¹		Met accretion at maximal marginal efficiency (% of y _{max})
	95% of y _{max} in Met accretion (g/bird in 14 d)	Maximum in marginal efficiency (g/bird in 14 d)	Maximum	At intake needed for 95% y _{max} in Met accretion	
LP	2.4	1.2	103	21	48
NP	2.5	1.0	95	19	38

¹Based on Figure 2.

ACKNOWLEDGMENT

This study was supported by the German Academic Exchange Office (DAAD) with a doctoral scholarship for Andrew A. Fatufe, which is gratefully acknowledged.

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