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Effect of Dietary Crude Protein, Lysine Level and Amino Acid Balance on Performance of Broilers 0 to 18 Days of Age

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Abstract: The present study was conducted to investigate the effects of dietary Crude Protein (CP), lysine and amino acid balance in male broiler diets on performance (weight gain and feed conversion, feed efficiency, protein efficiency ratio). Five test diet series were used: 1) the 23% CP diet; 2) the 21% CP diet; 3) the 21% CP diet plus additional amino acids (21.68% total CP) to provide at least as much of the essential amino acids as present in the 23% CP diet; 4) the 19% CP diet and 5) the 19% CP diet plus additional amino acids (20.31% total CP) to provide at least as much of the essential amino acids as present in the 23% CP diet. For each of the five test diets series, additional Lys was added to provide total Lys levels of 1.10, 1.15, 1.20, 1.25, 1.30, 1.35 and 1.40%. This resulted in a total of 35 final experimental treatments in a 5 x 7 factorial arrangement. Each treatment was fed to 6 replicate pens of 6 male broilers in electrically heated battery brooders from 1-18 days of age. Birds fed the low-protein diets (21%) supplemented with EAAs (21.68% total CP) showed significantly the highest BW and best FCR and FE. There were no significant differences in BW between birds fed control diet (23%) and 19% CP or 19% plus EAAs (20.31% total CP) and 21% CP. FC and PER were significantly affected by dietary protein levels. FCR and FE were significantly improved and BW increased significantly by increasing dietary lysine levels up to 1.25%. An interactions of CP with or without EAAs with dietary lysine level were significant for BW. FCR, FE, FC and PER not significantly influenced by interaction. Thus, our results suggest that maximum body weight could be obtained with a 21% low-CP plus EAA supplementation which was the same as that of the chicks fed high protein diet (23% CP). Optimum dietary lysine level for performance was affected by dietary protein level and amino acid balance.

Key words: Crude protein, lysine, amino acid balance, performance

INTRODUCTION

One of the important functions of nutritionists is to reduce the cost of feed while ensuring efficiency of utilization of supplemented low-protein diets with synthetic Amino Acids (AAs) to meet or exceed minimum amino acid standards suggested by NRC (1994). There is still controversy about this subject in the literature. Many studies have reported that young chicks fed a lowprotein diet supplemented with AAs produced equal performance to chicks fed positive control diets with 23% CP (Bornstein and Lipstein, 1975; Schutte, 1987; Parr and Summers, 1991; Han et al., 1992; Moran et al., 1992; Deschepper and de Groote, 1995). In addition, Ciftci and Ceylan (2004) reported that chicks fed a basal diet containing high CP (21.30%) showed poorer performance than those fed a low CP (19.13 and 17.97%) plus AAs during starter period. It is commonly agreed that greater performance in chicks can be achieved if the Essential Amino Acids (EAA) in low CP diets were equivalent to those needed in the higher CP diets and when a balance of AAs is maintained (Pinchasov et al., 1990; Ciftic and Ceylan, 2004). On the other hand, other studies (Parsons and Baker, 1982; Bregendahl et al., 2002; Si et al., 2004; Jiang et al., 2005; Waldroup et al., 2005) have reported impaired weight gain and feed efficiency when broilers were fed low protein amino acid-supplemented diets. The reasons for the impaired performance obtained from the above studies with regards to low protein supplemented amino acid diets remains unknown. Dietary lysine is usually the second limiting Amino Acid (AA) behind methionine for poultry fed corn-soybean meal diets and particularly important in improving performance, especially with regard to breast meat yield (Moran and Bilgili, 1990; Kidd et al., 1998; Kerr et al., 1999; Barboza et al., 2000; Labadan et al., 2001: Urdaneta-Rincon and Leeson 2004; Café and Waldroup, 2006). Moreover, Lys was used in the development of ideal amino acid ratios to express all other essential AA as a percentage of Lys, under the ideal protein concept (Baker and Han, 1994; Emmert and Baker, 1997; Baker et al., 2002). The relationship between dietary protein level and lysine requirements in commercial broiler diets were noted by Almquist (1952) who stated that the level of an essential amino acid required for optimum chick performance was a positive linear function of the dietary CP level. The amino acid requirements (% of CP) for maximum growth probably decrease with increasing dietary CP (Abebe and Morris, 1990a,b; Morris et al., 1999). However, Hurwitz et al. (1998) found an increase in weight gain

