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ORIGINAL ARTICLE

Free availability of high-energy foods led to energy over-ingestion and protein under-ingestion in choice-fed broilers

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ABSTRACT

The objective of this study was to compare energy and protein content of the diet selected by choice-fed broilers with that of broilers fed a balanced diet. One hundred and eighty 1-day-old male broilers were randomly assigned in groups of 10 to one of three experimental treatments ($n = 6$). Control broilers were fed a standard balanced diet, whereas choice-fed broilers were fed three foods which were more concentrated (Choice C+ treatment) or less concentrated (Choice C– treatment) in protein, carbohydrate or fat. We evaluated food intake behavior, nutrient intake, and performance parameters of broilers from 2 to 7 weeks of age. Choice C+ broilers showed enhanced preference for the high-fat food, which led to higher energy intake and lower protein intake than those of control broilers at 2 to 4 weeks of age. Body weight, weight gain and feed conversion efficiency were negatively affected by diet selection of Choice C+ broilers. Choice C– broilers selected a balanced diet, and showed performance parameters similar to those of control broilers. Our results supported the hypothesis that free availability of high-energy foods bias ingestive behavior of choice-fed broilers toward selecting a diet with higher energy and lower protein than needed for normal growth.

Key words: *choice feeding, diet selection, high-energy foods, ingestive behavior, nutritional balance.*

INTRODUCTION

Under free-choice feeding, animals have the capacity to select a combination of foods that better satisfies their nutritional requirements (Emmans 1991). They are able to learn the post-ingestive consequences of foods (Cabanac 1971; Provenza 1995), and modify their preferences according to nutritional needs (Gibson & Booth 1989; Villalba & Provenza 1999). As animals grow they choose diets with decreasing protein content and increasing energy content, consistent with increased energy needs for body fat deposition (Shariatmadari & Forbes 1993). However, it has also been shown that choice-fed animals can fail to select a combination of foods that meet their nutritional requirements (Forbes & Kyriazakis 1995; Duncan & Young 2002; Catanese *et al*. 2009).

Choice-fed broilers can select a diet with lower protein-to-energy ratio than required for normal growth and development (Cowan & Michie 1977; Siegel *et al*. 1997; Yo *et al*. 1998). A possible explanation for energy over-ingestion is the need to meet protein requirements (Simpson *et al*. 2003), since protein intake is more tightly regulated than energy intake (Sørensen *et al*. 2008; Felton *et al*. 2009). However, broilers can exceed energy intake even when fed food alternatives with adequate protein levels (Leeson & Caston 1993; Dana & Ogle 2002).

In the present study, we tested the hypothesis that free availability of high-energy foods bias ingestive behavior of choice-fed broilers toward selecting a diet with higher energy and lower protein than required for normal growth. To test this hypothesis we used the experimental framework proposed by Raubenheimer and Simpson (1997), in which animals are challenged to select a balanced diet by offering them foods that differ in the concentration of two or more nutrients. The nutritional composition of choice-fed foods was manipulated in order to create feeding environments

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of increasing energy and protein concentration. We evaluated food intake behavior, nutrient intake, and performance parameters (body weight, weight gain, feed conversion efficiency) in control broilers fed a standard balanced diet and in choice-fed broilers from 2 to 7 weeks of age.

MATERIALS AND METHODS

The experiment was conducted from June to December 2008 at the 'Centro de Recursos Naturales Renovables de la Zona Semiárida' (CERZOS), Bahía Blanca (38° 44′ S; 62° 16′ W), Argentina. All experimental protocols fulfilled animal welfare regulations of the Universidad Nacional del Sur (Bahía Blanca, Argentina), and adhere to the ASAB/ABS (2006) guidelines for the use of animals in research.

Animals and housing

One hundred and eighty 1-day-old male broiler chicks (Cobb 500™ breed) were wing-banded, weighed and housed in 18 floor pens (10 animals per pen). Each pen (1.5 m^2) was provided with three nipple drinkers, three 60 cm long feeding troughs, and sunflower hulls as litter material. Lighting program, room temperature and health care during the experimental period were according to recommendations of the breeder (Cobb-Vantress Inc 2004).

Experimental design and treatments

Floor pens (experimental units) were randomly assigned among a control and two choice-fed treatments ($n = 6$ floor pens). Control broilers were fed a standard balance diet to meet physiological requirements, as indicated by the breeder (Cobb-Vantress Inc 2004). The nutritional program included a 'starter' diet from 0 to 4 weeks of age (hereafter 'starter phase'), a 'grower' diet from 5 to 6 weeks of age (hereafter 'grower phase'), and a 'finisher' diet at 7 weeks of age (hereafter 'finisher phase'). Nutritional composition of each diet was assumed as the 'nutrient target' (*sensu* Simpson & Raubenheimer 1995) to be met by choice-fed broilers.

