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Broiler responses to digestible total sulphur amino acids at different ages: a neural network approach

A. Faridi^{a*}, A. Gitoee^b, N.K. Sakomura^c, D.C.Z. Donato^c, C. Angelica Gonsalves^c, M. Feire Sarcinelli^c,
M. Bernardino de Lima^c and J. France^d

^aDepartment of Animal Sciences, Center of Excellence, Ferdowsi University of Mashhad, Mashhad 91775-1163, Iran; ^bDepartment of Animal Science, College of Agriculture, University of Kurdistan, PO Box 416, Sanandaj, Iran; ^cDepartment of Animal Science, College of Agrarian and Veterinary Sciences, University Estadual Paulista, Jaboticabal, Sao Paulo 14884-900, Brazil; ^dDepartment of Animal and Poultry Science, Centre for Nutrition Modelling, University of Guelph, Guelph, ON N1G 2W1, Canada

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Three experiments were conducted with broiler chickens to evaluate the effects of digestible total sulphur amino acid (TSAA) on their performance at three different phases of starter (1–14 d), grower (15–28 d) and finisher (29–42 d). The measured traits included: average daily gain (ADG), feed intake, feed conversion ratio (FCR), carcass protein, body lipid (BL), feather weight gain, carcass plus feather protein, carcass TSAA deposition and nitrogen excretion (NE). A dilution technique was used to create seven diets (with eight replicates) increasing the TSAA content from 2.5 to 9.04 g/kg of diet for starter, 2.26 to 8.14 g/kg of diet for grower and 2.08 to 7.5 g/kg of diet for finisher. Data measured were imported to neural networks to predict the measured traits in response to dietary and intake levels of TSAA and find the optimal levels of TSAA that lead to the desired responses. Optimization results showed decreases in optimal dietary TSAA values with increasing age for all traits, while reverse was observed for intake values and requirements were increased as birds aged. The highest TSAA requirement (7.95, 7.2 and 6.6 g/kg and 283, 585 and 1150 mg/bird per d for starter, grower and finisher, respectively) were achieved for minimum BL and lowest (5.8, 5.2 and 4.9 g/kg and 201, 444 and 873 mg/bird per d for starter, grower and finisher, respectively) were suggested for minimum NE. Based on intake models, the optimal TSAA values for minimum FCR in phases 1–3 were 283, 585 and 1150 mg/bird per d while maximum ADGs were achieved with 201, 444 and 873 mg/bird per d of TSAA.

Keywords: broiler; total sulphur amino acid; neural network; optimization

1. Introduction

It has been well documented that broiler diets based upon soybean meal as the primary protein source are deficient in the total sulphur-containing amino acids (TSAA) methionine and cysteine (Almquist et al. 1942). Methionine is an essential amino acid (AA) in poultry nutrition that plays important roles including protein synthesis, methyl donor for methylation reaction, a precursor of important intermediates in metabolic pathways and synthesis of polyamines (Vazquez-Anon et al. 2006). There are many studies conducted to determine the TSAA requirement of broilers. Although, due to genetic improvement happened in recent years, it is necessary to update these requirements. Moreover, the TSAA requirement might change with the specific production goal such as either improving feed conversion or increasing breast meat yields (Pack et al. 2003). Schutte and Pack (1995) suggested that a TSAA requirement based on growth responses may not be sufficient for optimal carcass responses. Requirement for a specific nutrient, usually, is reported as either dietary

(% or g/kg of diet) or intake level of that specific nutrient. However, intake levels are more precise to be considered as they cover a part of variations due to different feed intake (FI) levels.

In recent years, application of soft-computing neural network (NN) modelling has gained much popularity in the poultry nutrition and their ability in predicting and optimization of poultry responses to nutrient has been well approved (Faridi & Golian 2011; Faridi et al. 2012; Faridi, Golian, Heravi-Mousavi et al. 2014; Faridi, Golian, France et al. 2014)

The aim of this study, therefore, was: (1) to design a set of experiments to evaluate the response of broiler chickens [average daily gain (ADG; g/bird per d), FI (g/bird per d), feed conversion ratio (FCR), carcass crude protein (CCP;%), body lipid (BL; g/kg of carcass), feather weight gain (FWG; g/bird per d), carcass plus feather crude protein (CFCP; g/bird per d), carcass TSAA deposition (CDTSAA; mg/bird per d) and nitrogen excretion (NE; g/bird per d)] to dietary and intake levels of digestible TSAA (g/kg of diet or g/bird per d for CP; mg/bird per d for TSAA) during phase 1 [starter

*Corresponding author. Email: ako_faridi@yahoo.com

(1–14 d)], phase 2 [grower (15–28 d)] and phase 3 [finisher (29–42 d)] of age; (2) to compare the accuracy of NN models to predict these responses based on dietary and intake levels of TSAA; and (3) to maximize or minimize the models constructed to find the dietary or intake levels of TSAA that optimize these outputs.