with increasing lysine supplementation on the 23% protein diet. More recently, Sterling (2002) and Sterling *et al.* (2003) concluded that the amino acid requirements of broilers are a constant proportion of CP levels at least in the range of CP levels commonly fed. Thus, the objective of our study was to test the effect of dietary protein, lysine and amino acid level on performance of broiler chicks fed low-protein diets to meet the minimum essential amino acids requirements according to NRC (1994) or plus additional essential amino acids identical as that of the chicks fed a high protein diet (23% CP).

MATERIALS AND METHODS

Three basal diets were formulated using ground yellow corn, dehulled solvent extracted soybean meal and corn gluten meal as the intact sources of protein. Diets were formulated to contain 19, 21, or 23% crude protein and to meet or exceed minimum amino acid standards suggested by NRC (1994) with the stipulation that Lys was fixed at 1.10% of the diet. A dietary electrolyte balance ((Na+K)-Cl) of 250 meg/kg was specified for all diets and was obtained by varying levels of feed grade sodium chloride and sodium bicarbonate. The crude protein and moisture content of the primary ingredients were determined prior to formulating the diets, with amino acid content and digestibility coefficients suggested by a major amino acid producer (Ajinomoto Heartland, Chicago IL) used in the formulation. Diets were supplemented with complete vitamin and trace mineral mixes. Composition and calculated nutrient content of the basal diets is shown in Table 1. Modifications were made to the basal diets to arrive at a total of five test diet series. These included 1) the 23% CP diet; 2) the 21% CP diet; 3) the 21% CP diet plus additional amino acids to provide at least as much of the essential amino acids as present in the 23% CP diet (21-PLUS); 4) the 19% CP diet and 5) the 19% CP diet plus additional amino acids to provide at least as much of the essential amino acids as present in the 23% CP diet (19-PLUS). A large batch of each of these basal diets sufficient for mixing the final test diets were made and aliquots used for further supplementation with lysine. For each of the five test diets series, additional Lys was added in the form of L-Lysine HCl to provide total Lys levels of 1.10, 1.15, 1.20, 1.25, 1.30, 1.35 and 1.40%. This resulted in a total of 35 final experimental treatments in a 5 x 7 factorial arrangement.

Male chicks of a commercial broiler strain (Cobb 500; Cobb-Vantress, Siloam Springs AR) were obtained from a local hatchery where they had been vaccinated in ovo for Marek's disease and had received vaccinations for Newcastle Disease and Infectious Bronchitis post hatch via a coarse spray. Six chicks were placed in each of 210 compartments in electrically heated battery brooders with raised wire floors. Continuous 24 h fluorescent lighting was used. Test diets fed in mash form and tap