Broilers exposed to free-choice treatments were fed with the starter diet during their first week of life. Then, from 2 to 7 weeks of age (experimental period) they were allowed to choose freely among three foods, each of them more concentrated (Choice C+ treatment) or less concentrated (Choice C– treatment) in protein, carbohydrate or fat. Within each choice-fed treatment, high-carbohydrate and high-fat foods were isocaloric and isonitrogenous in order to reduce biases in food preference due to energy or protein content.

Foods offered to choice-fed broilers were prepared from soybean meal, meat and bone meal, corn, sunflower oil and wheat bran, as described in Table 1. Since soybean meal is high in phytate, which represents an unavailable source of phosphorus for monogastric animals, we controlled for available phosphorus by supplementing the diets with a mineral mix (as indicated in Table 1). We also controlled for methionine and lysine ratios in the high-fat and high-carbohydrates diets by adding different amounts of DL-methionine and L-lysine to the diets as needed. Hence, nutrient combination of foods offered to choice-fed broilers define 'intake arrays' that fall on both sides of 'nutrient targets' for the starter, grower and finisher phases (see Raubenheimer & Simpson 1997). Therefore, for choice-fed treatments there was at least one combination of foods that meets broilers' requirements.

Foods were carefully ground into a mash form in order to avoid selection to be influenced by particle size (Yo *et al*. 1997). Foods also had similar color. They were offered *ad libitum* in separate feeding troughs, the position of which was daily randomized. Every day at 09.00 hours refusals were collected, weighed and new food provided. Broilers were weighed weekly during the experimental period. Broilers that died were weighed for adjustment of feed conversion efficiency.

Chemical analyses

Foods fed to broilers were sampled every time a new batch was prepared, and stored at −18°C until laboratory analyses. After defrosting, samples were pooled, homogenized and sub-samples taken to make duplicates for each determination. Sub-samples were freeze-dried to a constant weight for dry matter determination, and then ground to pass through a 1-mm screen. Ground sub-samples were analyzed for crude protein (nitrogen × 6.25; AOAC 1990), crude fiber and fat (Faithfull 2002), total starch (McCleary *et al*. 1994), and water-soluble carbohydrates (Thomas 1977). Crude protein was corrected by the apparent ileal digestibility coefficient (Huang *et al*. 2007), to estimate dietary protein effectively used by broilers (hereafter digestible protein) (Ravindran *et al*. 2005). Carbohydrate was represented by the sum of starch and water-soluble carbohydrates. Values for the rest of the nutritional properties shown in Table 1 were derived from National Research Council equations (NRC 1994).

Measurements and statistical analyses

We quantified weekly food and nutrient (digestible protein, carbohydrate, fat and apparent metabolizable energy, hereafter AME) intake, food preference (choice-fed treatments), body weight (g), weight gain (g/week), and feed conversion efficiency (g gain/g food consumed). Intake data were expressed as grams per gram of metabolic body weight (g/g $BW^{0.75}$) or as kJ/g of metabolic BW (kJ/g $BW^{0.75}$), as appropriate, to counteract differences in BW and metabolic active tissues of broilers.

Food preference, intake data and performance parameters were analyzed by the GLM procedure of SAS (SAS Institute Inc., Cary, NC, USA.), according a completely randomized design. For food preference analysis, preference for each food was the within-subject factor and pens the random factor. Model diagnostics included testing for a normal distribution of residuals and homogeneity of variance. When an effect was significant ($P < 0.05$), means were compared using the Tukey-Kramer Honestly Significant Difference test (Ruxton & Beauchamp 2008).

Geometric analysis of nutrient intake

The geometric framework (Simpson & Raubenheimer 1995) represents a tool to explore in a multi-dimensional nutrient space how animals solve the problem of satisfying multiple and changing nutrient needs when offered choices among two or more foods of different nutritional composition. Response variables used in this study were weekly intake of digestible protein, AME, carbohydrate, and fat during the starter (2–4 weeks of age), grower (5–6 weeks of age) and finisher phases (7 weeks of age). Because there were more dimensions than those possible to be graphically represented, data were integrated with a principal component analysis by using the correlation matrix between response variables (Jolliffe 2002), and then results were illustrated by biplot

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Table 1 Composition and nutrient content of the control diet offered at 2-4 weeks of age (starter diet), 5 and 6 weeks of age (grower diet), and at 7 weeks of age (finisher diet), and of the high-protein, high-carbohydrate, and high-fat foods offered free-choice to Choice C+ and Choice C– broilers over the entire experimental period (2-7 weeks of age). Choice C+ and Choice C– stand for choice-fed foods more or less concentrated in protein, carbohydrate or fat, respectively

