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ARTICLE INFORMATION	Fill in information in each box below
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Article Title (within 20 words without abbreviations)	Standardized ileal digestible methionine requirements for 22 to 29-d-old male broilers based on the growth performance and feather development
Running Title (within 10 words)	Standardized ileal digestible methionine requirement for broilers
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Ethics approval and consent to participate	The protocols for the present study were reviewed and approved by the Institutional Animal Care and Use Committee at Kyungpook National University (KNU2021-0217).

4

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6

Abstract The aim of the present experiment was to determine the standardized ileal digestible (SID) methionine (Met) requirement during the grower phase (22 to 29 days). A total of 192 Ross 308 male broilers (745 ± 50.5 g) were assigned to six dietary treatments in a randomized complete block design, with eight replicate cages per treatment. The experimental diets were formulated based on corn, soybean meal, and synthetic amino acids, with different levels of dietary SID Met ranging from 0.40% to 0.65%, in increments of 0.05%. Individual body weight and feed intake per cage were measured at the beginning and end of the experimental period. Weight gain (g/bird), feed intake (g/bird), gain-to-feed ratio (g/kg), feather weight (g), and relative feather percentage (% of body weight) were analyzed for linear or quadratic responses using orthogonal polynomial contrasts. Final body weight, weight gain, and feed intake increased quadratically as dietary SID Met concentration increased ($p < 0.05$). However, the composition of AA in the feathers showed no quadratic or linear effect based on dietary SID Met concentrations, except for Ile ($p = 0.05$; quadratic). The SID Met requirements for optimum and maximum weight gain of 22 to 29-days-old male broilers were estimated using the broken-line and quadratic-line models to 0.45% and 0.55%, respectively.

Keywords: Amino acid; Broiler; Methionine; Requirement; Standardized ileal digestible

Introduction

Methionine (Met) is an essential amino acid (AA) that is crucial in retaining body protein and promoting feather growth. In animal diets, particularly in poultry, Met is often the first-limiting AA when using corn- and soybean meal (SBM)-based diets [1]. Furthermore, Met is involved in various metabolic functions, including protein synthesis, immune response, acting as a methyl donor during DNA replication, and aiding in cysteine production through degradation. Dietary Met can lead to the de novo formation of cysteine (Cys), rendering Cys an indispensable amino acid (AA). This makes the total sulfur AA (TSAA; Met + Cys) a common representation for Met requirements. A dietary Cys deficiency may lead to overestimating the Met requirement in broilers. This is because dietary Met is utilized to meet the demand for Cys in case of insufficient dietary Cys. Consequently, converting Met into Cys to replenish insufficient levels may reduce the overall proportion of Met utilization.

34   Commercial diets are typically formulated based on TSAA to ensure adequate Met and Cys levels [2].

35   Understanding the specific growth response to dietary digestible Met concentrations in broilers is

36   crucial. This information is essential for accurately meeting the Met requirements of broilers to attain

37   production goals such as optimal and maximum breast meat yields. By optimizing Met levels,

38   productivity can be enhanced, and nutrient excretion into the environment reduced, benefiting both the

39   industry and environment. Numerous studies have concentrated on establishing TSAA requirements [3-

40   7], as well as the Met-to-Cys ratio [2]; however, only a few have specifically addressed Met

41   requirements [8-11]. Furthermore, decades of intensive selective breeding have increased voluntary

42   daily feed intake and enhanced digestive capacity, leading to rapid growth rates in modern broilers [12].

43   Therefore, continued research is essential to accurately determine Met requirements and appropriately

44   adjust dietary Met concentrations to align with the genetic improvements in broilers. The Met

45   requirements recommended by the National Research Council [13] and Rostagno et al. [14] differ from

46   those estimated using empirical methods to determine Met or TSAA requirements. Estimating Met

47   requirements for broilers is complex due to several interacting factors, including estimation

48   methodology [15], dietary Met sources [16], and animal-related factors, such as age [6,7], intestinal

49   growth [17], and onset of feathering [3]. Moreover, the individual AA needs for broilers may vary

50   depending on dietary nutrient concentrations, body size, and growth stage [18]. Additionally, the critical

51   role of feather development has been overlooked, despite its importance in protecting birds from

52   environmental risks and conserving heat [19]. Feather protein synthesis demands a substantial amount

53   of Cys [20,21] and constitutes a significant portion of the AA composition [3,22]. Therefore, the feather

54   growth rate might impact the necessary Met intake for optimal growth performance in broilers. This

55   study aims to determine Met requirements based on male broiler growth during the grower phase and

56   variations in Met concentrations in feathers using the ideal AA concept with diets containing different

57   dietary standardized ileal digestible (SID) Met concentrations.

58

59   **Materials and Methods**

60 *Animal ethics*

61   All the experimental procedures in this study were reviewed and approved by the Institutional

62 Animal Care and Use Committee of Kyungpook National University (KNU2021-0217).

63

64 *Animals and management*

65   A total of 192 22-day-old male broiler chickens (Ross 308) were used in this study. The birds

66 were allocated to six treatments in a randomized complete block design. To reduce the initial body

67 weight (BW) impact, a set of experimental treatments was categorized into groups (blocks). The initial

68 BW was regarded as a blocking factor. Each treatment having eight replicate cages containing four

69 birds per cage. The experimental diets were provided in mash form, and both water and feeds were

70 offered ad libitum. The environment was carefully controlled with continuous light and temperature.

71 The birds were housed in wire-floored cages, measuring $60 \, cm \times 60 \, cm \times 60 \, cm$, in an environmentally

72 controlled room. Following the Ross 308 broiler recommendations [23], the temperature in the room

73 was initially set at 33℃ for the first three days and then gradually decreased by 3℃ per week until it

74 reached 24℃ by day 29 and relative humidity was set at 65% for the first three days and gradually

75 decreased to 45% at the end of the experiment. This temperature and relative humidity were monitored

76 and maintained until the end of the experiment.