Table 1: Composition (g/kg) and calculated nutrient analysis of basal diets

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Ingredients	19% CP	21% CP	23% CP
Yellow corn	633.18	601.99	578.48
Soybean meal 47.5%	294.97	292.59	274.85
Com gluten meal 60%	0.00	39.10	87.41
Poultry oil	22.36	18.43	11.68
Defluorinated phosphate	18.37	18.16	18.00
Ground limestone	7.94	8.47	8.91
Vitamin premix ¹	5.00	5.00	5.00
Feed grade salt	2.36	2.54	2.24
MHA 84	2.24	1.13	0.39
Sodium bicarbonate	1.83	1.59	2.04
Mintrex P_Se ²	1.00	1.00	1.00
L-Lysine HCI	0.43	0.00	0.00
L-Threonine	0.32	0.00	0.00
Variable ³	10.00	10.00	10.00
Total	1000.00	1000.00	1000.00
Crude protein %	19.00	21.00	23.00
DEB meq/kg	250.00	250.00	250.00
ME kcal/kg	3085.00	3085.00	3085.00
Met	0.54	0.50	0.50
Lys	1.10	1.10	1.10
Trp	0.24	0.25	0.25
Thr	0.80	0.84	0.90
lle	0.82	0.90	0.97
His	0.54	0.58	0.61
Val	0.92	1.01	1.09
Leu	1.77	2.12	2.51
Arg	1.31	1.38	1.41
TSAA	0.90	0.90	0.94
Gly+Ser	1.78	1.74	1.64
Phe+Tyr	1.73	1.85	1.95
Sodium	0.25	0.25	0.25
Dig Met	0.52	0.47	0.47
Dig Lys	0.98	0.98	0.98
Dig Trp	0.20	0.22	0.22
Dig Thr	0.70	0.73	0.79
Dig Arg	1.21	1.28	1.32
Dig TSAA	0.82	0.82	0.84

Provides per kg of diet: vitamin A 7715 IU; cholecalciferol 5511 IU; vitamin E 16.53 IU; vitamin B12 0.013 mg; riboflavin 6.6 mg; niacin 39 mg; pantothenic acid 10 mg; menadione 1.5 mg; folic acid 0.9 mg; choline 1000 mg; thiamin 1.54 mg; pyridoxine 2.76 mg; d-biotin 0.066 mg; ethoxyquin 125 mg.

²Provides per kg of diet: Mn (as manganese methionine hydroxy analogue complex) 40 mg; Zn (as zinc methionine hydroxy analogue complex) 40 mg; Cu (as copper methionine hydroxy analogue complex) 20 mg; Se (as selenium yeast) 0.3 mg.

³Variable levels of supplemental amino acids

water were provided for ad libitum consumption. Six replicate pens, stratified across tiers of the battery, were assigned to each of the 35 dietary treatments. Mean body weights by pen were obtained at 1 and 18 d of age. Feed consumption during the test period was determined. Birds were checked twice daily for mortality; any bird that died was weighed to adjust feed conversion. Data were subjected to analysis of variance as a complete factorial arrangement with protein level, with and without amino acids supplementation and lysine level as main effects and two-way interactions.

Analysis used the general linear models procedure of SAS (SAS Institute, 1991). Mortality data were transformed to square root of n+1 prior to analysis; data are presented as natural numbers. All statements of probability are based on P<0.05.

RESULTS AND DISCUSSION

The main effects of dietary protein level and total lysine level are shown in Table 2. Birds fed the 21% CP diet supplemented with the EAAs (21.68% total CP) at levels equal to those in the 23% CP diet showed significantly the highest BW and best FCR and FE compared to the other treatment groups. Moreover, there were no significant differences in BW between birds fed control diet (23%) and 19% CP or 19% plus EAAs (20.31% total CP) and 21% CP. The FC and PER were significantly affected by dietary protein levels. Birds fed the high CP basal diet (23%) had lower FC and better PER compared to those fed on the low CP diet with or without EAAs supplemented, Table 2. These results are in agreement with those found by Waldroup et al. (2005) who reported that maximum growth rate of young chickens could be obtained with a 19% CP diet supplemented with crystalline EAA (21.4% total CP) that was equivalent to minimum requirements. In addition this diet improved protein utilization efficiency compared with a standard 23% protein diet.

Several workers have confirmed the same results (Bornstein and Lipstein, 1975; Summers and Leesson, 1985; Schutte, 1987; Stillborn and Waldroup, 1989; Parr and Summers, 1991; Han *et al.*, 1992; Moran *et al.*, 1992; and Descherpper and de Groote, 1995). Recently, Ciftci and Ceylan (2004) reported that a basal diet containing high CP (21.30%) resulted in poorer performance than a low CP (19.13 and 17.97%) during starter period when

the low-CP basal diets were supplemented with synthetic AAs to match AA content of the control diet.

