

Interaction between dietary protein and critical amino acids on performance of birds developed for free range farming during juvenile phase

S V RAMA RAO¹, A K PANDA², M V L N RAJU³, G SHYAM SUNDER⁴ and R P SHARMA⁵

Project Directorate on Poultry, Rajendranagar, Hyderabad, Andhra Pradesh 500 030 India

Received: 5 May 2009; Accepted: 23 December 2009

ABSTRACT

Vanaraja, a dual-purpose chicken variety needs intensive farm conditions to protect against cold, nutrient deficiencies and to provide immunity during the initial 6 weeks of age before letting them under free range conditions. Therefore, 2 separate experiments were conducted to identify optimum dietary levels of crude protein (CP), sulphur amino acids (SAA) and lysine (Lys) in diets of *Vanaraja* chicken from 1 to 42 days of age. Diets (9) were prepared with 3 levels of CP (14.5, 16 and 17.5%) and SAA (0.567, 0.626 and 0.685%) in a factorial manner and each diet was fed to 8 replicate groups of 6 chicks each in experiment 1. Similarly, 6 diets with 2 levels of CP (14.5 and 16%) and 3 levels of Lys (0.66, 0.73 and 0.79%) were prepared and each diet was fed to 12 replicate groups in experiment 2. The interaction between CP and SAA significantly influenced the body weight gain and feed conversion efficiency at 28 days of age. However, weight gain and feed efficiency at 14 and 42 days of age, relative weights of ready to cook yield (RTC), abdominal fat, gizzard, liver, giblet, intestine and lymphoid organs (bursa and spleen), and the antibody titres against sheep red blood cells (SRBC) and CBH response to phytohaemagglutinin-P (PHA-P) inoculation were not affected by the interaction between these 2 nutrients. Antibody production against SRBC was significantly higher in birds fed 17.5% CP compared to lower levels. Abdominal fat content decreased with increase in dietary CP. The interaction between CP and Lys did not influence weight gain, feed efficiency (at 14, 28 and 42d of age), RTC yield, abdominal fat, relative weights of gizzard, liver, giblet, bursa and spleen. Relative weight of abdominal fat and giblet decreased with increase in concentration of CP in diet. The relative weights of liver and giblet increased non-linearly with increase in Lys content in diet. Based on the results of both the experiments, it is concluded that *Vanaraja* chicks do not require more than 14.5% crude protein, 0.567% sulphur amino acids and 0.66% lysine for optimum performance and carcass traits during 1 to 42 days of age.

Key words: Amino acids, Immunity, Performance, Protein, *Vanaraja*

Vanaraja is a dual-purpose chicken variety, having multicolour feather pattern and good adaptability to diversified rearing conditions that are prevailing in free-range backyard farming. The protein and critical amino acid requirements suggested for commercial broilers and layer chicks may not be applicable for these birds as they are primarily dependant upon the genetic makeup and growth potential of birds (Simon 1989 and Fatufe *et al.* 2004). Rama Rao *et al.* (2005, 2006b) indicated lower requirement of energy and protein (2400 kcal/kg diet and 16%, respectively) for *Vanaraja* compared to that recommended for commercial broilers or layer chicks. Since, there is a close relation between the CP and amino acids in diet (Sterling *et al.* 2003 and Viera *et al.* 2004), and methionine and Lys are the first and second limiting amino acids in maize-soybean diet (Harms 1992), it is essential to find out optimum requirements

of sulphur amino acid (SAA) and Lys at different levels of CP in diet. Therefore, an attempt was made to determine the optimum concentrations of SAA and Lys for *Vanaraja* chicks at varied CP levels during juvenile phase.

MATERIALS AND METHODS

Two experiments were conducted to know the interaction between CP and SAA in experiment 1 and between CP and Lys in experiment 2. In both experiments, performance of chicks, their carcass traits and immune competence were recorded. All major feed ingredients were analysed for CP and crude fibre (AOAC, 1990). Analysed concentrations of amino acid and metabolizable energy (Rama Rao *et al.* 2006a) were considered while formulating the diets and the mash diets were fed *ad lib*. In experiment 1, 3 levels each of CP (14.5, 16 and 17.5%) and SAA (0.567, 0.626 and 0.685%) were considered using maize-deoiled rice bran-soybean meal based diet having constant levels of energy, calcium and

Present address: ¹Principal Scientist, ²Senior Scientist, ³Director.