77

78 *Dietary treatments*

79   Six experimental diets were formulated based on the corn-SBM-based diet, with varying SID

80 Met concentrations ranging from 0.40% to 0.65%. These diets were designed to contain graded levels

81 of dietary SID Met while maintaining sufficient Cys at 0.47% (**Table 1**). The ideal digestible AA ratios

82 relative to lysine concentrations, except for histidine and phenylalanine, for the grower phase (day 15

83 to 35 of age) were adopted from Hoehler et al. [24]. Relative histidine and phenylalanine ratios to lysine

84 were obtained from Rostagno et al [13]. Standardized ileal digestibility of AA in corn and SBM was

85 determined using equations described by Kong and Adeola [25]. Moreover, the SID AA in corn and

86 SBM for 25-day-old Ross 308 male broilers were derived from a previous study [26]. All indispensable

87 AA, except for Met, were included at 110% of recommended levels to prevent deficiencies; other AA

88   ratios; the recommended lysine level used in the present study was obtained from our previous study

89   [27], and the value was determined based on the weight gain of 21 to 28 days old male broilers using a

90   broken-line model. The dietary Met in each experimental diet served as the first-limiting AA. Analyzed

91   AA concentrations in the experimental diets were close to the calculated values (**Table 2**).

92

93   *Performance measurement and sampling*

94   During the experimental period, daily mortality for each cage was recorded. Individual BW,

95   feed supply, and leftovers per cage were also monitored to determine body weight gain (BWG), feed

96   intake, and gain-to-feed ratio (G:F) at the beginning and end of the experiment. Four birds from each

97   treatment group were selected based on their average BW for whole-body feather sampling. The AA

98   compositions in the broilers' feathers were determined using 29-day-old birds. Feathers were removed

99   from each carcass by dry plucking.

100

101   *Chemical analysis*

102   Dry matter and AA compositions in the experimental diets were analyzed. The samples were

103   ground using laboratory mill (CT293 Cyclotec, Foss, Denmark) for chemical analysis. Test feed

104   ingredients, commercial diet, and experimental diet samples were dried at 135℃ for 2 hours according

105   to the Association of Official Analytical Chemists (AOAC) International method 930.15. Ccorn, SBM,

106   commercial diet, and experimental diet samples were analyzed for AA using the AOAC International

107   methods 994.12 and 988.15 [28]. Similarly, the collected feather samples were ground and analyzed for

108   dry matter and AA composition using AOAC International methods 930.15, 994.12, and 988.15 [28].

109

110   *Data analysis*

111   Data for BWG and G:F were analyzed using the MIXED procedure in SAS (SAS Inst. Inc.,

112   Cary, NC, USA). The SID Lys requirements were estimated using one-slope broken-line and quadratic-

113   line models employing nonlinear regression analysis in SAS, based on the study by Robbins et al. [29].

114   Each experimental diet was treated as a fixed effect, while the blocks with replicates based on initial

BW per cage were considered a random effect. If the random effect was Final statistic model Each cage

served as an experimental unit, and statistical significance was set at $p = 0.05$.

Results

This experiment was conducted to determine the SID Met requirements of broilers in the

grower phase (22 to 29 days of age). Only one bird was dead during the experimental period and feed

intake of dead birds was calculated and eliminated from the final dataset.

Graded levels of dietary SID Met concentrations resulted in significant quadratic effects ($p <$

0.01) on final BW, BWG, and feed intake (**Table 3**). However, there was no significant differences in

G:F in response to dietary SID Met concentrations. Moreover, feather weight and relative feather

compositions (% of BW) were not influenced by dietary SID Met concentrations. The data in **Table 4**

indicate that dietary SID Met concentrations had no significant influence on weight and AA

composition in feathers (wing + body). The SID Met requirements for BWG and feed intake, which

were determined using a linear broken-line model, were 0.45% and 0.46%, respectively. The estimated

SID Met requirement for both BWG and feed intake, using a quadratic-line model, was 0.55%. In

addition, the calculated SID Met 95% of the upper asymptotic value of quadratic-line model for BWG

and feed intake was 0.52% (**Figures 1 and 2**).

Discussion

Various regression models can be used to determine the AA requirement. The selection of a

regression model is based on plotting the response of birds against dietary AA concentrations. A linear

broken-line model is appropriate if there is a plateau response after a certain point. On the other hand,

if there is an increase or decrease in responses after the breakpoint, a quadratic-line model is suitable to

determine the AA requirement or AA toxicity. Baker [31] suggested that estimated AA requirements

from a linear broken-line model represent the maximum response with a minimum amount of dietary

AA, regardless of economic considerations and safety margins. Moreover, the fitted broken-line model

provides an objective value rather than a subjective one. However, if there is an increase or decrease in

142 responses after the breakpoint, a quadratic-line model is suitable to determine the AA requirement or

143 AA toxicity. The estimated AA requirement does not yield objective breakpoints. Baker [30] proposed

144 that one must subjectively select, for example, 90% of the upper asymptote value of the quadratic line.

145 Therefore, this study provided the SID Met requirement based on the quadratic-line model using the

146 95% upper asymptote value.

147   The birds in this study had inadequate growth compared to the recommended growth rate

148 specified in the Ross 308 performance objectives [23]. Various factors, such as animal and

149 environmental conditions, can influence bird growth rate. It is challenging to explain why the birds did

150 not reach their target growth potential. However, one potential contributing factor could be the feed

151 forms used. Numerous studies have shown that different feed forms impact broiler chickens' growth

152 performance and feed efficiency. It is widely recognized that feeding pellets, rather than mash, enhance

153 broiler growth rate and increase feed intake [31;32]. In this study, the birds were fed crumble

154 commercial feed for 21 days, then after eight days, feeding mash-form the experimental diet. In terms

155 of BW, male broilers in this study at three weeks of age yielded results similar to previous studies that

156 aimed to determine the metabolic requirement of broilers [10; 20]. The lower BW observed in the birds

157 used in the present study after three weeks of age, compared to the performance potential of the Ross

158 308 breed, could be attributed to the transition from a crumble commercial to a mash-form experimental

159 diet.