The supplemental crystalline AA of the low CP basal diet (21%) equivalent to those in the 23% CP diet might have resulted in better overall AA availability and better performance because AA absorbed in free form are metabolized in the enterocytes to a larger extent than AA absorbed in peptide form (Bregendahl et al., 2002). Moreover, the crystalline AA were stated to be more bioavailable than intact protein-bound AA (Izquierdo et al., 1988; Han et al., 1990; Chung and Baker, 1991; Ciftci and Ceylan, 2004). Therefore, Izquierdo et al. (1988) and Chung and Baker (1992) reported that crystalline AAs are hypothesized to be 100% true and absorbed 100% into the enterocytes. These facts were confirmed by Rérat (1985) and Rérat et al. (1992), which showed differences between the appearance of AA in the portal blood after meals of free AA and intact protein or peptides.

Furthermore, Noy and Sklan (1995) and Tarvid (1995) suggested that the relatively low amino acid digestibility at 14 d for most feed ingredients may imply that the development of crude protein and amino acid digestion is incomplete at 14 d. This fact was confirmed by Nitsan et al. (1991) who demonstrated that trypsin and chymotrypsin activities (unit/kg body weight) in pancreas increased to maximum at d 11 post-hatching and was reduced at 23 d. Moreover, Noy and Sklan (2002) reported that the efficiency of protein retention decreased as dietary protein increased from 18, 23 and 28% CP during the first week posthatch.

The present results showed that the chicks fed 23% CP had lower feed intake compared to those fed a low-CP diet with or without supplemental AAs. The results were also previously confirmed by several studies (Parsons

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Parameters	•	Awt18	FCR	FE	FC	PER	Mort %
Protein	Lysine						
19	-	0.664 ^{bc}	1.459°	0.688₺	0.898ª	3.626 ^a	2.778
19+AA		0.673 ^b	1.435 ^{ab}	0.700ab	0.900°	3.435 ^b	2.778
21		0.671⁵	1.425₺	0.705°	0.884 ^{ab}	3.368⁵	3.969
21+AA		0.688°	1.408 ^b	0.714°	0.896°	3.305 ^c	3.373
23		0.659°	1.436ab	0. 701 ^{ab}	0.875⁵	3.048 ^d	4.365
SEM		0.0043	0.0011	0.0053	0.0.502	0.0243	0.8423
P-∨alue		<0.0001	0.0279	0.0172	0.0069	<0.0001	0.5852
	1.10	0.629 ^d	1.476°	0.680≎	0.857₺	3.257 [℃]	3.334
	1.15	0.658°	1.464 ^{ab}	0.687bc	0.892ª	3.289bc	4.444
	1.20	0.678ab	1.413⁵	0.710°	0.892ª	3.393°	2.500
	1.25	0.691°	1.418 ^ε	0.709°	0.912ª	3.390°	4.444
	1.30	0.686ab	1.407₺	0.715°	0.897ª	3.411ª	3.056
	1.35	0.681ab	1.420⁵	0.708°	0.893°	3.401°	3.334
1.40	1.40	0.673⁵	1.432 ^{bc}	0.702ab	0.893°	3.355 ^{ab}	3.056
SEM		0.0551	0.0131	0.0062	0.0082	0.0287	0.0165
P-∨alue		<0.0001	0.0005	0.0003	0.0005	0.0002	0.7777

abMeans with different superscripts in the same column are significantly different (P<0.05).