2. Materials and methods

2.1. Birds, diets and measurements

Three experiments designed to evaluate the response of broiler chickens to dietary TSAA during the three phases of starter, grower and finisher were conducted at the College of Agrarian and Veterinary Sciences, University Estadual Paulista, Jaboticabal (Sao Paulo, Brazil). The Institution's Ethics and Animal Welfare Commission approved all procedures.

In each phase, 1120 Cobb 500 broilers were distributed across 56 pens with 20 birds in each (10 of each sex). A completely random design with seven dietary treatments and eight replicates was used. At the beginning of each trial, birds were individually weighed and distributed such that each experimental unit carried the same weight. Experimental diets were formulated using the dilution technique. A high protein summit diet was formulated (Table 1) and diluted (phase 1: 100%; phase 2: 90%; phase 3: 83% of summit diet) to contain approximately 1.2 times the TSAA level suggested by Rostagno et al. (2005) for broilers during the respective phases, with all other essential AAs set at a minimum of 1.4 times their suggested levels. These summit diets were diluted sequentially with iso-energetic, protein-free diets (Fisher & Morris 1970) to create a range of feeds increasing in TSAA content (starter: 2.5–9.04; grower: 2.26–8.14; and finisher: 2.08–7.5 g/kg of diet). The birds used in phase 2 (15–28 d) were fed with a standard commercial starter diet from 1 to 14 d of age while birds used in phase 3 (29–42 d) were fed with starter and grower diets from 1 to 14 and 15 to 28 d of age (Table 1).

Digestible AA contents of the summit diets were determined through a digestibility trial using the method described by Sakomura and Rostagno (2007). Total AA concentrations in the diets and the excreta were determined by high-performance liquid chromatography (HPLC). The weight gain, FI and FCR were calculated from the measurements of body weight and feed consumed during each growth phase.

Protein and lipid (g/d) in the feather-free body and in the feathers were determined using the comparative slaughter technique. The number of birds from each experimental unit sampled at the beginning and end of a phase was six and two birds, respectively.

The birds were killed by CO₂ asphyxiation, de-feathered, eviscerated and weighed. The samples were

Table 1. Ingredient and nutrient composition of the feeds used in the trial.

	Diets			Nitrogen free
	Starter	Grower	Summit	
<i>Ingredient (g/kg)</i>				
Corn	551.8	586.1	339	–
Soybean meal (45%)	378.6	337	510	–
Corn gluten meal (60%)	–	–	37.3	–
Soy oil	30.9	39.25	65	100
Dicalcium phosphate	19.1	17.5	20.3	27
Limestone	9.3	8.8	9.5	5.1
Salt	5.15	4.9	4.5	5.15
L-Lys HCl	0.44	1.44	4.9	–
DL-Met	1.9	2.1	3.28	–
L-Thr	0	0.25	2.01	–
L-Valine	–	–	1.4	–
L-Arginine	–	–	0.54	–
Mineral premix ^a	0.5	0.5	0.5	0.5
Vitamin premix ^b	0.5	0.5	0.5	0.5
Choline chloride (60%)	0.7	0.7	1	1
Potassium chloride	–	–	–	11.5
Starch	–	–	–	428
Sugar	–	–	–	150
Rice husk	–	–	–	150
Inert	0.7	0.7	–	121
<i>Calculated nutrients (g/kg)</i>				
ME (kcal/kg)	3000	3100	3050	3050
Calcium	9.4	8.7	10	10
Available phosphorus	4.7	4.3	5	5
Crude protein	218.7	203.8	294	–
Met+Cys	7.9	7.75	9.04	–
Met	5	5	5.82	–
Lysine	11.1	10.9	15.8	–
Threonine	7.4	7.12	10.2	–

^aContent/kg of product: Mn = 150,000 mg, Fe = 100,000 mg, Zn = 100,000 mg, Cu = 16,000 mg, I = 1500 mg.