Table 1. Ingredient and nutrient composition (%) of basal diets with different protein content

Ingredients	Crude protein (%)		
	14.5	16.0	17.5
Maize	38.60	36.40	34.00
De-oiled rice bran	50.40	47.95	46.20
Soybean meal	7.30	12.00	16.20
Shell grit	1.40	1.40	1.40
Dicalcium phosphate	1.40	1.35	1.30
Vitamin premix ¹	0.20	0.20	0.20
Trace mineral mix ²	0.10	0.10	0.10
Choline chloride 50%	0.05	0.05	0.05
Common salt	0.35	0.35	0.35
Toxin binder	0.10	0.10	0.10
Antibiotic ³	0.05	0.05	0.05
Coccidiostat ⁴	0.05	0.05	0.05
<i>Nutrient composition %</i>			
ME, kcal/kg	2404	2402	2397
Crude protein	14.52	16.07	17.50
Calcium	0.862	0.864	0.865
Available phosphorus	0.439	0.432	0.427
Lysine*	0.570	0.669	0.771
SAA**	0.498	0.541	0.579

¹Vitamin premix provided (mg/kg diet): thiamin 1; pyridoxine, 2; cyanocobalamin, 0.01; niacin, 1.5; pantothenic acid, 10; tocopherol, 10; riboflavin, 5; menadione, 1; retinal, acetate, 8250 IU, and cholecalciferol, 1200 ICU.

²Trace mineral premix provided (mg/kg): Zn, 60; Mn, 60; Fe, 30; Cu, 4.3 g.

³Furazolidone: 22.4% w/w.

⁴Coban TM (monensin sodium 10% w/w).

*Synthetic DL-methionine and L-lysine HCl were supplemented to the basal diet to obtain desired levels of amino acids in the test diets

**SAA, sulphur amino acids.

available phosphorus (Table 1). The metabolizable energy concentration in basal diet was 2400 kcal/kg. The concentration of Lys in the diets with different CP concentrations was modified according to the CP level in diet so as to maintain a constant ratio between CP and Lys (4.78% of CP). In experiment 2, two levels of CP (14.5 and 16%) and 3 levels of Lys (0.657, 0.725 and 0.793%) were considered. The concentration of SAA in the diets was maintained constant (0.567%) in experiment 2, which was found adequate as per the data generated from experiment 1. The desired concentrations of CP and amino acids were obtained by adjusting the levels of the major feed ingredients, synthetic DL-methionine and L-lysine HCl.

Day-old straight run *Vanaraja* chicks were housed in raised wire floor galvanised iron battery brooders with 6 birds in each replicate pen (47.5 inch length, 29.5 inch breadth, 17

inch height). Chicks were fed ground maize on day one followed by respective experimental diet. Each experimental diet was fed *ad lib.* to 8 and 12 replicates, respectively, in experiment 1 and 2. Completely randomised design was followed while allotting the diets to replicates. Measured quantity of feed was offered 2 times a day depending on intake by the group of birds in a pen to avoid any chance of feed wastage. Birds were protected against Marek's disease (HVT), Newcastle disease (Lasota) and infectious bursal disease (Georgia) by giving the respective vaccine at 1, 8 and 15 days of age. The brooder temperature was maintained at 34±1°C up to 7 days of age and gradually decreased to 26±1°C by 21 days of age, after which, chicks were kept at ambient room temperature (22 to 27°C). Light (20 lux) was provided continuously throughout the experimental period using incandescent bulbs.

Weight gain and feed intake were recorded at bi-weekly intervals. Feed efficiency was calculated as feed intake/weight gain. On day 42, one bird from each replicate was picked up at random and slaughtered by cervical dislocation to study the relative weight of ready to cook (RTC) yield, gizzard, abdominal fat, liver, giblet and lymphoid organs (bursa and spleen).

The antibody titres against sheep red blood cells (SRBC) and cutaneous basophilic hypersensitivity (CBH) against phytohemagglutinin-P (PHA-P) were recorded (experiment 1). On day 30, six birds from each treatment in experiment 1, and 12 birds per treatment in experiment 2 were inoculated intravenously with 0.1 ml of 0.5% suspension of SRBC (about 2 billion/bird). Two to three ml of blood was drawn from brachial vein from each bird at 35 and 40 days of age (i.e. 5 and 10 days post inoculation), which were inoculated with SRBC to measure the antibody production (log2) against SRBC antigen by employing microtiter haemagglutination procedure (Wegmann and Smithies 1966).