160   When assessing the AA requirements for broilers, growth response indicators such as BWG

161 and feed efficiency are traditionally employed [33]. In this study, the impact of dietary SID Met

162 concentrations on feed efficiency was not significant. However, both BWG and feed intake showed

163 significant results. Feed intake is the main factor driving the growth rate of broilers. Therefore, the

164 estimated SID Met requirement was determined based on both the BWG and feed intake of birds. The

165 estimated Met requireme nts for 22 to 29-day-old male broilers in the present study were within the

166 reported range of values, ranging from 0.46% for 21 to 42-day-old [20] to 0.59% for 21 to 42-day-old

167 [10]. The optimal dietary Met requirement (using linear-broken line model) based on weight gain

168 (0.45%) and feed intake (0.46%), as estimated in this study, was similar to the estimation for weight

169 gain and G:F (0.46%) provided Moran [34]. Although growth response and feed intake of birds

170 increased quadratically, but the G:F is not influenced by dietary SID Met concentration from 0.40% to

171 0.65%. Therefore, in the present study, SID Met requirements for 22 to 29-day old male broilers were

172 determined based on the weight gain and feed intake; and linear-broken and quadratic-line models were

173 employed to determine the SID Met requirements. The estimated Met requirement was lower than the

174 value recommended by the NRC [13], which was 0.38% for three to six-week-old broilers, but similar

175 to the value of 0.46% for 22 to 33-day-old broilers reported by Rostagno et al. [14].

176   Feather AA composition may change depending on dietary Met concentrations. A deficiency

177 of dietary Cys can induce the conversion of Met into Cys; therefore, dietary L-Cys supplement replaces

178 Cys and increases the proportion of digestible Met concentrations used for protein synthesis to increase

179 body mass [2]. Moreover, the contribution of TSAA in the integument is greater than that in muscle

180 tissue, and TSAA concentration is a major factor influencing the synthesis of feather keratin [3].

181 Consequently, an insufficiency in TSAA can lead to abnormal feathering, potentially impacting the

182 nutritional requirement for growth due to its effects on the amino acid pool. Kalinowski et al. [20]

183 evaluated the Met requirement for 0-to-3-week-old broilers by increasing DL-Met concentrations from

184 0.35% to 0.50% while maintaining a constant Cys level (0.50%) but failed to elicit a weight gain

185 response. Nevertheless, they observed improved FCR and N retention, irrespective of the birds'

186 feathering rate. As a result, they concluded that the Met requirement is not influenced by the feathering

187 rate, establishing it at 0.46% based on N retention using the quadratic model. However, they confirmed

188 variations in Cys to TSAA) requirement depending on strains with genetically distinct feathering rates,

189 such as 44% and 47% in slow- and fast-feathering birds, respectively. Therefore, the feathering degree

190 may confound results, as mature broilers require higher N retention and AA for feathering than younger

191 counterparts [20]. Fisher et al. [35] highlighted how dietary specifications could impact feather growth

192 and AA composition. In contrast, Conde-Aguilera et al. [36] observed no differences in feather AA

193 composition and retained Met and Cys contents in response to dietary TSAA concentrations, which

194 conform to our results. In addition, the AA composition in feathers in the present study was similar to

195 the previous studies [37-39]. In this context, birds may be more sensitive to dietary AA depend on the

196 contribution on feather synthesis compared to the dietary Met, given its relatively higher proportion

197 (7.33%) deposited in feather compared to Met (0.57%) [40].

198   Feather mass in birds corresponds to the excreted N within the consumed AA. Vargas et al.

199   [41] reported that feather weight increased from 1.4 g to 151.3 g as BW increased from 44 g to 4,188 g

200   while maintaining a constant relative weight (3.43%) from 1 to 56 days. Feather weight and deposited

201   AA were measured to verify whether dietary Met contributes to feather AA composition. Similar

202   outcomes were observed in the present study, where dietary SID Met concentrations did not impact

203   feather weight (g), and relative feather weight (% of BW) averaged 1.91% at an average BW of 1,103

204   g at 29 days old. This result suggests that the lowest dietary SID Met levels (0.40%) are not limiting for

205   feather protein synthesis. However, the relative feather weight to BW (1.91% vs. 3.43%) from the

206   present study was lower than in Vargas et al. [41] with a similar BW. Differences between the studies

207   could be attributed to the shorter feeding duration in this study (seven days) and different genetic

208   backgrounds.

209   Numerous factors influence feather growth and AA composition, such as dietary specificatio

210   ns [3,35], limiting AA [42], and bird age [34,43,44]. The absence of differences in feather AA

211   composition based on dietary SID Met concentrations in the current study may be attributed to adequate

212   dietary AA, except for Met, which was supplied to meet broiler requirements according to the ideal AA

213   ratio. This is because the experimental diets were formulated to increase Met concentrations from

214   deficient to excess levels. The limitation in AA utilization is determined by the amount of dietary

215   second-limiting AA as the dietary first-limiting AA is increased [45]. Therefore, the results of the

216   present study suggest that balanced dietary AA supplementation is crucial in terms of AA

217   concentrations and supporting protein accretion for growth and feather conditions.

218   In conclusion, the dietary SID Met concentration ranged from 0.40% to 0.65%, and the

219   constant dietary protein ratio did not affect the feather AA composition of 29-days old male broilers.