	Control diet			High-protein food		High-fat food		High-carbohydrate food	
	Starter	Grower	Finisher	Choice C–	Choice C+	Choice C–	Choice C+	Choice C–	Choice C+
Diet or food composition (g/100 g)									
Corn	59.06	68.40	70.05	44.10	29.40	50.97	45.00	78.26	85.80
Soybean meal	30.90	21.71	19.50	47.90	62.64	9.81	3.57	8.90	3.05
Meat and bone meal	6.56	6.50	6.50	7.04	7.00	8.80	5.26	10.54	8.00
Sunflower oil	2.25	2.24	2.80	0.00	0.00	6.18	8.40	1.10	1.19
Wheat bran	0.00	0.00	0.00	0.00	0.00	22.29	34.96	0.00	0.00
Trace mineral-vitamin premix†	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Mineral mix‡	0.55	0.36	0.37	0.36	0.36	0.67	1.30	0.00	0.36
Sodium chloride	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
DL-methionine	0.18	0.19	0.18	0.10	0.10	0.36	0.41	0.20	0.45
L-lysine	0.00	0.10	0.10	0.00	0.00	0.42	0.60	0.50	0.65
Nutritional composition									
AME (MJ/kg)	12.78	13.24	13.51	11.48	10.81	13.48	13.74	13.48	13.72
Crude protein (g/100 g)	22.95	19.90	18.81	29.00	34.00	18.08	15.01	17.95	15.01
Digestible protein (g/100 g)	17.96	15.46	14.78	22.99	27.15	14.10	11.27	14.05	11.31
Fat (g/100 g)	5.86	6.00	6.58	3.45	3.20	10.27	12.02	5.54	5.52
Carbohydrate (g/100 g)	40.11	45.40	46.29	31.80	23.51	38.67	36.61	50.75	55.14
Crude fiber (g/100 g)	2.90	2.71	2.64	3.33	3.65	4.24	5.16	2.39	2.31
Calcium (g/100 g)	0.92	0.85	0.84	0.97	1.01	0.97	0.95	0.98	0.94
Available phosphorus (g/100 g)	0.46	0.43	0.42	0.49	0.51	0.60	0.48	0.53	0.47
Methionine (g/100 g)	0.53	0.50	0.47	0.54	0.87	0.62	0.62	0.60	0.67
Methionine + cystine (g/100 g)	0.90	0.82	0.77	1.01	1.14	0.90	0.90	0.91	0.91
Lysine (g/100 g)	1.17	1.05	0.97	1.60	2.08	1.18	1.16	1.17	1.17
Digestible protein/AME (g/kJ)	1.41	1.17	1.09	2.00	2.51	1.05	0.82	1.04	0.82

†Provided per kg of diet or food: antioxidant (ethoxyquin), 100 mg; biotin, 0.2 mg; calcium D-pantothenate, 12.8 mg; cholecalciferol, 60 g; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; trans-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; DL-tocopheryl acetate, 60 mg; choline chloride, 638 mg; cobalt, 0.3 mg; copper, 3 mg; iron, 25 mg; iodine, 1 mg; manganese, 125 mg; molybdenum, 0.5 mg; selenium, 200 g; zinc, 60 mg. ‡Mineral mix was composed by 33% of dicalcium phosphate and 66% of calcium carbonate. AME, apparent metabolizable energy.

graphic display (Gabriel 1971). The analysis was performed with the PRINCOMP procedure of SAS (SAS Institute Inc., Cary, NC, USA.).

RESULTS

Food preference

Food preference values are in Table 2. Control broilers showed no preference for feeding trough position over the entire experimental period. Preference of Choice C– broilers for the high-fat food was higher, lower or similar than preference for the high-carbohydrate food at 2–3 weeks of age, 4 weeks of age and 5 to 7 weeks of age, respectively, whereas their preference for the high-protein food was lower than preference for the high-fat and the high-carbohydrate foods over the whole experimental period. Preference of Choice C+ broilers for the high-fat food was higher than preference for the high-carbohydrate and the high-protein foods during the entire experimental period, whereas preference for the high-carbohydrate food was higher than preference for the high-protein food at 2-4 weeks of age only. Average preference of Choice C+ broilers was highest for the high-fat food, intermediate for the high-carbohydrate food and lowest for the high-protein food.

Total food intake and nutrient intake

Total food and nutrient intake data are in Table 3. Total food intake of Choice C+ broilers was higher than total food intake of control broilers over the entire experimental period, except at 5 weeks of age, whereas total food intake of Choice C– broilers was higher than that of control broilers at 2-4 weeks of age only. Average total food intake of Choice C– broilers was similar to total food intake of control and Choice C+ broilers.

