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Male and Female Broilers Response to Different Ideal Amino Acid Ratios During the Second and Third Weeks Posthatch

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Abstract: A chick bioassay with chemically defined amino acid (AA) diets was conducted to compare four different AA profiles: the NRC (1994), Feedstuff, Rhone Poulenc Animal Nutrition (RPAN) and Illinois Ideal Chick Protein (IICP) AA profiles. The ideal ratios of IICP, RPAN and Feedstuff calculated using digestible AA requirements and total AA requirements used for NRC. This battery study involved male and female chicks during 7 to 21 days of age. Indispensable AA were rationed to lysine according to requirement ratios presented in the four profiles. Digestible lysine set at 1.07 and 0.98 % of diet for male and female, respectively. All diets were kept isonitrogenous (2.6 % N) by varying levels of L-glutamic acid. All diets were checked to have at least 0.3 % proline and 0.6 % glycine. Diets for all profiles contained 3200 kcal ME/kg and a positive control diet was used according to NRC (1994) recommendations. Chicks fed a common corn-soybean meal diet for 160 h and were raised in the floor pen. Then chicks weighed individually and allocated to battery pens so that most uniformity among pens occurs. Four battery pens of five chicks were fed one of four different profiles or positive control diet in both sexes. Weight gain and feed intake were measured for each pen at day 21 and then feed:gain was calculated. Results indicate that chicks fed positive control diet weighed more ($p < 0.5$). Among semi purified diets chicks fed diets formulated with NRC (1994) ideal AA ratios had significantly ($P < 0.5$) better weight gain and feed:gain in both sexes relative to IICP and RPAN, but not to Feedstuff. RPAN had worst weight gain and feed:gain in females. Results of this experiment suggest that new ideal ratio of threonine (relative to lysine) in IICP for starter period may be under-estimated.

Key words: Ideal amino acid ratios, isonitrogenous, isocaloric, broiler

Introduction

Feeding strategy in pig and poultry production has been given a new perception with the advent environmental problems related to pollution of nitrogen (N) from animal manure. Formerly, dietary adjustments to pig and poultry requirements were aimed at maximizing production performance without special concern for nutrient oversupply, especially protein and amino acids. The recent environmental constraints have forced to base protein/amino acid feeding not only on terms of N retained in animal products, but also in terms of non-utilized fraction of N ingested. Therefore, a sound management of protein feeding in poultry and pigs necessitates nowadays a close adjustment of protein/amino acid supply to the requirements so as to obtain the lowest level of N output. Many factors can influence amino acid (AA) requirements of chicks (and other animals) at any given growth stage (Baker *et al.*, 2002; Baker, 1997; Baker and Han, 1994), i.e., dietary factors (e.g., protein level, energy level, and presence of protease inhibitors), environmental factors (e.g., disease, crowding, feeder space, and heat or cold stress), and genetic factors (e.g., sex and capacity for lean vs. fat growth). Thus AA requirements cannot apply to all birds under all dietary, environmental, and body compositional conditions. Pig nutritionists were the first to address these problems by expressing AA requirements as ratios to Lys for different weight

categories (Wang and Fuller, 1989). Although AA requirements change due to the factors mentioned above, the ideal ratios remain similar, and thus only an accurate requirement for Lys needs to be established. The benefit of ideal AA ratios is that once a ratio is established for a certain age period, one can concentrate on accurately determining the Lys requirement under a variety of conditions and then calculate the requirement for all other AA under this condition based on the Lys requirement and ideal ratios. Lysine was chosen as the reference AA because it is used almost exclusively for protein accretion, it is a limiting AA in reduced protein corn-soybean meal broiler diets, and the analysis for Lys is uncomplicated (Baker, 1997). Thus, estimating an accurate Lys requirement is essential because it is the basis for setting requirements for all other indispensable AA. Certain steps need to be taken in order to accurately determine AA ratios to Lys. The objectives of this study was :1) to evaluate the response of male and female broiler chicks to different ideal amino acid ratios with regard to differences are between male and female from body compositional aspect 2) evaluation of the new ideal ratios reported by Baker *et al.* (2002).