220   The estiamted SID Met requirements for 22-29 days old broilers vary depending on the statistical

221   methods. The different SID Met requirements for broilers can be used for various target groups of

222   animals. The optimum (brokne-line model) SID Met requirements based on the weight gain and feed

223   intake were estimated to be 0.45% and 0.46%, respectively. The estimated maximum (quadratic-line

224   model) SID Met requirements for BWG and feed intake was 0.55%. In addition, the calculated SID Met

225   requirements for BWG and feed intake, which is 95% asymptotic value of quadratic-line model was

226   0.52%.

227

228   **Acknowledgments**

232

233

References

235   1.   Fernandez SR, Aoyagi S, Han Y, Parsons C M, Baker DH. Limiting order of amino acids in corn
236   and soybean meal for growth of the chicks. Poult Sci. 1994;73:1887-96.
237   https://doi.org/10.3382/ps.0731887

238   2.   Pacheco LG, Sakomura NK, Suzuki RM, Dorigam J, Viana GS, van Milgen J, and Denadai JC.
239   Methionine to cystine ratio in the total sulfur amino acid requirements and sulfur amino acid
240   metabolism using labelled amino acid approach for broilers. BMC Vet Res. 2018;14:364.
241   https://doi.org/10.1186/s12917-018-1677-8

242   3.   Wheeler, K. B., and T. D. Latshaw. Sulfur amino acid requirements and interactions in broilers
243   during two growth periods. Poult Sci. 1981;60:228-36. https://doi.org/ 10.3382/ps.0600228

244   4.   Pack M, Schutte JB. Sulfur amino acid requirement of broiler chicks from fourteen to thirty-eight
245   days of age: 2. Economic evaluation. Poult Sci. 1995;74:488-93. https://doi.org/
246   10.3382/ps.0740488

247   5.   Baker DH, Fernandez SR, Webel DM, Parsons CM. Sulfur amino acid requirement and cystine
248   replacement value of broiler broilers during the period three to six weeks posthatching. Poult Sci.
249   1996;75:737-42. https://doi.org/ 10.3382/ps.0750737

250   6.   Aftab U, Ashraf M. Methionine+cystine requirement of broiler chickens fed low density diets
251   under tropical conditions. Trop Anim Health Prod. 2009;41:363-9.
252   https://doi.org/10.1007/s11250-008-9197-3

253   7.   Goulart CDC, Costa FGP, Silva JHV, Souza JG, Rodrigues VP, Oliveira CFS. Requirements of
254   digestible methionine+cystine for broiler chickens at 1 to 42 days of age. Rev Bras Zootec.
255   2011;40:797-803. https://doi.org/10.1590/S1516-35982011000400013

256   8.   Kim JH, Cho WT, Yang CJ, Shin IS, Han IK. Partition of amino acids requirement for maintenance
257   and growth of broilers II. Methionine. Asian Australas J Anim Sci. 1997;10:277-83.
258   https://doi.org/10.5713/ajas.1997.277

259   9.   Chamruspollert M, Pesti GM, Bakalli RI. Determination of the methionine requirement of male
260   and female broiler chicks using an indirect amino acid oxidation method. Poult Sci. 2002;81:1004-
261   13. https://doi.org/10.1093/ps/81.7.1004

262   10.   Mulyantini NGA. Digestible methionine requirement for performance and carcass yield of broiler
263   finisher. In: Proceedings of the 5th International Seminar on Tropical Animal Production; 2010;
264   Yogyakarta, Indonesia.

265   11.   Li LL, Abouelezz KFM, Zhonggang C, Gad-Elkareem AEG, Fan Q, Ding F, Gao J, Jiang S, Jiang
266   Z. Modelling methionine requirements of fast-and slow-growing chinese yellow-feathered
267   chickens during the starter phase. Animals. 2020;10:443. https://doi.org/10.3390/ani10030443

268 12. Tallentire, CW, Leinonen I, Kyriazakis I. Breeding for efficiency in the broiler chickens: A review.
269   Agron Sustain Dev. 2016;36:66. https://doi.org/10.1007/s13593-016-0398-2

270 13. NRC. 1994. Nutrient requirements of poultry, 9th rev. ed. Natl. Acad. Press, Washington, DC.
271   USA.

272 14. Rostagno HS, Albino LFT, Hannas MI, Donzele JL, Sakomura NK, Perazzo FG, Saraiva A, Abreu
273   MLT, Rodrigues PB, Oliveira RF, Barreto SLT, Brito CO. Brazilian tables for poultry and swine :
274   composition of feedstuffs and nutritional requirements, 4 ed. Viçosa: Departamento de Zootecnia,
275   UFV, Viçosa, MG, Brazil.

276 15. Featherston WR, Rogler JC. Methionine-cystine interrelations in chicks fed diets containing
277   suboptimal levels of methionine. J Nutr. 1978;108:1954-8. https://doi.org/10.1093/jn/108.12.1954

278 16. Ullrich C, Langeheine M, Brehm R, Taube V, Rosillo Galera M, Rohn K, Popp J, and Visscher C.
279   Influence of different methionine sources on performance and slaughter characteristics of broilers.
280   Animals. 2019;9:984. https://doi.org/10.3390/ani9110984

281 17. Sklan D, Noy Y. Crude protein and essential amino acid requirements in chicks during the first
282   week posthatch. Br Poult Sci. 2003;44:266-74. https://doi.org/ 10.1080/0007166031000124586

283 18. Mbajiorgu CA, Ng`ambi JW, Norris DD. Voluntary feed intake and nutrient composition in
284   chickens. Asian J Anim Vet Adv. 2011;6:20-8. https://doi.org/ 10.3923/ajava.2011.20.28

285 19. Deschutter A, Leeson S. Feather growth and development. Worlds Poult Sci. 1986;42:259-67.
286   https://doi.org/10.1079/WPS19860020

287 20. Kalinowski A, Jr. Moran ET, Wyatt CL. Methionine and cystine requirements of slow- and fast-
288   feathering broiler males from three to six weeks of age. Poult Sci. 2003;82:1428-37.
289   https://doi.org/ 10.1093/ps/82.9.1428