At 43 days of age, 6 birds in experiment 1 and 12 birds per treatment in experiment 2 were injected intradermally in right wattle with 100 µg of PHA-P in 0.1 ml of normal saline solution to measure the cutaneous basophil hypersensitivity (CBH) reaction. The thickness of the wattles was measured using a micrometer before inoculation and at 24 h PI. The CBH response was expressed as unit increase in wattle thickness due to inoculation of PHA-P considering the normal saline injected wattle as control.

The data were subjected to factorial analysis (Snedecor and Cochran 1989) (CP vs SAA in experiment 1, CP vs lys in experiment 2). When the interaction between the factors was not significant, the effect of main factors was compared using one-way analysis of variance (Snedecor and Cochran 1989). The differences among the means were compared with Duncan multiple range test (Duncan 1955). Contrast analysis was run to know the trend (linear/non-linear) of main factors on different variables.

Table 2. Performance, relative weights of organs (g/kg live weight), antibody titres against sheep red blood cells in *Vanaraja* chicks fed different levels of CP and SAA-experiment 1

CP%	SAA	Body weight gain, g		Feed intake/weight gain			RTC	Abd. fat	Gizzard	SRBC titre, log2
		1–14d	1–42d	1–14d	1–28d	1–42d				
<i>Interaction</i>										
14.5	0.567	102	605	2.44	2.56 ^{bc}	2.97	706	8.98	28.0	7.67
14.5	0.626	90	572	2.57	2.70 ^a	3.13	700	12.46	28.4	7.33
14.5	0.685	95	577	2.50	2.67 ^{ab}	2.97	703	8.10	30.9	6.67
16	0.567	103	614	2.57	2.63 ^{ab}	3.02	697	9.92	27.2	6.67
16	0.626	96	638	2.33	2.43 ^{cd}	2.92	672	7.76	29.3	7.00
16	0.685	108	622	2.28	2.49 ^{cd}	2.96	694	7.18	27.9	7.33
17.5	0.567	113	654	2.27	2.46 ^{cd}	2.92	697	2.68	27.5	8.67
17.5	0.626	124	638	2.08	2.40 ^d	3.00	694	5.16	27.8	8.83
17.5	0.685	117	637	2.16	2.43 ^{cd}	2.88	706	8.22	28.3	9.83
<i>Main factors</i>										
14.5		96 ^b	584 ^b	2.50 ^a	2.64 ^a	3.02	703 ^a	9.85 ^a	29.1 ^b	7.22 ^b
16		102 ^b	625 ^a	2.40 ^a	2.52 ^b	2.97	688 ^b	8.29 ^b	28.1 ^a	7.00 ^b
17.5		118 ^a	643 ^a	2.17 ^b	2.43 ^c	2.93	699 ^a	5.35 ^c	27.8 ^b	9.11 ^a
	0.567	106	624	2.43	2.55	2.97	700	7.19	27.5	7.67
	0.626	103	616	2.33	2.51	3.02	689	8.46	28.5	7.72
	0.685	107	612	2.31	2.53	2.94	701	7.83	29.0	7.94
SEM		2.35	4.99	0.032	0.019	0.020	2.22	0.766	0.41	0.306

Means having common superscript in a column/sub-column do not vary significantly ($P < 0.05$).

RESULTS AND DISCUSSION

Experiment 1

The interaction between CP and SAA significantly influenced the body weight gain ($P < 0.05$) and feed efficiency ($P < 0.01$) at 28 days of age (Table 2). However, the weight gain and feed efficiency at 14 and 42 days of age were not affected ($P > 0.05$) by the interaction. At 14.5% CP, the body weight gain ($P < 0.05$) and feed efficiency ($P < 0.01$) decreased significantly by increasing the SAA from 0.567 to 0.626%. However, such depression was not observed with further increase in SAA to 0.685%. At 16% CP, the performance increased significantly with increase in SAA from 0.567 to 0.626% and no further improvement was observed at 0.685%. At 17.5% CP, variation in concentration of SAA did not influence the weight gain. At the lowest level of CP (14.5%) higher levels of SAA depressed weight gain but improvement was observed at 16% CP with increase in level of SAA from 0.567 to 0.626%. The depressed weight gain at 14.5% CP might be due to higher concentrations of SAA per unit of protein (0.043 to 0.047). Body weight gain and feed efficiency were improved with increase in levels of SAA from 0.567 to 0.626% at 16% but not at 17.5% CP in diet. The weight gain and feed efficiency in groups fed 16% CP-0.626% SAA and 17.5% CP-0.567% SAA were similar, indicating lower requirement of SAA (0.567%) when the diet contained higher concentration of CP (17.5%). Similar to these observations Morris *et al.* (1987) reported higher SAA requirement per unit of protein as the protein concentration reduced to 16.0%. It is possible that functions of SAA other