^bContent/kg of product: Folic acid = 1000 mg, pantothenic acid = 15,000 mg, Niacin = 40,000 mg, Biotin = 60 mg, vitamin B1 = 1800 mg, vitamin B12 = 12,000 mg, vitamin B2 = 6000 mg, vitamin B6 = 2800 mg, vitamin D3 = 2,000,000 UI, vitamin E = 15,000 mg, vitamin K3 = 1800 mg, Se = 300 mg, antioxidant = 500 mg.

then placed in individual bags and frozen. Upon thawing, the components were ground in an industrial meat grinder (98 BT CAF Rio Claro, Sao Paulo, Brazil) to obtain homogeneous samples, which were freeze-dried (SuperModulyo Freeze Dryer, Thermo Electron Corporation, Edwards, Asheville, NC) at –50°C to determine water content, and thereafter, ground again in a micro-mill (Ika A11 Basic, Ika Works do Brazil Ltda, Taquara, RJ, Brazil). These dried samples (100 g per each bird)

were analysed for CP by the Kjeldahl method (#954.01) according to AOAC (1995) procedures. The AA contents of protein in the feather-free body and in the feathers were measured in birds sampled at the beginning and end of each phase and were analysed by HPLC from which the amount of each AA deposited during each phase was calculated.

2.2. NN models: development and optimization

After data collection, the first step in a NN design is appropriate selection of the network architecture. In this study, 51 ensemble feed-forward multiplier perceptron NN models (27 and 24 models based on dietary and intake TSAA levels, respectively) were developed to predict the broiler responses (ADG, FI, FCR, CCP, BL, FWG, CFCP, CDTSAA and NE) to different levels of TSAA. In all the models developed, the hyperbolic tangent was used as the activation function and the quasi-Newton method as a training algorithm. Two different random data groups, namely training ($n = 34$) and testing ($n = 22$) sets, were used for model development (StatSoft 2011).

To overcome the limited number of data points available for each trait ($n = 56$) and to take full advantage of them, a bootstrapping method was used to generate 50 different sets for training and testing (Faridi, Golian, Heravi-Mousavi et al. 2014). In order words, for each of the responses investigated in each phase, 50 different random training and testing sets were generated from the main data and the corresponding NN models were ensemble to construct the final model (see Figure 1). Statistica Neural Networks software version 10.0 was used to re-sample the data, construct and train the NN models and ensemble them (StatSoft 2011). Predictive ability of

the constructed NN models was assessed using common criteria such as R^2 , mean square error (MSE) and bias.

An important aspect of modelling a biological system using NNs is optimizing the models developed to find a set of values for the independent variable (TSAA) for which the constructed model yields the optimum (maximum or minimum) response (ADG, FI, FCR, CCP, BL, CFCP, CDTSAA and NE). With the random search approach (as the optimization algorithm) applied here, for each selected set of independent values, the model prediction is evaluated and compared with the desired response. This process is then repeated until a set of independent values is discovered for which the model prediction is equal or as close as possible to the desired response value (StatSoft 2011).

3. Results

Dietary levels of TSAA and CP along with the corresponding broiler responses (ADG, FI, FCR, CCP, BL, FWG, CFCP, CDTSAA and NE) in phases 1–3 are summarized in Tables 2–4, respectively. Accuracy of bootstrapped NN models developed to predict the responses of broilers to dietary and intake TSAA levels are summarized in Tables 5 and 6, respectively. Results showed that the overall accuracy of all developed models in all responses (except for CCP) in all phases were acceptable. Based on the results presented in Tables 5 and 6, the highest accuracy of prediction was achieved with FCR models followed by NE, ADG and CFCP, respectively. It seems that there is a tendency to decrease in accuracy of models developed as birds aged. Comparing Tables 5 and 6, models developed based on the intake TSAA levels (mg/bird per d) provided higher accuracy of prediction (higher R^2 and lower MSE values) compared to models developed based on dietary levels (g/kg of diet). Based on bias values, our models showed little overestimation or underestimation of broiler responses to TSAA during different phases of breeding. Scatter plots for bootstrapped NN models of actual vs. predicted values of ADG in response to dietary and intake TSAA levels in different phases are shown in Figure 2. Higher accuracy of NN models developed based on intake levels, compared to dietary levels, is obvious. Random search method results for optimization of NN models developed to predict the response of broilers are summarized in Tables 7 and 8, respectively. Results showed that, as expected, the TSAA requirements decreased based on dietary levels while increased based on intake levels as birds aged. Optimization results showed appreciable effects of traits studied on TSAA requirement of broilers during different phases. For example, the TSAA required to minimize FCR in first phase achieved as 7.8 g/kg of diet while this value for minimizing NE was 5.8 g/kg of diet. Based on both

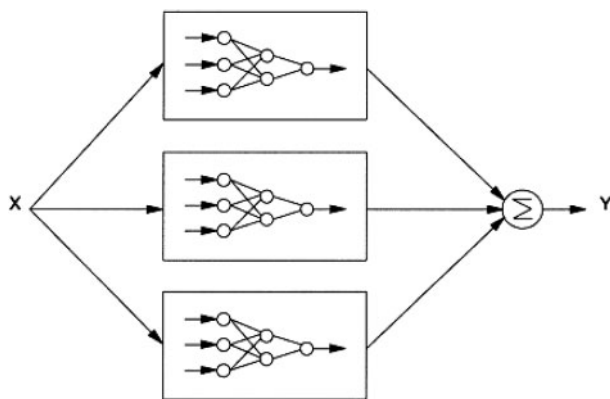


Figure 1. Schematic diagram of a bootstrapped NN model where X is the input variable (TSAA), Y is the output (investigated traits), and each rectangle stands as a separate NN. Herein 50 separate NN models were developed. Source: Zhang (1999).