AME intake of Choice C+ broilers was higher than AME intake of control broilers over the whole experimental period, except at 5 weeks of age. AME intake of Choice C– broilers was higher than that of control broilers at 2 to 4 weeks of age only. Average AME intake of Choice C+ and Choice C– broilers was similar, and greater than that of control broilers.

Digestible protein intake was highest for control broilers, intermediate for Choice C– broilers, and

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Table 2 Weekly and mean preference index for feeding trough position by control broilers ($n = 6$), and for each of the foods offered to choice-fed Choice C+ ($n = 6$) and Choice C– ($n = 6$) broilers. Control broilers were fed a standard balanced diet, whereas Choice C+ and Choice C– broilers were choice-fed foods more or less concentrated in protein, carbohydrate or fat, respectively. The preference index is calculated as the amount of food ingested from a particular feeding trough (control broilers) or as the amount of a particular type of food ingested (choice-fed broilers) divided by the total amount of food ingested

Treatment		Age of broilers (weeks)						Mean
		2	3	4	5	6	7	
Control	Feeding trough 1	0.31	0.35	0.31	0.31	0.33	0.37	0.33
	Feeding trough 2	0.36	0.36	0.33	0.34	0.30	0.28	0.33
	Feeding trough 3	0.33	0.29	0.36	0.35	0.37	0.35	0.34
s.e.		0.018	0.019	0.020	0.012	0.019	0.026	0.020
P		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Choice C–	High-protein food	0.13c	0.09c	0.11c	0.10b	0.08b	0.09b	0.10b
	High-carbohydrate food	0.25b	0.41b	0.51a	0.42a	0.47a	0.48a	0.42a
	High-fat food	0.62a	0.51a	0.38b	0.48a	0.45a	0.43a	0.48a
s.e.		0.054	0.046	0.043	0.043	0.045	0.042	0.030
P		***	***	***	***	***	***	***
Choice C +	High-protein food	0.20c	0.14c	0.17c	0.20b	0.24b	0.19b	0.19c
	High-carbohydrate food	0.30b	0.36b	0.37b	0.30b	0.30b	0.29b	0.31b
	High-fat food	0.50a	0.50a	0.46a	0.50a	0.48a	0.52a	0.50a
s.e.		0.033	0.042	0.034	0.037	0.028	0.041	0.040
P		***	***	***	***	***	***	***

Within treatment, means in a column with no letters (a, b, c) or with letters in common are not different ($P > 0.05$). n.s. non-significant ($P > 0.05$), *** $P < 0.001$.

lowest for Choice C+ broilers at 2 to 4 weeks of age only. At 7 weeks of age digestible protein intake of control broilers was lower than digestible protein intake of both Choice C+ and Choice C– broilers. Average digestible protein intake was highest for control broilers, intermediate for Choice C– broilers, and lowest for Choice C+ broilers.

Carbohydrate intake was similar among treatments at 2 to 4 weeks of age; thereafter, carbohydrate intake of control broilers was higher than that of Choice C+ and Choice C– broilers. Average carbohydrate intake was similar among experimental treatments.

Fat intake of Choice C+ and Choice C– broilers was higher than fat intake of control broilers over the entire experimental period. Average fat intake was highest for Choice C+ broilers, intermediate for Choice C– broilers, and lowest for control broilers.

Digestible protein/AME intake ratio of control broilers was higher than that of Choice C+ broilers at 2-5 weeks of age. Average digestible protein/AME ratio was highest for control broilers, intermediate for Choice C– broilers and lowest for Choice C+ broilers.

BW, weight gain and feed conversion efficiency

Data on body weight, weight gain, and feed conversion efficiency are in Table 4. Choice C+ broilers had lower body weight than control and Choice C– broilers over the whole experimental period. Weekly weight gains were higher for control and Choice C– broilers than for Choice C+ broilers at 2-5 weeks of age. Control and Choice C– broilers had similar weekly weight gains over the entire experimental period, except at 7 weeks of age when weight gain of Choice C– and Choice C+ broilers was higher than weight gain of control broilers.

Control broilers had greater feed conversion efficiency than Choice C+ broilers at 2-5 weeks of age, and similar to Choice C– broilers over the entire experimental period, except at 3 weeks of age when Choice C– broilers had lower feed conversion efficiency than control broilers. At 6 and 7 weeks of age feed conversion efficiency was similar among treatments.

Principal component analysis of nutrient intake

Principal component (PC) analysis of nutrient intake showed that the first PC accounted for 71%, 58%, 68% and 60% of total variance, whereas the second PC accounted for 22%, 38%, 28% and 30% of total variance, in the starter (2-4 weeks of age), grower (5-6 weeks of age) and finisher (7 weeks of age) phases, and for the cumulative data (2-7 weeks of age), respectively. For all phases, variables were properly reconstructed (more than 90% of reconstruction) with the first two PCs.