Materials and Methods

Ideal ratios: The NRC (1994), Feedstuff (1999) and Rhone-Poulenc Animal Nutrition (RPAN, 1993) have

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Table 1: Amino acid ratios in IICP, NRC, RPAN and Feedstuff

Amino Acid	IICP	NRC	RPAN	Feedstuff
Lys	100	100	100	100
Met+Cys	72	78	79	74
Met	36	46	44	42
Cys	36	36	35	32
Thr	56 ¹	73	65	65
Val	77 ¹	82	84	82
Arg	105	114	117	108
Trp	16 ¹	18	19	20
Ile	64 ¹	73	78	68
Leu	104	109	150	103
His	35	32	35 ²	30
Phe+Tyr	105	122	105 ²	128

¹New ratios reported by Baker *et al.* (2002). ²RPAN did not specify ratios for this AA, so the ratios used in IICP was used also for RPAN.

been published. The AA in these profiles were calculated as percentages of listed lysine requirement (i.e., 1.10 % total Lys for NRC (1994), 0.82% Digestible Lys for Feedstuff and 1.0% Digestible Lys for RPAN). The resulting ratios are presented in Table 1. The new ratios for threonine (The), valine (Val), tryptophan (Trp) and isoleucine (Ile) published by Baker *et al.* (2002) were used for Illinois Ideal Chick Protein (IICP) profile. Digestible Lys used as basis to calculate other AA requirements were 1.07 and 0.98% of diet for male and female, respectively.

Diets: The dietary variables were AA profiles and glutamate level. All AA supplied as L-isomers except methionine, which was supplied as DL-isomers. Feed-grade sources were used for lysine. HCL (98.5%) and methionine (99%); the remaining AA were pharmaceutical grade. The true digestibility of free AA was assumed to be 100%. AA content of corn and soybean meal (SBM) determined by using HPLC. RPAN digestibility coefficient were used to estimate digestible AA content of corn and SBM. Digestible AA requirements were estimated by using ideal ratios in the tested profiles and digestible lysine requirement (1.07 and 0.98 for male and female, respectively). All the diets were kept isonitrogenous (by varying levels of glutamate) and isocaloric (3200 Kcal ME/Kg of diet). Also, when profiles were compared lysine level remained constant. All the diets were checked to have at least 0.3% of diet proline and 0.6% of diet glycine. A positive control diet was also formulated by using NRC (1994) recommendations (3200 Kcal ME, 1.1% Tot Lys and 0.9% SAA). The diets used in this bioassay are presented in Table 2.

Chicks and feeding regimen: 300 male and female feather sexed (150 male and 150 female) chicks (Ross *Ross) were fed a standard 23% CP corn-soybean meal diet until 160 h posthatching. On day 8 posthatching after being subjected to an overnight period of feed withdrawal, chicks were weighed individually and 200(100 male and 100 female) of chicks with similar weight selected and randomly allotted to pens such that each pen had a similar initial weight and weight distribution. Four battery pens of five chicks were fed one of four different profiles or positive control diet in both sexes. Weight gain, feed intake and feed efficiency measured for each pen at day 21 posthatch.

Statistical analysis: Values presented as mean±SE. data from growth assay were subjected to GLM procedures appropriate for completely randomized block designs. Data were analyzed as a 2 x 5 factorial, with main effect of AA profile (IICP, NRC, RPAN and Feedstuff) and Sex (Male and female) by using SAS software.

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Table 2: Composition of experimental diets