than protein synthesis also might have contributed to the higher SAA requirement per unit of protein at the lower protein concentrations. Birds fed 16% CP-0.567% SAA diet recorded significantly ($P < 0.05$) low body weight gain compared to those fed 17.5% CP-0.567% SAA, suggesting that the SAA requirement per unit of CP reduced as the concentration of CP increased in diet.

Body weight gain at 14 and 42 days of age and feed efficiency at 14 days of age improved non-linearly ($P < 0.01$) with increase in CP concentration in the diet. At 42 days of age, the feed efficiency was not affected by the concentration of CP in diet. Weight gain at 42 days of age was not affected by the CP concentration in diet suggesting that the minimum CP level tested (14.5%) is adequate to support the growth during 1 to 42 days of age. Variation in dietary concentration of SAA did not influence ($P > 0.05$) body weight gain and feed efficiency (Table 2). Though the concentrations of CP and SAA significantly influenced weight gain and feed efficiency at 28 days of age, they were not affected at the end of experiment (1 to 42 days of age), which implies that the minimum concentrations of CP and SAA (14.5% CP and 0.567%, respectively) used in the trial appeared to be adequate for *Vanaraja* chicks from 1 to 42 days of age. The lower requirement of CP and SAA in diet might be due to low growth potential of the birds. The calculated ratio between SAA and CP was 0.039, which is in close agreement with the levels (0.036) recommended by Bornstein and Lipstein (1966) for broilers.

The relative weights of RTC, abdominal fat, gizzard, liver, gible, intestine and lymphoid organs (bursa and spleen) were

Table 3. Feed efficiency, organ weights (g/kg weight) and antibody titres against SRBC in *Vanaraja* chicks fed different lysine levels at 2 levels of crude protein (CP)- experiment 2

CP	SAA	Feed intake/weight gain		Abd. fat	Liver	Giblet	SRBC titre, log 2	
	%	1–28d	1–42d				5d PI	10d PI
<i>Interaction</i>								
14.5	0.66	2.87	2.95	10.0	24.9	58.0	6.50 ^b	6.17 ^a
14.5	0.73	2.88	2.93	10.6	23.6	56.4	6.58 ^{ab}	6.42 ^a
14.5	0.79	2.86	2.97	11.2	27.0	60.5	6.00 ^{bc}	5.42 ^{ab}
16	0.66	2.80	2.90	6.14	24.5	55.0	8.17 ^a	6.92 ^a
16	0.73	2.85	2.90	8.21	23.3	50.4	4.83 ^c	4.08 ^b
16	0.79	2.79	3.02	4.76	24.9	57.2	7.33 ^{ab}	6.33 ^a
<i>Main factors</i>								
14.5		2.88 ^a	2.96	10.6 ^a	25.2	58.3 ^a	6.36	6.00
16		2.82 ^b	2.95	6.4 ^b	24.2	54.2 ^b	6.78	5.78
	0.66	2.84	2.92 ^b	9.4	23.4 ^b	53.4 ^b	5.71 ^b	5.25
	0.73	2.83	3.00 ^a	8.0	25.9 ^a	58.8 ^a	6.67 ^{ab}	5.88
SEM	0.79	0.013	0.013	0.667	0.375	0.740	0.245	0.281

Mean having common superscript in a sub-column do not vary significantly ($P < 0.05$); PI, post inoculation.

not affected ($P > 0.05$) by the interaction between CP and SAA concentrations in diet (Table 2). The RTC and abdominal fat contents decreased non-linearly ($P < 0.05$) with increase in levels of CP in diet. Similar to these findings, Ciftci and Ceylan (2004) and Wijtten *et al.* (2004) also reported reduced abdominal fat in chicks fed higher dietary concentrations of CPI amino acids compared to those fed lower protein diet. The dietary variations in concentration of CP did not influence the other carcass parameters studied. Variation in dietary concentration of SAA did not influence ($P > 0.05$) carcass parameters except the RTC yield ($P < 0.05$), which increased non-linearly with increase in SAA concentration in diet (Table 3).