Table 2. Mean values (SD) of digestible total sulphur amino acid (DTSAA; g/kg of diet) and protein (CP; g/kg of diet) and corresponding broiler responses at 1–14 d of age.

Input		Investigated traits								
CP	DTSAA	ADG	FI	FCR	CCP	FWG	CFCP	BL	CDTSAA	NE
74.3	2.5	10.03 (1.7)	22.18 (3.6)	2.2 (0.04)	14.04 (0.95)	0.32 (0.15)	1.42 (0.29)	159.3 (21.8)	42.9 (8.6)	36.4 (19.7)
106.2	3.6	19.3 (2.5)	32 (3.8)	1.7 (0.03)	15.2 (0.55)	0.53 (0.12)	2.96 (0.45)	141.6 (14.5)	87.5 (12)	69.4 (17.5)
138	4.7	25.6 (1.1)	35.5 (1.5)	1.4 (0.02)	14.5 (0.39)	0.82 (0.19)	3.8 (0.3)	125.9 (8.6)	110.9 (9.5)	184.7 (44.2)
169.9	5.8	28.6 (0.94)	36.2 (0.95)	1.3 (0.02)	14.8 (0.89)	0.88 (0.08)	4.4 (0.22)	101.1 (5.5)	134.6 (8.07)	273.8 (26.9)
201.7	6.9	29.4 (1.49)	35.3 (1)	1.2 (0.03)	16.1 (0.32)	0.97 (0.2)	4.9 (0.29)	61.8 (7)	146.6 (12.1)	349 (37)
233.5	8	29.4 (1.6)	34.7 (1.3)	1.18 (0.02)	15.2 (0.61)	1.05 (0.22)	4.75 (0.24)	57.1 (5.8)	147 (5.8)	538 (42.1)
265.4	9.04	28.1 (1.3)	32.6 (1.1)	1.16 (0.01)	15.9 (0.78)	1.07 (0.21)	4.7 (0.1)	48.9 (8.5)	141.2 (7.03)	634 (53.9)

ADG, average daily gain (g/bird per d); FI, feed intake (g/bird per d); FCR, feed conversion ratio; CCP, carcass protein (%); BL, body lipid (g/kg of carcass); FWG, feather weight gain (g/bird per d); CFCP, carcass plus feather protein (g/bird per d); CDTSAA, carcass TSAA deposited (mg/bird per d); NE, nitrogen excretion (mg/bird per d).

Table 3. Mean values (SD) of DTSAA (g/kg of diet) and protein (CP; g/kg of diet) and corresponding broiler responses at 15–28 d of age.

Input		Investigated traits								
CP	DTSAA	ADG	FI	FCR	CCP	FWG	CFCP	BL	CDTSAA	NE
66.35	2.26	30.4 (0.98)	86.4 (2.6)	2.84 (0.05)	15.7 (0.93)	1.08 (0.33)	5 (0.51)	169.8 (17.32)	136.5 (20.93)	115.6 (73.8)
95.5	3.25	44.9 (2.35)	93.3 (4.14)	2.08 (0.04)	15.6 (0.57)	1.53 (0.44)	7.4 (0.39)	147.5 (5.44)	199.2 (19)	240.5 (71)
124.7	4.25	53.9 (2.96)	93.1 (4.37)	1.73 (0.03)	15.6 (0.61)	1.73 (0.34)	8.7 (0.49)	130.8 (11.34)	238.1 (12.6)	464.2 (97)
153.9	5.24	58.7 (4.2)	91 (4.45)	1.55 (0.04)	15.2 (0.57)	2.56 (0.33)	9.8 (0.8)	104 (5)	278.1 (24.2)	673.3 (66)
180.5	6.15	59.3 (3.2)	83.9 (3.75)	1.41 (0.04)	15.5 (0.37)	2.54 (0.23)	9.8 (0.34)	84.8 (11.32)	267.5 (18.3)	860.5 (106.1)
209.7	7.14	61.3 (4.25)	81.8 (4.37)	1.33 (0.03)	15.6 (0.87)	3 (0.45)	10.7 (0.26)	67.4 (12.8)	312.7 (16.6)	1038.5 (171.8)
238.9	8.14	59.8 (4.48)	77.6 (4.39)	1.3 (0.04)	16 (0.36)	2.74 (0.45)	10.6 (0.71)	51.9 (8.28)	294.2 (17.87)	1273.8 (131.4)