¹Awt18 = Average weight at 18 day; FCR = Feed Conversion Ratio; FE = Feed Efficiency; FC = Feed Consumption; PER = Protein Efficiency Ratio; Mort = Mortality

and Baker, 1982; Nakhata and Anderson, 1982; Pesti and Fletcher, 1984; Fancher and Jensen, 1989b; Aletor et al., 2000; Sklan and Plavnik, 2002). These studies reported decreased feed intake with increased CP. In addition, Fancher and Jensen (1989a) suggested that specific amino acids rather than CP per se are instrumental in depressing the feed intake. Moreover, the increased feed intake was often observed in literature in chickens fed low-CP amino acid supplemented diets (Aletor et al., 2000; Rosebrough and McMurtry, 1993; Fancher and Jensen, 1989a,b; Rosebrough and Steele, 1985). These results were explained by Aletor et al. (2000) who demonstrated that wider energy/protein ratios of low-CP diets might increase consumption of dietary energy, as broilers eat to meet dietary protein amino acid requirements. Moreover, they reported improved energy and protein efficiency in low-CP broiler diets compared to the control diet.

In contrast, other studies (Parsons and Baker, 1982; Bregendahl *et al.*, 2002; Si *et al.*, 2004; Jiang *et al.*, 2005; Waldroup *et al.*, 2005) reported impaired weight gain and feed efficiency when broilers were fed low protein amino acid-supplemented diets. This was explained by Aftab *et al.* (2006) who suggested that supplemented free amino acids to low-CP might cause a sudden influx of AAs that increase the catabolism of AAs from muscles or those absorbed from diet in order to maintain homeostasis of plasma AAs profile. Further, they reasoned impairment of performance of chicks fed low-CP diets with supplemented AAs might be consequent to increased catabolism of AAs.

BW increased significantly as dietary lysine levels increased (Table 2). Moreover, the highest BW was obtained for chickens fed diets containing 1.25 % total dietary lysine. In the same trend, increased lysine supplementation in basal diet showed the best significant (p<0.01) values of FCR and FE. However, 1.1% and lysine level in chickens diets showed the lower and best values FC and PER, respectively compared to other dietary lysine levels. Our results showed that the optimum dietary lysine levels are in the range of 1.2-1.35% for BW, FCR and FE for broilers up to 18 day of age and indicated that the optimum of dietary lysine level is higher than 1.10% as suggested by NRC (1994) during starter period 0-3 week of age.

These results are similar to those obtained by Labadan et al. (2001) who reported that lysine requirements for maximum weight gain, breast muscle weight and feed efficiency were 1.28, 1.32 and 1.21% lysine, respectively for 0-2 week old broilers. Dietary lysine levels of 1.17% for maximal weight gain and 1.41% for maximal feed efficiency were estimated for broiler chicks fed a diet containing 23% CP to 3 week of age (Han and Baker, 1991). Moreover, Holsheimer and Ruesink (1993) demonstrated that higher breast muscle yields at 49 day

of age were obtained with dietary lysine levels higher than 1.15% in broiler diets 0 to 14 day of age irrespective of dietary lysine level in the range of 1.10-1.30% from 15-49 day of age. Kidd et al. (1997) reported that increasing total lysine from 1.1-1.2% improved daily weight gain and feed efficiency of broiler chicks 1-18 day of age. In addition, several studies suggested that dietary lysine requirements of broilers are higher than indicated by NRC (1994) during the starter period for maximum weight gain, breast muscle weight and feed efficiency (Morris et al., 1987; Morris and Abebe, 1990; Surisdiarto and Farrell, 1991).

The interaction of CP levels and lysine level are shown in Table 3. An interaction of CP with or without EAAs with dietary lysine level were significant for BW. Increasing lysine from 1.1-1.40% in diets increased BW for chickens fed the low-protein 21% CP plus EAAs at levels to equal those in the 23% CP diet. Moreover, chicks given 1.25% lysine on the low CP 21% plus EAAs diet had highest BW compared to other treatment groups. The BW increased with up to 1.25% lysine on the low CP 19% or 19% plus EAAs diets. The BW increased up to 1.35 and 1.40% lysine for chicks fed on high 23% CP diet. However, chicks given 1.1 and 1.15% lysine on the high CP 23% diet had lower BW than other treatment groups. The FCR, FE, FC and PER showed no significant interactions between CP levels and lysine levels. Mortality rate was not significant among chicks receiving all experimental diets.