Starter phase

Treatment groups selected diets that differed mainly in fat and digestible protein content (Fig. 1). Choice C+ broilers selected a diet higher in fat and lower in digestible protein than that selected by Choice

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Table 3 Weekly and mean individual intake of total food, apparent metabolizable energy, digestible protein, carbohydrate and fat, and dietary digestible protein/apparent metabolizable energy ratio of control ($n = 6$), Choice C+ ($n = 6$) and Choice C– ($n = 6$) broilers. Control broilers were fed standard balanced diets, whereas Choice C+ and Choice C– broilers were choice-fed foods more or less concentrated in protein, carbohydrate or fat, respectively

Treatment	Age of broilers (weeks)						Mean
	2	3	4	5	6	7	
	Total food intake (g/g $LW^{0.75}$)						
Control	3.96b	3.95b	4.14b	4.36	3.86b	3.60b	3.98b
Choice C–	4.30a	4.28a	4.40a	4.07	3.92b	3.84b	4.14ab
Choice C+	4.25a	4.21a	4.47a	4.41	4.19a	4.21a	4.27a
s.e.	0.05	0.06	0.06	0.08	0.05	0.08	0.07
P	**	*	*	n.s.	**	**	**
	Apparent metabolizable energy intake (kJ/g $LW^{0.75}$)						
Control	50.61b	50.57b	53.00b	57.75	51.03b	48.69b	51.95b
Choice C–	56.60a	56.77a	58.02a	53.74	51.95ab	50.78b	54.63a
Choice C+	56.18a	56.39a	59.53a	56.47	54.93a	55.68a	56.56a
s.e.	0.83	1.16	1.21	1.64	0.92	1.08	0.98
P	**	**	**	n.s.	*	**	**
	Digestible protein intake (g/g $LW^{0.75}$)						
Control	0.71a	0.71a	0.74a	0.67	0.60	0.53b	0.66a
Choice C–	0.67b	0.65b	0.67b	0.63	0.60	0.59a	0.63b
Choice C+	0.62c	0.57c	0.63c	0.62	0.63	0.60a	0.61c
s.e.	0.01	0.01	0.01	0.02	0.01	0.01	0.01
P	***	***	***	n.s.	n.s.	**	***
	Carbohydrate intake (g/g $LW^{0.75}$)						
Control	1.59	1.59	1.66	1.96a	1.73a	1.71a	1.71
Choice C–	1.61	1.69	1.78	1.61b	1.58b	1.55b	1.64
Choice C+	1.62	1.68	1.77	1.68b	1.57b	1.60b	1.65
s.e.	0.02	0.02	0.03	0.05	0.03	0.03	0.03
P	n.s.	n.s.	n.s.	**	**	*	n.s.
	Fat intake (g/g $LW^{0.75}$)						
Control	0.22b	0.22b	0.23c	0.26b	0.23c	0.21c	0.23c
Choice C–	0.35a	0.33a	0.32b	0.31a	0.30b	0.29b	0.32b
Choice C+	0.35a	0.36a	0.37a	0.37a	0.34a	0.36a	0.36a
s.e.	0.02	0.02	0.02	0.01	0.01	0.02	0.01
P	**	***	***	***	***	***	***
	Digestible protein/apparent metabolizable energy intake ratio × 100						
Control	1.41a	1.41a	1.41a	1.17a	1.17	1.09	1.27a
Choice C–	1.18b	1.14b	1.15b	1.17a	1.15	1.16	1.15b
Choice C+	1.10c	1.01c	1.06c	1.10b	1.15	1.08	1.08c
s.e.	0.02	0.01	0.02	0.01	0.02	0.07	0.03
P	***	***	***	**	n.s.	n.s.	***

For each variable, means in a column with no letters (a, b, c) or with letters in common are not different ($P > 0.05$). n.s. non-significant ($P > 0.05$), $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. LW, live weight.

C– broilers, whereas the latter chose a diet higher in fat and lower in digestible protein than the standard balanced diet offered to control broilers. There was a negative correlation between fat intake and digestible protein intake ($r = -0.75$, $P < 0.002$), whereas AME intake was positively correlated with carbohydrate and fat intake ($r = 0.81$, $P < 0.014$ and $r = 0.93$, $P < 0.001$; respectively).

Grower phase

Treatment groups selected diets that differed mainly in fat and carbohydrate content (Fig. 1). Choice C+ broilers selected a diet with more fat than that selected by Choice C– broilers, whereas the latter selected a diet with more fat than the standard balanced diet offered to control broilers. Choice-fed broilers selected a diet with less carbohydrate than that offered to control broilers. However, dietary AME and digestible protein content was similar among treatments. There was a negative correlation between fat intake and carbohydrate intake ($r = -0.59$, $P < 0.01$).