ADG, average daily gain (g/bird per d); FI, feed intake (g/bird per d); FCR, feed conversion ratio; CCP, carcass protein (%); BL, body lipid (g/kg of carcass); FWG, feather weight gain (g/bird per d); CFCP, carcass plus feather protein (g/bird per d); CDTSAA, carcass TSAA deposited (mg/bird per d); NE, nitrogen excretion (mg/bird per d).

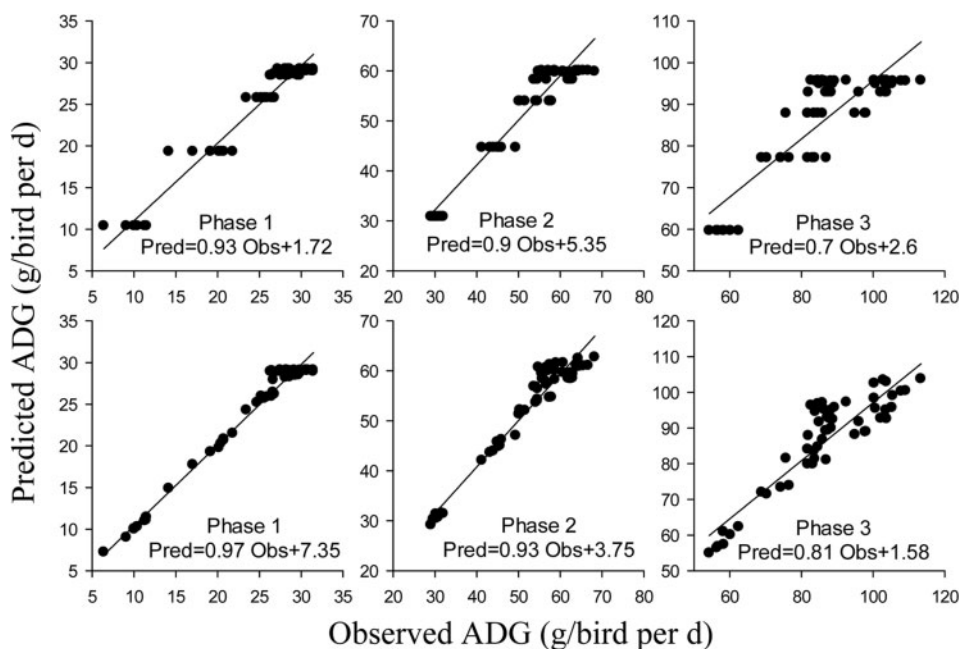


Figure 2. Scatter plots for bootstrapped NN models of actual vs. predicted values of ADG (g/bird per day) in response to dietary (top row) and intake (down row) levels of TSAAs in different phases.

Table 4. Mean values (SD) of DTSAA (g/kg of diet) and protein (CP; g/kg of diet) and corresponding broiler responses at 29–42 d of age.

Input		Investigated traits								
CP	DTSAA	ADG	FI	FCR	CCP	FWG	CFCP	BL	CDTSAA	NE
61.04	2.08	58.1 (2.5)	193.1 (13.6)	3.32 (0.14)	17.2 (0.82)	2.6 (0.55)	9.27 (0.55)	195.8 (14.4)	280 (39.3)	403.3 (206.5)
87.6	2.98	78.2 (6.7)	200 (17.9)	2.56 (0.06)	15.9 (0.95)	2.54 (0.64)	13.13 (0.96)	151.9 (19.2)	318.9 (64.1)	701.2 (225.8)
114.1	3.89	87.8 (8.2)	191.6 (15.4)	2.19 (0.05)	16.5 (0.78)	3.5 (0.4)	15.4 (1.3)	145.7 (11.6)	427.7 (19.3)	1014.4 (197.9)
140.7	4.79	93.8 (8.7)	184.5 (14.5)	1.97 (0.03)	16.5 (0.77)	4.57 (0.45)	15.7 (1.3)	138.8 (23)	489.6 (63.4)	1580.6 (187.5)
167.2	5.69	95.5 (9)	177.4 (13)	1.86 (0.05)	17.4 (0.58)	4.2 (0.52)	16 (1.8)	109.1 (19.9)	478.7 (65.9)	2183.8 (264.3)
193.7	6.6	96.3 (10.4)	172.4 (12.7)	1.8 (0.07)	16.7 (0.78)	4.98 (1.09)	16.4 (1.8)	118.8 (21.5)	502.5 (89.1)	2693.7 (165.3)
220.3	7.5	95.8 (11)	165.5 (12.9)	1.74 (0.08)	16.8 (1.02)	4.64 (0.53)	16.8 (2.2)	122.2 (12.9)	464.7 (84.4)	3152.1 (260.8)