290 21. van Emous RA, van Krimpen MM. The Poultry Integument in Health and Welfare, Effects of
291   nutritional interventions on feathering of poultry - a review. In: Olukosi OA, Olori VE, Helmbrecht
292   A, Lambton S, French NA. Poultry Feathers and Skin. Cambridge, England, CABI; 2019. p. 133-
293   50. https://doi.org/10.1079/9781786395115.0133

294 22. Leeson S, Walsh T. Feathering in commercial poultry II. Factors influencing feather growth and
295   feather loss. Worlds Poult Sci. 2004;60:52-63. https://doi.org/ 10.1079/WPS20034

296 23. Aviagen. Ross 308 Broiler: Performance Objectives [Internet]. 2019 [cited 15 Apr 2023]
297   http://eu.aviagen.com/tech-center/download/1339/Ross308-308FF-BroilerPO2019-EN.pdf

298 24. Hoehler D, Lemme A, Ravindran V, Bryden WL, Rostagno HH. Feed formulation in broiler
299   chickens based on standardized ileal amino acid digestibility. In: Proceedings of Advances in

300   Poultry Nutrition, VIII. International Symposium on Aquatic Nutrition; 2006; UANL, Monterrey,
301   New Lion, Mexico.

302   25. Kong C, Adeola O. Evaluation of amino acid and energy utilization in feedstuff for swine and
303   poultry   diets.   Asian-Australas   J   Anim   Sci.   2014;27:917-25.
304   https://doi.org/10.5713%2Fajas.2014.r.02

305   26. An SH, Sung JY. Kang HK, Kong C. Additivity of ileal amino acid digestibility in diets containing
306   corn, soybean meal, and corn distillers dried grains with solubles for male broilers. Animals.
307   2020;10:933. https://doi.org/10.3390/ani10060933

308   27. An SH, Kang H-K, Kong C. Standardized ileal digestible lysine requirements of 21–28 days old
309   male   broilers.   Anim   Feed   Sci   Technol.   2020;292:115409.
310   https://doi.org/10.1016/j.anifeedsci.2022.115409

311   28. AOAC International. Official methods of analysis. 18th ed. Gaithersburg, MD, USA: Association
312   of Officissssal Analytical Chemist International, 2006.

313   29. Robbins KR, Saxton AM, Southern LL. Estimation of nutrient requirements using broken-line
314   regression   analysis.   J   Anim   Sci.   2006;84:E155-65.   https://doi.org/
315   10.2527/2006.8413_supple155x

316   30. Baker, D.H. Ideal amino acid patterns for broiler chicks. In: D'Mello, J.P.F, editor. Amino acids
317   in animal nutrition. Wallingford: CABI Publishing; 2003. p. 223-35.

318   31. Nir I, Hillel R, Shefet G, Nitsan Z. Effect of grain particle size on performance. 2. Grain texture
319   interactions. Poult Sci. 1994; Abdollahi MR, Zaefarian F, Ravindran V. Feed intake response of
320   broilers: impact of feed processing. Anim. Feed. Sci. Technol. 2018;237:154-65.
321   https://doi.org/10.3382/ps.0730781

322   32. Hamungalu O, Zaefarian F, Abdollahi MR, Ravindran V. Performance response of broilers to
323   feeding pelleted diets is influenced by dietary nutrient density. Anim Feed Sci Technol.
324   2020;268:114613

325   33. D'Mello J.P.F. Responses of growing poultry to amino acids. In: D'Mello, J.P.F, editor. Amino
326   acids in animal nutrition. Wallingford: CABI Publishing; 2003. p. 237-263

327   34. Moran ET. Cystine requirement of feather-sexed broiler chickens with sex and age. Poult Sci.
328   1981;60:1056-61. https://doi.org/10.3382/ps.0601056

329   35. Fisher M, Leeson S, Morrison WD, Summers JD. Feather growth and feather composition of
330   broiler chickens. Can J Anim Sci. 1981;61:769-73. https://doi.org/10.4141/cjas81-093

331   36. Conde-Aguilera JA, Cobo-Ortega C, Tesseraud S, Lessire M, Mercier Y, van Milgen J. Changes

332 in body composition in broilers by a sulfur amino acid deficiency during growth. Poult Sci.
333 2013;92:1266-75. https://doi.org/10.3382/ps.2012-02796

334 37. Adler SA, Slizyte R, Honkapää K, Løes A-K. 2018. In vitro pepsin digestibility and amino acid
335 composition in soluble and residual fractions of hydrolyzed chicken feathers. Poult Sci.
336 2018;97:3343-57. https://doi.org/10.3382/ps/pey175

337 38. Wecke C, Khan DR, Sünder A, Liebert F. Age and gender depending growth of feathers and
338 feather-free body in modern fast growing Meat-Type Chickens. Open J Anim Sci. 2017;7:376-92.
339 https://doi.org/10.4236/ojas.2017.74029

340 39. Greenhalgh S, Chrystal PV, Selle PH, Liu SY. Reduced-crude protein diets in chicken-meat
341 production: Justification for an imperative. Worlds Poult Sci J. 2020;76:537-48.
342 https://doi.org/10.1080/00439339.2020.1789024

343 40. Selle PH, Macelline SP, Chrystal PV, Liu SY. A reappraisal of amino acids in broiler chicken
344 nutrition. Worlds Poult Sci J. 2023. https://doi.org/10.1080/00439339.2023.2234342

345 41. Vargas L, Sakomura NK, Leme BB, Antayhua F, Reis M, Gous R, Fisher C. A description of the
346 potential growth and body composition of two commercial broiler strains. Br Poult Sci.
347 2020;61:266-73. https://doi.org/10.1080/00071668.2020.1716300

348 42. Farran MT, Thomas OP. Valine deficiency: 1. The effect of feeding a valine-deficient diet during
349 the starter period on performance and feather structure of male broiler chicks. Poult Sci.
350 1992;71:1879-84. https://doi.org/10.3382/ps.0711879