The antibody titres against SRBC (6.67 to 9.83) and CBH response (0.53 to 0.82) to PHA-P inoculation were not affected ($P > 0.05$) by the interaction between CP and SAA concentrations in diet. However, the antibody titre against SRBC inoculation increased non-linearly with the level of CP in diet. Increased antibody production against SRBC challenge with increased CP in diet contradicts our previous findings (Rama Rao *et al.* 1999). However, it is essential to note that growth potential of birds and levels of CP used in the present and earlier studies varied greatly. Though the exact mechanism of influence of protein on antibody production is not known, since antibodies are protein it is presumed that, higher concentration of protein (amino acids) is required for immunity compared to growth. The requirements of protein/amino acids for immune competence were reported to be higher than for optimum weight gain (Latshaw, 1991, Rama Rao *et al.* 1999, 2003). The CBH response to PHA-P inoculation was not affected by the CP concentration in diet.

The requirement of protein and methionine also depends on genetic makeup of the bird (Simon 1989, Fatufe *et al.*

2004), perhaps due to differences in the contribution of different protein fractions to whole body protein. Since, dietary protein/critical amino acid requirements were linearly related to protein accretion (weight gain) (Baker *et al.* 1996), slow growing *Vanaraja* performed well even at the lowest concentrations of CP and SAA tested in the present experiment.

Experiment 2

Interaction between CP and lysine or the concentrations of the individual nutrients did not influence ($P > 0.05$) weight gain (68.8 – 71.6 g, 260.5–274.1 g, 557–584 g, respectively) or feed efficiency (2.41–2.54, 2.79–2.88, 2.90–3.02, respectively) at 14, 28 and 42 days of age (data not shown). Though, feed efficiency improved ($P < 0.05$) at higher CP (16%) in diet at 28 days of age, such benefit was not observed at 42 days of age (Table 3). The feed efficiency at 42 days decreased linearly ($P < 0.05$) with increase in Lys content in diet. Decreased feed efficiency with increased concentrations of Lys contradicts the reports of Baker *et al.* (1996) and Mack *et al.* (1999), who observed improved feed efficiency at higher levels of amino acids in diet above the required concentrations for growth. These authors found that increased levels of amino acids in the diet marginally decreased feed intake without fall in body weight gain, which led to improve feed efficiency. However, such reduction in feed intake was not observed in this trial. Carcass variables, except relative weights of liver ($P < 0.05$) and giblet ($P < 0.01$), were not affected by the concentrations of Lys studied. The relative weight of liver and giblet increased non-linearly with increase in concentration of Lys in the diet.

The interaction between CP and Lys or their concentrations in diet did not influence the RTC yield (660–703 g/kg), relative weights of gizzard (26.2–28.8 g/kg), bursa

(0.90–1.10 g/kg) and spleen (2.16–2.53 g/kg). Similarly, the relative weights of abdominal fat, liver and gizzard were affected by the interaction (Table 3). The relative weights of abdominal fat and gizzard decreased significantly with increase in concentration of CP from 14.5 to 16% in diet. The relative weights of liver and gizzard were highest in group fed 0.79% Lys followed by 0.66 and 0.70%. Though, the interaction between CP and Lys significantly affected antibody titre against SRBC at 5 and 10 days PI, the trend was not clear to attribute the changes to treatment effect.

Absence of response in weight gain and feed efficiency at 42 days of age and in majority of carcass parameters with the increase in concentrations of CP (14.5 to 16%) and Lys (0.66 to 0.79%) in diets suggest that the minimum levels of CP and Lys used in the present study (14.5 and 0.66%) appear to be adequate for *Vanaraja* chicks during 1 to 42 days of age. The levels of CP and Lys are considerably lower than the requirements suggested for even egg type layer chicks during the same age. The ratio between Lys and CP found optimum in this study (0.046) was nearer to 0.050 suggested by Barbour *et al.* (1993).

Based on the results of both the experiments, it can be concluded that *Vanaraja* chicks require not more than 14.5% crude protein, 0.567% sulphur amino acids and 0.66% lysine for optimum performance and carcass traits during 1 to 42 days of age.