ADG, average daily gain (g/bird per d); FI, feed intake (g/bird per d); FCR, feed conversion ratio; CCP, carcass protein (%); BL, body lipid (g/kg of carcass); FWG, feather weight gain (g/bird per d); CFCP, carcass plus feather protein (g/bird per d); CDTSAA, carcass TSAA deposited (mg/bird per d); NE, nitrogen excretion (mg/bird per d).

Table 5. Accuracy of bootstrapped NN models in response to dietary levels of DTSAA and protein.

Phase	Investigated traits								
	ADG	FI	FCR	CCP	FWG	CFCP	BL	CDTSAA	NE
<i>Phase 1 (1–14 d)</i>									
R^2	0.95	0.82	0.99	0.38	0.71	0.94	0.93	0.95	0.97
MSE	2.3	4.8	0	0.56	0.03	0.08	131	74	1455
Bias	-0.03	-0.16	0	-0.03	-0.01	0	-0.44	0.03	1.7
<i>Phase 2 (15–28 d)</i>									
R^2	0.91	0.66	0.99	0.10	0.74	0.92	0.94	0.88	0.93
MSE	10.4	15.4	0	0.38	0.15	0.28	109	403	10745
Bias	0.14	0.1	0	0.01	-0.01	-0.02	-0.41	-0.73	6.2
<i>Phase 3 (29–42 d)</i>									
R^2	0.73	0.4	0.98	0.17	0.67	0.76	0.67	0.64	0.96
MSE	64	189	0.01	0.72	0.39	2	329	3624	42266
Bias	0.27	1.45	0	-0.03	-0.01	0.02	-0.73	0.87	8.8

ADG, average daily gain (g/bird per d); FI, feed intake (g/bird per d); FCR, feed conversion ratio; CCP, carcass protein (%); BL, body lipid (g/kg of carcass); FWG, feather weight gain (g/bird per d); CFCP, carcass plus feather protein (g/bird per d); CDTSAA, carcass TSAA deposited (mg/bird per d); NE, nitrogen excretion (g/bird per d).

Table 6. Accuracy of bootstrapped NN models in response to intake levels of DTSAA and protein.

Phase	Investigated traits							
	ADG	FCR	CCP	FWG	CFCP	BL	CDTSAA	NE
<i>Phase 1 (1–14 d)</i>								
R^2	0.98	0.99	0.39	0.71	0.97	0.93	0.97	0.98
MSE	1.11	0	0.56	0.03	0.05	131	50	1135
Bias	0.04	0	-0.01	-0.01	0.01	-0.42	0.02	-0.56
<i>Phase 2 (15–28 d)</i>								
R^2	0.94	0.99	0.15	0.75	0.94	0.94	0.88	0.97
MSE	6.8	0	0.35	0.14	0.22	111.4	404	5643
Bias	0.15	0	0	-0.01	-0.02	-0.1	0.64	0.9
<i>Phase 3 (29–42 d)</i>								
R^2	0.84	0.97	0.19	0.67	0.82	0.76	0.67	0.98
MSE	38	0.01	0.7	0.39	1.5	262	3367	36768
Bias	0.36	0	-0.03	-0.01	-0.03	-0.15	0.7	-1.63

ADG, average daily gain (g/bird per d); FI, feed intake (g/bird per d); FCR, feed conversion ratio; CCP, carcass protein (%); BL, body lipid (g/kg of carcass); FWG, feather weight gain (g/bird per d); CFCP, carcass plus feather protein (g/bird per d); CDTSAA, carcass TSAA deposited (mg/bird per d); NE, nitrogen excretion (g/bird per d).

Table 7. Optimization results for the bootstrapped NN models to achieve optimal broiler performance in response to dietary levels of DTSAA.