Feed Ingredient (%)	IICP		NRC		Feedstuff		RPAN	
	Male	Female	Male	Female	Male	Female	Male	Female
Corn	76.19	76.5	76.38	76.25	75.43	76.1	80.13	80.88
Soybean Meal	11.19	7.85	11.19	7.85	12.03	7.85	9.85	4.84
Dicalcium Phosphate	2.04	2.38	2.04	2.38	2.03	2.38	2.04	2.10
Oyster shell	1.36	1.23	1.37	1.23	1.37	1.46	1.38	1.38
Sun Flower Oil	1.50	1.50	1.50	0.92	1.70	1.50	0.70	-
NaHCO ₃	0.56	0.74	0.66	0.66	0.66	0.7	0.66	0.66
Min premix ¹	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Vit premix ²	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
L-Glutamate	2.42	4.93	1.39	3.69	2	4.55	0.81	3.86
L-Lys.HCL	0.87	0.85	0.86	0.85	0.84	0.85	0.84	0.93
DL-Met	0.42	0.39	0.49	0.45	0.45	0.41	0.48	0.48
L-Thr	0.65	0.35	0.79	0.72	0.38	0.39	0.28	0.64
L-Arg	0.68	0.65	0.77	0.74	0.69	0.68	0.63	0.83
L-Leu	0.59	0.57	0.56	0.56	0.51	0.51	0.43	1.04
L-Val	0.39	0.38	0.44	0.43	0.43	0.43	0.4	0.49
L-Ile	0.23	0.21	0.35	0.31	0.28	0.27	0.42	0.39
L-Phe	0.06	0.06	0.24	0.22	0.28	0.3	0.02	0.21
L-Gly	0.19	0.32	0.18	0.31	0.16	0.32	0.16	0.4
L-Trp	0.07	0.07	0.09	0.09	0.11	0.11	0.10	0.11
L-His	0.11	0.11	0.08	0.08	0.05	0.05	0.10	0.16
Sand	-	1.58	-	-	-	0.53	-	-
Calculated Nutrient								
(%)ME (Kcal/Kg)	3200	3200	3200	3200	3200	3200	3200	3200
CP	16.25	16.25	16.25	16.25	16.25	16.25	16.25	16.25
Ca	1	1	1	1	1	1	1	1
Ava.Phosphorus	0.45	0.45	0.45	0.45	0.45	0.5	0.45	0.45
Cl	0.25	0.20	0.20	0.20	0.20	0.20	0.20	0.21
Na	0.20	0.22	0.20	0.20	0.20	0.21	0.20	0.20
Arg	1.12	1.02	1.21	1.11	1.15	1.05	1.25	1.14
Gly	0.80	0.89	0.80	0.30	0.78	0.89	0.63	0.96
His	0.37	0.34	0.34	0.31	0.32	0.29	0.37	0.37
Ile	0.66	0.60	0.78	0.71	0.72	0.66	0.83	0.76
Leu	1.16	1.06	1.16	1.06	1.10	1	1.40	1.47
Lys	1.07	0.98	1.07	0.98	1.07	0.98	1.07	0.98
Met	0.59	0.54	0.65	0.59	0.61	0.56	0.66	0.62
Met+Cys	0.77	0.70	0.83	0.76	0.80	0.72	0.84	0.77
Phe	0.71	0.65	0.89	0.81	0.94	0.88	0.46	0.77
Phe+Tyr	1.12	1.02	1.30	1.19	1.36	1.25	1.12	1.02
Thr	0.60	0.55	0.78	0.71	0.69	0.63	0.69	0.63
Trp	0.17	0.15	0.19	0.17	0.21	0.19	0.20	0.18
Val	0.82	0.75	0.87	0.80	0.87	0.80	0.89	0.82
DCAD	136	138	141	131	150	128	143	133 ¹

Provided (per kilogram of diet): Ca₃(PO₄)₂, 28.0 g; K₂HPO₄, 9.0 g; NaCl, 8.89 g; MgSO₄·7H₂O, 3.5 g; ZnCO₃, 0.10g; CaCO₃, 3.0 g; MnSO₄·H₂O, 0.65 g; FeSO₄·7H₂O, 0.42 g; KI, 40 mg; CuSO₄·5H₂O, 20mg; Na₂MoO₄·2H₂O, 9 mg; H₃BO₃, 9 mg; CoSO₄·7H₂O, 1 mg; Na₂SeO₃, 0.22 mg.

²Provided (per kilogram of diet): thiamin_HCL, 20 mg; niacin, 50 mg; riboflavin, 10 mg; D-Ca-pantothenate, 30 mg; vitamin B₁₂, 0.04 mg; pyridoxine_HCL, 6 mg; D-biotin, 0.6 mg; folic acid, 4 mg; menadione dimethylpyrimidinol bisulfate, 2 mg; cholecalciferol, 15 µg; retinyl acetate, 1, 789 µg; ascorbic acid, 250 mg.

Results and Discussion

Feed: gain and weight gain (WG) are presented in Table 3 (interaction between sex and diet was not significant and so don't included in Table). In the male broilers,

among different ideal amino acid ratios, NRC (1994) had better feed:gain (P<0.05) than other ratios. Feedstuff had lower feed:gain in comparison to IICP and RPAN, but the differences were not significant. In the female

Table 3: Male and female broilers performance with different ideal amino acid ratios

Sex	Ideal ratio	Feed intake (g)	Feed: gain	Weight gain (g)
Male	IICP	554.75±10.25 ^a	1.78±0.15 ^a	309.75±4.77 ^{cd}
	NRC	553.25±6.18 ^a	1.53±0.12 ^c	362±1.61 ^{ab}
	Feedstuff	607.25±7.05 ^a	1.78±0.19 ^{ab}	340±2.08 ^{bc}
	RPAN	577.25±7.99 ^a	1.96±0.05 ^a	292.25±3.55 ^d
	Control	597.75±6.96 ^a	1.59±0.15 ^{bc}	374.25±1.16 ^a
	Mean	575.05	1.72	335.65
	SE	1.66	0.048	0.009
Female	IICP	482.30±7.91 ^b	2.01±0.12 ^{ab}	236±3.40 ^c
	NRC	537.70±3.90 ^b	1.81±0.12 ^b	296.50±2.62 ^b
	Feedstuff	529.80±12.50 ^b	1.87±0.30 ^b	282.80 ±2.82 ^b
	RPAN	246.30±3.67 ^c	2.36±0.81 ^a	104±3.75 ^d
	Control	673.50±9.44 ^a	1.90±0.23 ^b	352±1.93 ^a
	Mean	493.90	2.02	254.25
	SE	3.58	0.10	0.02