351 43. Stilborn HL, Moran ET, Gous RM, Harrison MD. Effect of age on feather amino acid content in
352 two broiler strain crosses and sexes. J Appl Poult Res. 1997;6:205-9.
353 https://doi.org/10.1093/japr/6.2.205

354 44. Reis, MDP, Sakomura NK, Teixeira IAMA, da Silva EP, Kebreab E. Partitioning the efficiency of
355 utilization of amino acids in growing broiler: Multiple linear regression and multivariate
356 approaches. PLos ONE. 2018;13:e0208488. https://doi.org/10.1371/journal.pone.0208488

357 45. Abebe S, Morris TR. Note on the effects of protein con- centration on response dietary lysine by
358 chicks. Br Poult Sci. 1990;31:255-60. https://doi.org/10.1080/00071669008417255

359

Tables and Figures

361 **Table 1.** Ingredient and chemical compositions (%) of experimental diets for broilers aged 22 to 29

362 days (as-fed basis)

	Dietary SID Met concentration[1], %					
Inclusion rate, g/kg	0.40	0.45	0.50	0.55	0.60	0.65
Corn	42.50	42.50	42.50	42.50	42.50	42.50
Soybean meal	24.50	24.50	24.50	24.50	24.50	24.50
Cornstarch	9.92	9.92	9.92	9.92	9.92	9.92
Sucrose	9.92	9.92	9.92	9.92	9.92	9.92
Glutamic acid	4.37	4.32	4.27	4.22	4.17	4.12
Soybean oil	1.80	1.80	1.80	1.80	1.80	1.80
L-Arginine	0.30	0.30	0.30	0.30	0.30	0.30
L-Histidine	0.08	0.08	0.08	0.08	0.08	0.08
L-Isoleucine	0.22	0.22	0.22	0.22	0.22	0.22
L-Leucine	0.04	0.04	0.04	0.04	0.04	0.04
L-Lysine-HCl	0.51	0.51	0.51	0.51	0.51	0.51
L-Methionine	0.21	0.26	0.31	0.36	0.41	0.46
L-Cysteine	0.27	0.27	0.27	0.27	0.27	0.27
L-Phenylalanine	0.01	0.01	0.01	0.01	0.01	0.01
L-Threonine	0.20	0.20	0.20	0.20	0.20	0.20
L-Tryptophan	0.02	0.02	0.02	0.02	0.02	0.02
L-Valine	0.28	0.28	0.28	0.28	0.28	0.28
Limestone	1.30	1.30	1.30	1.30	1.30	1.30
Dicalcium phosphate	1.51	1.51	1.51	1.51	1.51	1.51
Salt	0.40	0.40	0.40	0.40	0.40	0.40
Sodium bicarbonate	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix[2]	0.50	0.50	0.50	0.50	0.50	0.50
Mineral premix[3]	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.14	0.14	0.14	0.14	0.14	0.14
Total	100.0	100.0	100.0	100.0	100.0	100.0
Calculated value, %						
Metabolizable energy, kcal/kg	3,132	3,132	3,132	3,133	3,133	3,134
Crude protein	19.0	19.0	19.0	19.0	19.0	19.0
Calcium	0.810	0.810	0.810	0.810	0.810	0.810
Non-phytate P	0.415	0.415	0.415	0.415	0.415	0.415
SID amino acids, g/kg						
Arginine	11.85	11.85	11.85	11.85	11.85	11.85
Histidine	4.18	4.18	4.18	4.18	4.18	4.18
Isoleucine	7.90	7.90	7.90	7.90	7.90	7.90
Leucine	12.08	12.08	12.08	12.08	12.08	12.08
Lysine	11.29	11.29	11.29	11.29	11.29	11.29
Methionine	4.00	4.50	5.00	5.50	6.00	6.50
Cysteine	4.73	4.73	4.73	4.73	4.73	4.73
Phenylalanine	7.11	7.11	7.11	7.11	7.11	7.11
Threonine	7.34	7.34	7.34	7.34	7.34	7.34
Tryptophan	1.81	1.81	1.81	1.81	1.81	1.81
Valine	9.03	9.03	9.03	9.03	9.03	9.03

363 [1]SID = Standardized ileal digestible.

364   [2]Supplies the following per kilogram of diet: vitamin A, 24,000 IU; vitamin D_3, 8,000 IU; vitamin E, 160

365   mg/kg; vitamin K_3, 8 mg/kg; vitamin B_1, 8 mg/kg; vitamin B_2, 20 mg/kg; vitamin B_6, 12 mg/kg; pantothenic

366   acid, 40 mg/kg; folic acid, 4 mg/kg; niacin, 12 mg/kg.

367   [3]Supplies the following per kilogram of diet: Fe, 120 mg/kg; Cu, 320 mg/kg; Zn, 200 mg/kg; Mn, 240 mg/kg;

368   Co, 2 mg/kg; Se, 0.6 mg/kg; I, 2.5 mg/kg.

369   **Table 2.** Analyzed amino acid compositions of experimental diets (%)

Inclusion rate, %	Dietary SID Met concentration[1], %					
	0.40	0.45	0.50	0.55	0.60	0.65
Indispensable amino acid						
Arginine	1.19	1.24	1.23	1.19	1.17	1.21
Histidine	0.45	0.46	0.45	0.45	0.44	0.44
Isoleucine	0.75	0.78	0.78	0.76	0.75	0.77
Leucine	1.23	1.25	1.31	1.24	1.20	1.23
Lysine	1.14	1.18	1.19	1.20	1.17	1.17
Methionine	0.39	0.43	0.50	0.55	0.59	0.61
Phenylalanine	0.70	0.74	0.76	0.74	0.71	0.73
Threonine	0.70	0.71	0.75	0.73	0.70	0.71
Valine	0.94	0.95	0.96	0.94	0.92	0.94
Dispensable amino acid						
Alanine	0.69	0.72	0.74	0.72	0.69	0.71
Aspartic acid	1.38	1.46	1.53	1.46	1.39	1.45
Cysteine	0.46	0.43	0.43	0.44	0.45	0.44
Glutamic acid	6.26	6.34	6.19	6.24	6.27	6.22
Glycine	0.57	0.60	0.62	0.60	0.58	0.60
Proline	0.86	0.89	0.90	0.88	0.84	0.87
Serine	0.66	0.68	0.76	0.73	0.65	0.68
Tyrosine	0.46	0.48	0.50	0.47	0.43	0.48

370   [1]SID = standardized ileal digestible.