Phase	Investigated traits ^a							
	ADG	FI	FCR	CCP	CFCP	BL	CDTSAA	NE
<i>Phase 1 (1–14 d)</i>								
TSAA (g/kg)	7.3	5.8	7.8	7.4	7.8	7.95	7.7	5.8
Optimum response	29.2	35.7	1.18	15.6	4.8	55.5	146.1	270.5
<i>Phase 2 (15–28 d)</i>								
TSAA (g/kg)	6.8	5.3	7.2	7	7.15	7.2	7.15	5.2
Optimum response	60.1	88	1.34	15.6	10.4	67.7	294.7	666
<i>Phase 3 (29–42 d)</i>								
TSAA (g/kg)	6.2	5	6.6	6.2	6.6	6.6	6.3	4.9
Optimum response	95.6	182.3	1.79	16.9	16.4	119	490	1620.6

^aOptimization was conducted to maximize ADG, CCP, CFCP and DTSAA while minimize FCR, BL and NE.

ADG, average daily gain (g/bird per d); FI, feed intake (g/bird per d); FCR, feed conversion ratio; CCP, carcass protein (%); BL, body lipid (g/kg of carcass); CFCP, carcass plus feather protein (g/bird per d); CDTSAA, carcass TSAA deposited (mg/bird per d); NE, nitrogen excretion (g/bird per d).

Table 8. Optimization results for the bootstrapped NN models to achieve optimal broiler performance in response to intake levels of DTSAA.

Phase	Investigated traits ^a						
	ADG	FCR	CCP	CFCP	BL	CDTSAA	NE
<i>Phase 1 (1–14 d)</i>							
TSAA (mg/bird per d)	255	275	264	276	283	269	201
Optimum response	29.2	1.18	15.6	4.8	56.7	146	244.2
<i>Phase 2 (15–28 d)</i>							
TSAA (mg/bird per d)	574	584	551	587	585	578	444.5
Optimum response	60.4	1.34	15.6	10.43	66.4	296.2	615
<i>Phase 3 (29–42 d)</i>							
TSAA (mg/bird per d)	1118	1139	1123	1147	1150	1146	873
Optimum response	96	1.78	16.9	16.6	1170	489.5	1628

^aOptimization was conducted to maximize ADG, CCP, CFCP and DTSAA while minimize FCR, BL and NE.

ADG, average daily gain (g/bird per d); FCR, feed conversion ratio; CCP, carcass protein (%); BL, body lipid (g/kg of carcass); CFCP, carcass plus feather protein (g/bird per d); CDTSAA, carcass TSAA deposited (mg/bird per d); NE, nitrogen excretion (g/bird per d).

dietary and intake levels, results showed that maximum TSAA requirement in the first phase were needed to minimize BL (7.95 g/kg of diet; 283 mg/bird per d) and FCR (7.89 g/kg of diet; 275 mg/bird per d) or maximize CDTSAA (7.5 g/kg of diet; 269 mg/bird per d) responses. Almost the same trend can be seen for other phases regarding to different responses. Based on the overall optimization results, it seems that considering the TSAA requirement for minimum BL or FCR responses can cover the TSAA requirement for all other responses (Tables 7 and 8).

4. Discussion

Predictive models have been serving as a useful option in poultry nutrition for nutritional and management decisions. These models are developed based on data achieved from actual experiments. The data can be pre-processed and expressed in a set of rules, such as it is

often the case in knowledge-based expert systems, or serve as training data for statistical and machine learning models. Among the options in the latter category, the most popular are linear regression and NN models (Dreiseitl & Ohno-Machado 2002). NNs are algorithms that can be used to perform non-linear modelling and provide an alternative to linear regression models, the most commonly used models for predictive models in poultry nutrition.

In recent years NN models were developed to predict the response of broilers to different nutrients, especially AAs. These models were used to find the optimal levels of nutrients studied which may be considered as requirements (Ahmadi & Golian 2011; Mehri 2012; Faridi et al. 2013, Faridi, Golian, Heravi-Mousavi et al. 2014; Faridi, Golian, France et al. 2014).

These models have the ability to 'learn' the relationships between input and output variables. This is achieved by training the models with a training data-set

while a testing data-set is used to evaluate the model performance and check for the possible over-fitting. Therefore, it is clear how data cleaving (train and test groups) is important in model constructions. Statistical resampling, the bootstrapped method, can serve as a useful option in data partitioning as using this method, each data line can participate in both training and testing sets. This is especially important in poultry nutrition as obtaining data in this area is very expensive and time consuming. Despite the importance of bootstrapping, to our knowledge, only one study has used this method in developing NN models in poultry nutrition. Faridi, Golian, Heravi-Mousavi et al. (2014) used bootstrapped NN models to predict the response of broilers to dietary CP and branched chain AAs. Their results indicated that the bootstrapped NN models were more accurate in 76 and 57 cases (out of 100) compared with separated NN models (Faridi, Golian, Heravi-Mousavi et al. 2014).