broilers, RPAN had the highest feed:gain ($P<0.05$). NRC (1994) had lower but not significant feed:gain than IICP and feedstuff. Chicks fed positive control diet had a higher WG ($P<0.01$) in both male and female. Among the tested ratios, NRC had higher WG ($P<0.05$) compare to IICP and RPAN in both sexes. RPAN also gained less than other ratios both in male and female.

Adverse effects of AA imbalance on the utilization of nutrient are well documented. It expected that with AA imbalance, protein utilization efficiency decrease dramatically. Experiments in rats indicated detrimental effects of AA imbalance on utilization of first limiting AA. Excess AA contributes to AA imbalance, stimulate AA catabolism pathway which will result in AA, especially, limiting AA catabolism (Kumta *et al.*, 1962). D'Mello and Lewis (1970) reported that supplementation of diet with excess Leu, inhibited response of chicks to first limiting AA, namely Met, and decreased efficiency of protein utilization. It seems that in the present study, high level's of Leu due to inappropriate ideal ratio between Lys and Leu (100 to 150) in RPAN caused an AA imbalance and resulted in AA catabolism and lower feed efficiency. IICP also had a higher gain:feed in comparison to NRC(1994). It seems that the ratio between Lys and Thr in IICP resulted in higher gain:feed. Comparison of IICP and NRC (1994) ideal ratios shows two major difference, the ratio between Lys:SAA and Lys:Thr. Results of other experiments suggest that the Lys:SAA is not under-estimated in IICP, because Austic (1994) and Dutch Bureau of Livestock Feeding (1994) reported 72 and 73 for the Lys:SAA, respectively. But, in the case of Thr, Baker and Han (1994) reported 67 for the Lys:Thr which decreased to 55.7 in the current reevaluation (Baker *et al.*, 2002) of this ratio. In addition, Austic (1994) and Dutch Bureau of Livestock Feeding (1994) reported 62,65 for the ratio between Lys and Thr, respectively. Also, Mack *et al.* (1999) reported 59 for the ratio between Lys:Thr for the 20 to 40 days of age which it should be higher for the Starter period. With respect to what

mentioned above it can be conclude that threonine level in IICP wasn't sufficient. Barkely and Wallis (2001) indicated an improvement in feed efficiency by increasing threonine level from 5.7 to 7.2 g/Kg of diet. It seems that lower Thr:Lys reported by Baker *et al.* (2002) in their reevaluation, which used for IICP in our study, may be the major cause of higher feed:gain for IICP than NRC (1994). Chicks fed positive control diet had the highest weight gain in both sexes. Han *et al.* (1992), Parr and Summers (1991) reported the same performance for the chicks fed conventional diet or low protein diet supplemented with synthetic AA, but contrary Edmonds *et al.* (1985), Pinchasov *et al.* (1990) show lower performance for the chicks fed low protein diet supplemented with synthetic AA in comparison to conventional diet. Higher WG of NRC (1994) in comparison to IICP is in contradiction with findings of Baker and Han (1994). It seems that the contradiction is due to Thr:Lys ideal ratio used in their study (67:100) and the present study (56:100). Broilers fed inadequate threonine have decreased live performance (Dozier *et al.*, 1999, 2000). Mucus production by the intestinal epithelium contributes to its "unstirred water layer," which, in turn, influences nutrient recovery from the lumen during digestion (Specian and Oliver, 1991). Mucus consists of high molecular weight glycoproteins (Specian and Oliver, 1991), with threonine representing about 40% of the protein (Carlstedt *et al.*, 1993). Mucins are largely undigestible, and the associated threonine cannot be recovered (Fuller, 1994). Approximately 50% of the dietary requirement for threonine is utilized by the intestinal mucosa of pigs (Stoll *et al.*, 1998; Wu, 1998). Because threonine is highly concentrated in proteins directly associated with the gastrointestinal mucosa, as well as digestive enzymes in the lumen necessary for effective nutrient recovery, diets low in threonine may adversely affect efficiency of nutrient recovery. It can be concluded that Thr deficiency due to low ideal ratio in IICP is the reason of lower weight gain in this treatment.

The same discussions can be made about the differences in females weight gain. Results of this experiment suggest that new ideal ratio of threonine (relative to lysine) in IICP for starter period may be under-estimated.

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