Finisher phase

Choice-fed broilers selected a diet lower in carbohydrate but higher in fat, digestible protein, and AME than the standard balanced diet fed to control broilers (Fig. 1). Choice C+ broilers selected a diet with more fat, digestible protein and AME than that selected by Choice C– broilers. There was a positive correlation between fat intake and AME intake ($r = 0.88$,

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Table 4 Weekly body weight, weight gain and feed conversion efficiency of control ($n = 6$), Choice C+ ($n = 6$) and Choice C– ($n = 6$) broilers. Control broilers were fed standard balanced diets, whereas Choice C+ and Choice C– broilers were choice-fed foods more or less concentrated in protein, carbohydrate or fat, respectively

Treatment	Age of broilers (weeks)					
	2	3	4	5	6	7
	Body weight (g)					
Control	283a	512a	972a	1527a	2095a	2678a
Choice C–	293a	550a	964a	1475a	2033a	2675a
Choice C+	257b	448b	763b	1145b	1671b	2285b
s.e.	5.87	12.41	23.84	32.56	48.36	67.93
P	**	**	***	***	***	**
	Weight gain (g/week)					
Control	155a	230a	459a	540a	568	552b
Choice C–	171a	256a	417a	510a	558	642a
Choice C+	137b	191b	315b	382b	503	612a
s.e.	4.48	9.88	14.87	13.57	18.25	19.01
P	**	**	***	**	n.s.	*
	Feed conversion efficiency (gain/feed)					
Control	0.56a	0.53a	0.63a	0.53a	0.47	0.41
Choice C–	0.56a	0.53a	0.55b	0.50ab	0.47	0.45
Choice C+	0.50b	0.46b	0.48c	0.45b	0.46	0.44
s.e.	0.04	0.06	0.04	0.06	0.06	0.07
P	**	**	***	*	n.s.	n.s.

For each variable, means in a column with no letters (a, b, c) or with letters in common are not different ($P > 0.05$). n.s. non-significant ($P > 0.05$), *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

$P < 0.001$), and between fat intake and digestible protein intake ($r = 0.70$, $P < 0.001$).

Cumulative data

Choice-fed broilers selected a diet lower in digestible protein and carbohydrate but higher in fat and AME than that offered to control broilers (Fig. 1). Choice C+ broilers selected a diet with the highest fat and AME content and the lowest carbohydrate and digestible protein content. There was a positive correlation between fat intake and AME intake ($r = 0.90$, $P < 0.001$), and a negative correlation between fat intake and digestible protein intake ($r = -0.68$, $P < 0.021$).

DISCUSSION

The traditional paradigm of diet selection states that animals are able to combine foods with different nutrient compositions in order to reach a particular nutritional balance defined by their individual and dynamic requirements (Emmans 1991). However, nutrient composition of food options may have an important influence on nutrient balancing. Our results supported the hypothesis that free availability of high-energy foods bias ingestive behavior of choice-fed broilers toward selecting a diet with higher energy and lower protein than needed for normal growth.

During the starter phase (2-4 weeks of age), choice-fed broilers ingested more AME than control broilers fed a standard balanced diet, mainly because of enhanced preference for the energy-dense foods (high-carbohydrate and high-fat foods). Broiler preference for energy-dense foods has been previously shown, although it was not clearly related to excessive energy intake (Cerrate *et al.* 2007). Conversely, choice-fed broilers ingested less digestible protein than control broilers. Protein intake is the most influential variable in growing broiler performance (Ahmadi & Golian 2010). Failures to meet protein requirements often impair weight gain and feed conversion efficiency (Bregendahl *et al.* 2002), as it was observed for Choice C+ broilers in the present study. Contrarily, productive performance of Choice C– broilers was not affected by dietary selection. Protein intake of Choice C– broilers was intermediate between control and Choice C+ broilers, and likely closer to adequate than to deficient levels, since no differences in weight gains and feed conversion efficiency were observed between control and Choice C– broilers during this phase. Most of the research related to broilers' protein requirements has been conducted using crude protein as a proxy for truly required dietary protein, which may overestimate protein requirements (Ravindran *et al.* 2005). Parr and Summers (1991) observed that reducing crude protein content from 23% to 21% in the starter diet had no effect on BW of broilers.