Effects of methionine or TSAA on broiler performance has been well documented before. However, little information is available on the effects of TSAA on different broiler traits during different phases. In this study NN models were developed to predict the different responses of broilers to dietary and intake levels of TSAA during starter, grower and finisher phases. Results showed an acceptable accuracy of response prediction by NN models. In general, accuracy of models developed based on intake levels were higher than dietary levels indicating that considering the FI may cover a part of variations in responses. Results showed that the accuracy of models were decreased as birds aged. This may show the importance of proper nutrition in early stages of breeding. In an experiment conducted to evaluate the effects of TSAA on broiler performance during 1–42 days of age, Rakangtong and Bunchasak (2011) stated that the young broilers (up to 21 d of age) were more sensitive to the TSAA supplementation than were the older broilers (22–42 d of age).

One of the important aspects for application of predictive models (including NN) is to optimize the models to find the optimal values for independent (input) variables. In this study, NN models were subjected to a random search optimization process. Ability of this approach in determining optimum nutrient values (especially AAs) has been approved before (Faridi et al. 2013).

Results showed that the optimal TSAA levels were affected by the traits studied. The optimal dietary TSAA levels during the starter phase ranged from 5.8 to 7.95 g/kg of diet. These values for grower and finisher phases are from 5.2 to 7.2 and from 4.9 to 6.6 g/kg of diets, respectively. The same changes were observed in optimal intake levels and the requirements were affected by the response (Table 8). Our optimization results showed that, based on both dietary and intake TSAA levels, the highest requirement was achieved for minimum

BL, minimum FCR, maximum CFCP and maximum CDTSAA. The lowest requirement was achieved for minimum NE (Tables 7 and 8).

Our results showed that the TSAA requirement (both dietary and intake levels) for minimum FCR is higher than for maximum ADG. In agreement with these results, Schutte and Pack (1995) showed that the TSAA requirement was found to be higher for minimum FCR and breast meat yield than for obtaining maximum ADG. They further reported that based on FCR response, the requirement for TSAA was estimated to be 8.8 g/kg of diet for the age period of 14 to 34 or 38 d. The estimated TSAA requirement was equivalent to approximately 7.5 g/kg of diet apparent digestible TSAA during this period (Schutte & Pack 1995). In contrast, Knowles and Southern (1998) obtained a requirement of 6.6 g/kg digestible TSAA for maximum ADG, and 6.3 g/kg for FE in broiler birds from 0 to 15 d of age.

Dozier and Mercier (2013) after conducting two experiments reported that the digestible TSAA intake during 1–15 d of age that optimized FCR ranged from 296 to 306 mg/bird per d, which was higher than reported in previous research. They claimed that the higher estimates of TSAA of their research may be supported by faster growth translating to higher AA needs of the modern broiler (Dozier & Mercier 2013). However, these recommendations were higher than what we reported here (201–283 mg/bird per d) which can be explained by difference in breeding period (1–14 d vs. 1–15 d) and strains (Cobb 500 vs. Ross × Ross 708 and Hubbard × Cobb 500).

Our results showed that the TSAA requirements (dietary and intake levels) in all phases for maximum CFCP was higher than that for CCP. This may indicate the importance of TSAA in feather growth and development. Kalinowski et al. (2003) stated that a methionine requirement approximating 5 g/kg of diet is appropriate for broilers during first 21 d of age, regardless of feather rate; however, the estimated cystine requirement for slow-feathering males (3.9 g/kg) was less than for fast-feathering (4.4 g/kg) males.

Based on the optimization results, the highest TSAA requirement (dietary and intake levels) was achieved for minimum BL. An important aspect of the protein and methionine interrelationship is the ability of both to act as lipotropic agents. Vieira et al. (2004) reported that TSAA supplementation strongly affected abdominal fat and decreased it by 30%.

Through the optimization process, the minimum TSAA requirement was achieved for minimum NE. Since nitrogen excreted comes from the unused nitrogen in food, several nutritional strategies have been proposed to improve its use and thus reduce excretion. Reducing the protein content of the diet reduces NE, although the level of essential AAs must be maintained by

supplementation with crystalline AAs if this strategy is to be effective and production rates are not to be harmed (Namroud et al. 2008). However, some studies have indicated that performance rates will inevitably worsen (Ferguson et al. 1998).

The overall results of this study showed that bootstrapped NN model is a promising option in predicting the response of broilers to TSAA levels. It was concluded that developing models based on nutrient intake levels may lead to more precise models compared to dietary nutrient levels. Optimization results indicated that the TSAA requirements may differ based on the traits studied. Through the results obtained here, the TSAA requirement for minimum FCR was higher than that for maximum ADG. Moreover, the highest and lowest TSAA requirements were achieved for minimum BL and NE, respectively.

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Disclosure statement

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