371

372 **Table 3.** Growth performance of male broilers aged 22- to 29-days old fed diets containing different
373 dietary standardized ileal digestible methionine concentrations[1]

Item[2]	Dietary SID Methionine concentration[2], %						SEM[3]	P values	
	0.40	0.45	0.50	0.55	0.60	0.65		Linear	Quadratic
Initial B W (at d 22), g	745	744	745	745	745	745	1.08	0.942	0.529
Final BW (at d 29), g	1,061	1,108	1,122	1,116	1,117	1,098	40.47	0.107	0.006
Weight gain, g/bird	316.0	364.5	377.2	370.7	372.2	353.3	40.58	0.105	0.006
Feed intake, g/bird	534.8	583.8	588.6	600.4	593.9	573.9	41.79	0.062	0.007
G:F, g/kg	594.8	625.5	640.5	616.1	624.8	613.6	17.09	0.641	0.143
Feather weight, g	21.6	21.5	23.1	23.5	19.0	22.6	1.45	0.863	0.671
Feathers, % of BW	1.95	1.90	2.09	2.03	1.60	1.91	0.11	0.226	0.525

374 [1]Values are least squares means of eight replicate cages, with four birds per cage.

375 [2]BW = body weight; G:F = gain to feed ratio. SID = standardized ileal digestible.

376 [3]SEM = standard error of the mean.

Table 4. Analyzed amino acid compositions in feather of 29-day-old male broilers fed different dietary methionine concentrations (as-is basis)[1]

Item	Dietary SID Methionine concentration[2], %						SEM[2]	P-value	
	0.40	0.45	0.50	0.55	0.60	0.65		Linear	Quadratic
Indispensable amino acid									
Arginine	6.5	6.4	6.7	6.3	5.1	6.5	0.64	0.44	0.80
Histidine	0.7	0.8	0.7	0.7	0.7	0.7	0.05	0.68	0.60
Isoleucine	4.4	4.4	4.4	4.2	4.3	4.4	0.06	0.92	0.05
Leucine	7.6	7.6	8.1	7.6	7.9	7.7	0.24	0.69	0.40
Lysine	2.2	2.3	2.1	2.2	2.1	2.2	0.16	0.67	0.80
Methionine	0.6	0.6	0.6	0.6	0.6	0.6	0.04	0.73	0.44
Phenylalanine	4.4	4.4	4.6	4.3	4.6	4.5	0.10	0.79	0.71
Threonine	4.5	4.5	4.5	4.3	4.5	4.5	0.06	0.87	0.16
Valine	6.9	6.8	7.1	6.7	7.0	6.9	0.16	0.82	0.93
Dispensable amino acid									
Alanine	4.1	4.0	4.4	4.1	4.3	4.1	0.15	0.65	0.29
Aspartic acid	6.5	6.5	6.6	6.4	6.6	6.5	0.07	0.90	0.92
Cysteine	6.3	6.2	6.4	6.0	6.5	6.1	0.18	0.78	0.74
Glutamic acid	10.4	10.4	10.3	10.1	10.4	10.4	0.14	0.72	0.25
Glycine	6.6	6.6	7.0	6.6	6.9	6.7	0.20	0.59	0.53
Proline	9.1	9.0	9.4	8.7	9.2	9.2	0.25	0.76	0.68
Serine	9.9	9.7	10.2	9.6	10.1	10.0	0.34	0.84	0.83
Tyrosine	2.7	2.8	2.7	2.5	2.7	2.7	0.13	0.48	0.62

[1]Values are least squares means of four replicate cages, with one bird per cage.

[2]SID = Standarized ileal digestible; SEM = standard error of the mean.
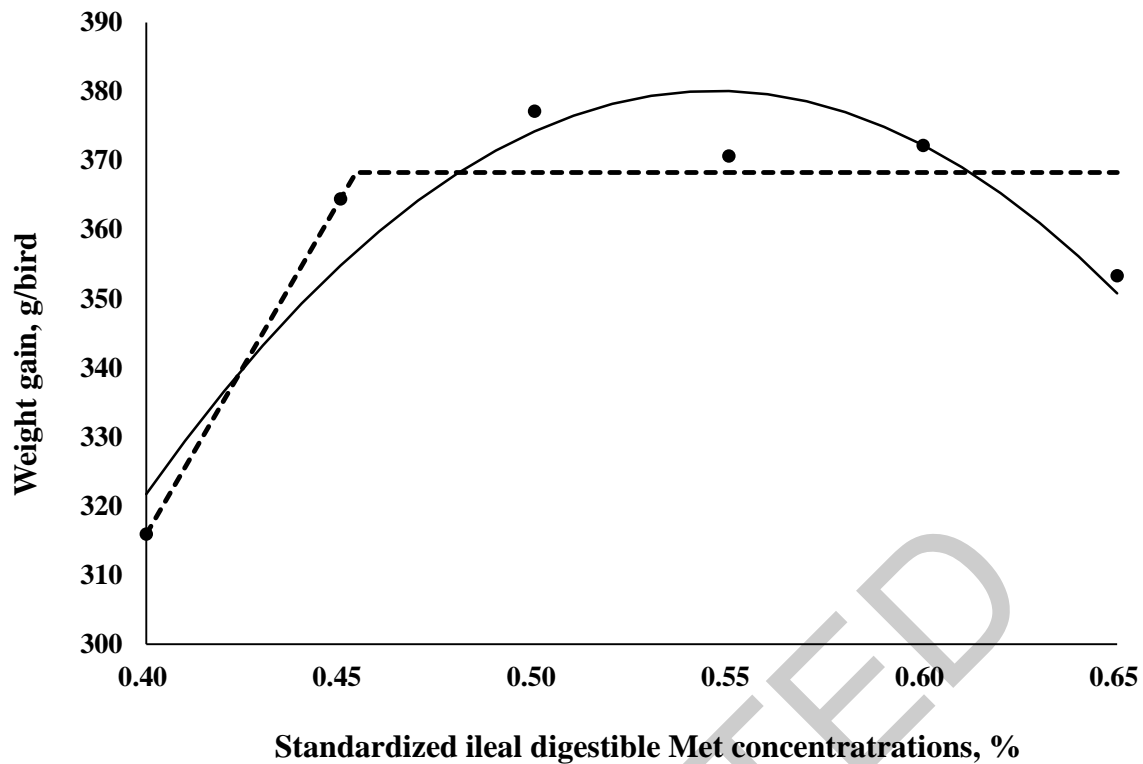
Figure 1. Fitted broken (dot) and quadratic line (solid) of growth performance on body weight gain (g)