At 4 and 5 weeks of age, control broilers were fed with the grower diet, which contained higher levels of AME and lower levels of digestible protein than those of the starter diet. In the grower phase, digestible protein intake of choice-fed broilers was similar to that of control broilers. However, at 5 weeks of age, Choice C+ broilers had lower weight gain and feed conversion

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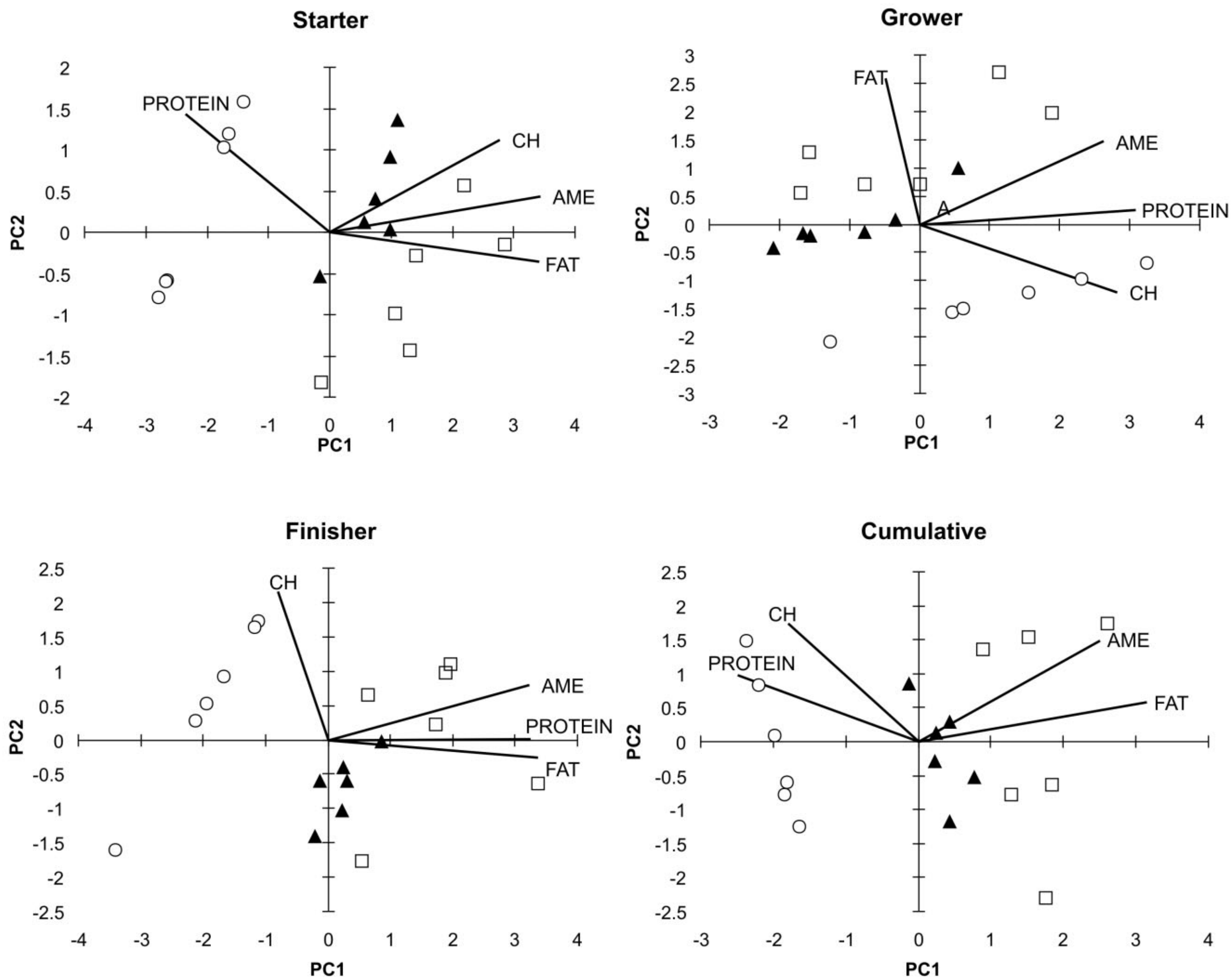

Figure 1 Biplot of the first and second principal component (PC1 and PC2, respectively) representing intake of digestible protein (PROTEIN), apparent metabolizable energy (AME), carbohydrate (CH) and fat (FAT); and individual nutrient selection of control ($n = 6$; open circles), Choice C+ ($n = 6$; open squares) and Choice C– ($n = 6$; filled triangles) broilers during the starter (2-4 weeks of age), grower (5–6 weeks of age), and finisher (7 weeks of age) phases of the traditional broiler feeding program, and for cumulative data (2-7 weeks of age). Control broilers were fed a standard balanced diet, whereas choice-fed broilers selected among foods more concentrated (Choice C+) or less concentrated (Choice C–) in protein, carbohydrate or fat.

efficiency than control and Choice C– broilers. These results can be explained by the relatively lower digestible protein/AME intake ratio of Choice C+ broilers. Protein/AME intake ratio is a main determinant of *de novo* lipogenesis in broilers (Rosebrough *et al.* 1989), and it was found to be negatively correlated with the proportion of energy stored as fat (MacLeod 1991). Increments in fat deposition are commonly related to reduced growth efficiency in broilers (Bartov *et al.* 1974; Bregendahl *et al.* 2002). Average daily weight of ostrich chicks is lower when fed a high-energy than a medium-energy diet (Cooper 2004).