REFERENCES

- AOAC 1990. *Official Methods of Analysis*. 15th edn. Association of Official Analytical Chemists, Washington, DC.
- Baker D H, Fernandez S R, Webel D M and Parsons C M. 1996. Sulphur amino acid requirement and cystine replacement value of broiler chicks during the period three to six weeks posthatching. *Poultry Science* **75**: 737–42.
- Barbour G, Latshaw J D and Bishop B. 1993. Lysine requirement of broiler chicks as affected by protein source and method of statistical evaluation. *British Poultry Science* **34**: 747–56.
- Bornstein S and Lipstein B. 1966. Methionine supplementation of practical broiler ratio. The value of added methionine in broiler finisher rations. *British Poultry Science* **7**: 274.
- Ciftci I and Ceylan N. 2004. Effects of dietary threonine and crude protein on growth performance, carcass and meat composition of broiler chickens. *British Poultry Science* **45**: 280–89.
- Duncan D B. 1955. Multiple range and multiple 'f' test. *Biometrics* **11**: 1–42.
- Fatufe A A, Timmler R and Rodehutsord M. 2004. Response to lysine intake in composition of body weight gain and efficiency of lysine utilization of growing male chickens from two genotypes. *Poultry Science* **83**: 1314–24.
- Harms R H. 1992. A determination of the order of limitation of amino acids in a broiler breeder diet. *Journal of Applied Poultry Research* **1**: 410–14.
- Latshaw J D. 1991. Nutrition - mechanisms of immunosuppression. *Veterinary Immunology and Immunopathology* **30**: 111–20.
- Mack S, Bercovici D, De Groote G, Leclerc B, Lippens M, Pack M, Schutte J B and Van Cauwenberghe S. 1999. Ideal amino acid profile and dietary lysine specification for broiler chickens of 20 to 40 days of age. *British Poultry Science* **40**: 257–65.
- Morris T R, Al-Azzawi I, Gous R M and Simpson G L. 1987. Effects of protein concentration on responses to dietary lysine by chicks. *Poultry Science* **28**: 185–95.
- Rama Rao S V, Panda A K, Raju M V L N, Shyam Sunder G, Bhanja S K and Sharma R P. 2005. Performance of *Vanaraja* chicken on diets containing different concentrations of metabolizable energy at constant ratio with other essential nutrients during juvenile phase. *Indian Journal of Poultry Science* **40**: 245–48.
- Rama Rao S V, Praharaj N K, Reddy M R and Panda A K. 2003. Interaction between genotype and dietary concentrations of methionine for immune function in commercial broilers. *British Poultry Science* **44**: 104–12.
- Rama Rao S V, Praharaj N K, Reddy M R and Sridevi B. 1999. Immune competence, resistance to *Escherichia coli* and growth in male broiler parent chicks fed different levels of crude protein. *Veterinary Research Communications* **23**: 323–36.
- Rama Rao S V, Raju M V L N, Reddy M R, Panda A K and Shyam Sunder G. 2006a. Sunflower seed meal as a substitute for soybean meal in commercial broiler chicken diets. *British Poultry Science* **47**: 592–98.
- Rama Rao S V, Raju M V L N, Reddy M R, Panda A K and Shyam Sunder G. 2006b. Performance of *Vanaraja* chicks fed diets containing different concentrations of protein. *Indian Journal of Animal Nutrition* **23**: 83–87.
- Simon O. 1989. Metabolism of proteins and amino acids. Protein Metabolism in Farm Animals (Eds) Boack H D, Eggum B O, Low A G, Simon O, and Zebrowska T, ed. Oxford University Press, Oxford.
- Snedecor G W and Cochran W G. 1989. *Statistical Methods*. Oxford and IBH Publishing Company, New Delhi
- Sterling K G, Pesti G M and Bakalli R I. 2003. Performance of broiler chicks fed various levels of dietary lysine and crude protein. *Poultry Science* **82**: 1939–47.
- Viera S L, Lemme A, Goldenberg D B and Brugalli I. 2004. Responses of Growing Broilers to Diets with Increased Sulfur amino acids to lysine ratios at two dietary protein levels. *Poultry Science* **83**: 1307–13.
- Wegmann T G and Simthies O. 1966. A simple hemagglutination system requiring small amounts of red blood cells and antibodies. *Transfusion* **6**: 67–73.
- Wijten P J A, Park R, Lemme A and Langhout D J. 2004. Effect of different dietary ideal protein concentrations on broiler performance. *British Poultry Science* **45**: 504–11.