for 22 to 29-day-old broilers as function of standardized ileal digestible (SID) Met in the diet. Data

points express means of 8 cages per treatment. The one-slope broken-line model shows that the SID

Met requirement is 0.45% (Y =368.3–970.3 [0.45-X] [X < 0.45]; P = 0.05; R^2 = 0.873). The quadratic-

line model shows that the SID Met requirement is 0.55% (Y = 380.1–2,726 [0.55–X] [0.55–X] [X <

0.55]; P = 0.03; R^2 = 0.910) and 95% of the upper asymptotic value of the quadratic-line model indicates

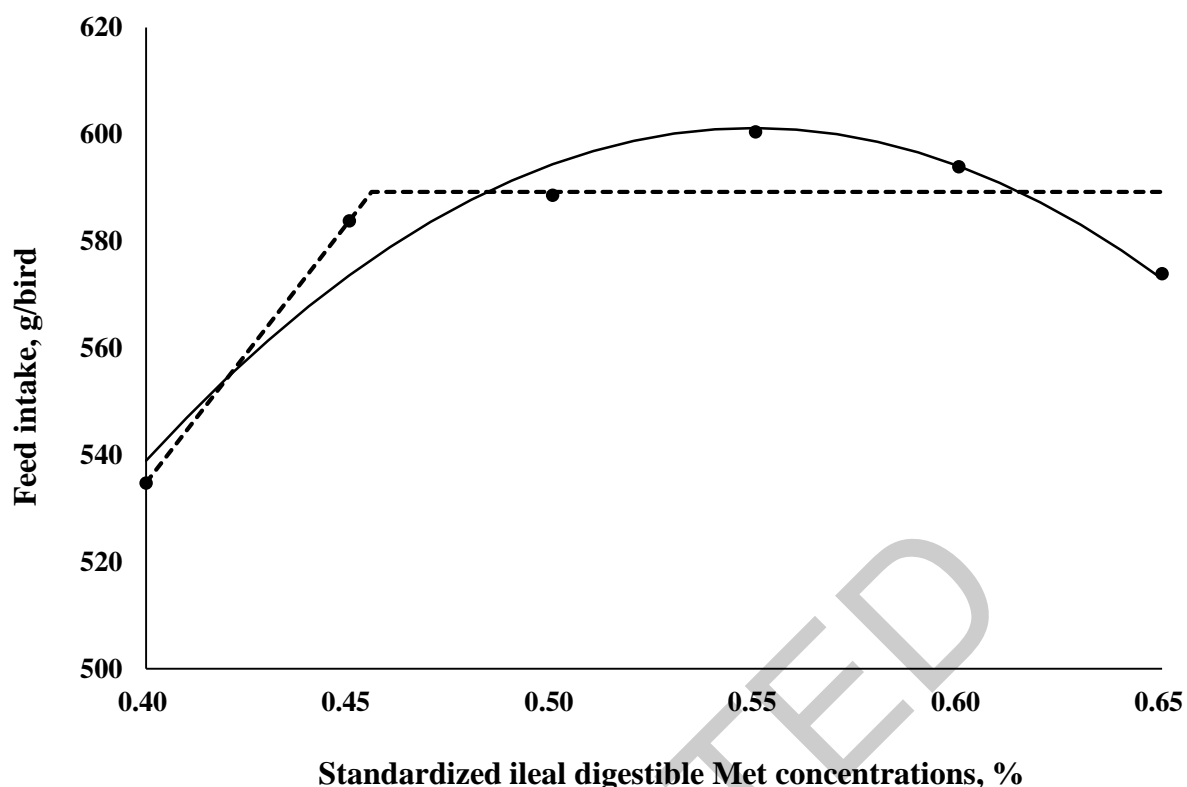that the SID Met requirement is 0.52%.

390

Figure 2. Fitted broken (dot) and quadratic line (solid) of growth performance on feed intake for 22 to

29-day-old broilers as function of standardized ileal digestible (SID) Met in the diet. Data points express

means of 8 cages per treatment. The one-slope broken-line model shows that the SID Met requirement

is 0.46% (Y = 589.2–980.9 [0.46-X] [X < 0.46]; P = 0.05; R^2 = 0.863). The quadratic-line model shows

that the SID Met requirement is 0.55% (Y = 601.2–2,790 [0.55–X] [0.55–X] [X < 0.55]; P = 0.01; R^2

= 0.944) and 95% of the upper asymptotic value of the quadratic-line model indicates that the SID Met

requirement is 0.52%.