During the finisher phase (7 weeks of age), choice-fed broilers ingested more digestible protein than control broilers fed the finisher diet, which contained higher levels of AME and lower levels of digestible protein than those in the grower diet. This may explain the higher weight gain and the tendency toward greater feed conversion efficiency of choice-fed broilers ($P = 0.096$). These results suggest that choice-fed broilers had a nutritional target different from that set by the commercial finisher diet. Optimal body weight and feed conversion efficiency were observed in broilers fed the starter diet (relatively high in digestible protein and low in AME) over the entire feeding period, or when they were fed a combination of starter diet at the beginning and of grower diet toward the end of the feeding period (Roush *et al.* 2004). The implementation of a dietary strategy based on optimizing economic outputs may not necessarily agree with the biological needs of broilers.

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Choice-fed broilers had a greater preference for fat over carbohydrate as an energy source over the whole experimental period, which was more accentuated in Choice C+ broilers (Tables 2 and 3). Moreover, cumulative data on nutrient intake revealed a positive correlation between fat and AME intake, and a negative correlation between fat and digestible protein intake. These results suggest that the presence of high-fat foods in the alimentary environment of broilers impairs their ability to select a nutritionally balanced diet, particularly when protein requirements are elevated (i.e. during the starter phase). It has been suggested that animals may over-ingest energy in order to satisfy protein requirements (Simpson *et al*. 2003; Simpson & Raubenheimer 2005; Morrison *et al*. 2012). However, in the present study the fact that a high-protein food was always available for choice-fed broilers excludes the possibility that broilers over-ingested energy in an attempt to meet protein requirements. Other authors have also observed that choice-fed broilers can under-ingest protein, even in the presence of high-protein foods (Yo *et al*. 1998; Dana & Ogle 2002). Since high-fat diets in the present study contained wheat bran, a source a fiber with positive impact on gut function, it can be argued that broilers may have preferred high-fat diets for the fiber component. However, fiber can improve digestion but only if levels are low (which was not the case in our study), otherwise fiber is considered as an antinutritive factor for broilers (Smits *et al*. 1998). Therefore, broilers in C– and C+ chose to eat the high-fat diet even at the cost of eating high levels of fiber.

The tendency of choice-fed broilers to over-ingest energy and under-ingest protein has also been observed in cattle (Catanese *et al*. 2009), rats (Smith *et al*. 1997) and humans (Simpson & Raubenheimer 2005), which suggests a possible evolutionary link. Neel's 'Thrifty genotype hypothesis' (Neel 1962) proposes the evolution of metabolic and behavioral adaptations to take advantages of surplus periods, in order to ensure energy reserves (i.e. fat deposits) for subsequent famines. This may explain why high-energy foods, like the high-fat food used in the present study, present as attractive and preferable over less energy-dense foods (Galef 1996). However, these adaptations can fail to work properly in environments that differ from those where they were originally shaped (Prentice & Jebb 2003). Animals exposed to artificial environments characterized by continuous availability of high-energy foods may fail to down-regulate their intake in order to prevent energy over-ingestion, as it was observed for Choice C+ broilers in the present study (see also Simpson & Raubenheimer 2005). But, as the energetic content of foods offered on choice is reduced, diet selection would eventually lead to a nutritionally balanced diet adequate to normal growth, as it was observed for Choice C– broilers. Broilers given a choice between a starter and a finisher diet (reduced range of energetic content between alternatives) showed similar performance than broilers fed the sequence of starter-grower-finisher diets (Cerrate *et al*. 2007); similarly, broilers choice-fed different combinations of isoenergetic foods with different protein concentrations were able to select a nutritionally balanced diet (Shariatmadari & Forbes 1993).

In conclusion, our results supported the hypothesis that free availability of high-energy foods bias ingestive behavior of choice-fed broilers toward selecting a diet with higher energy and lower protein than required for normal growth. This was particularly true when choice-fed broilers had available the food with the highest AME concentration (Choice C+ broilers), and for the period when protein requirement was the highest (starter phase). Energy over-ingestion and protein under-ingestion were mainly explained by enhanced preference for fat over carbohydrate as an energy source. Contrarily, in the presence of foods of lower energy concentration, choice-fed broilers selected a more balanced diet, and showed performance parameters comparable to those of broilers fed the standard balanced diet (control broilers). A way to confirm these results in a future study could be by using one single diet but formulated according the nutrient balance selected by broilers in the present study.

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