Results of the present study indicate that optimum lysine level was not a constant proportion of CP levels with or without AAs supplementation. These results were confirmed by Abebe and Morris, 1990a; Surisdiarto and Farrell, 1991; Morris et al., 1992; Morris et al., 1999, who reported that amino acid requirements (% of CP) for maximum growth decrease with increasing dietary CP level. Moreover, Hurwitz et al. (1998) found increases in weight gain with increasing lysine supplementation on the 23% protein diet. In addition, Sterling et al. (2006) reported significant interaction between dietary protein level and lysine for body weight and feed efficiency during the starter period, with Cobb broilers showing the best performance at the highest lysine level at 23% CP. The results of this study showed the best significant BW value related to increased lysine level up to 1.25% for chicks fed low-CP (21% and 19%) plus AAs supplementation and (21% and 19% CP) without added AAs. These results might be a consequence of improvement in bioavailability of free EAAs balance that resulted in decreased optimum lysine level for broiler growth compared to the intact protein in the control diet. This fact was confirmed by Hurwitz et al. (1998) who suggested that adding single amino acids to low CP diet was more effective in improving amino acid balance than adding high protein diets, resulting in a decrease in the requirements of lysine and arginine.

Table 3: Growth performance of broiler chicks as affected by interaction between dietary crude Protein, lysine and amino acid balance at 18 d of age¹

Parameters		Awt18	FCR	FE	FC	PER	Mort %
Protein %	Lysine %						
19	1.10	0.655 ^{efghi}	1.473	0.680	0.896	3.583	1.389
	1.15	0.665 ^{cdefgh}	1.441	0.696	0.888	3.665	2.778
	1.20	0.676 ^{bcdefgh}	1.444	0.695	0.914	3.634	4.167
	1.25	0.680 ^{abcdefgh}	1.466	0.685	0.919	3.631	2.778
	1.30	0.676 bodefgh	1.466	0.685	0.922	3.607	2.778
	1.35	0.651 ^{fghi}	1.438	0.670	0.866	3.682	2.778
	1.40	0.644 ^{hijk}	1.483	0.678	0.879	3.578	2.778
19+AA	1.10	0.647 ^{ghij}	1.446	0.693	0.871	3.396	0.000
	1.15	0.681 ^{abcdefgh}	1.444	0.694	0.915	3.420	2.778
	1.20	0.662 ^{cdefghi}	1.414	0.709	0.869	3.493	1.389
	1.25	0.693 ^{abcde}	1.422	0.708	0925	3.467	6.944
	1.30	0.679 ^{bcdefgh}	1.444	0.696	0.915	3.411	4.167
	1.35	0.689 ^{abcdef}	1.436	0.701	0.919	3.452	2.778
	1.40	0.660 ^{c defghi}	1.440	0.698	0.889	3.405	1.389
21	1.10	0.627 ^{ijkl}	1.475	0.681	0.854	3.244	5.556
	1.15	0.657 ^{efghi}	1.447	0.694	0.879	3.306	2.778
	1.20	0.685 ^{abcdefg}	1.395	0.719	0.888	3.433	2.778
	1.25	0.688abcdef	1.426	0.703	0.912	3.350	4.167
	1.30	0.683 ^{abcdefgh}	1.392	0.721	0.880	3.445	2.778
	1.35	0.689 ^{abcdef}	1.412	0.712	0.896	3.415	5.556
	1.40	0.671 ^{bcdefgh}	1.427	0.705	0.882	3.382	4.167
21+AA	1.10	0.615 ^{jkl}	1.483	0.678	0.835	3.156	2.778
	1.15	0.678 ^{bcdefgh}	1.431	0.701	0.903	3.232	6.944
	1.20	0.695 ^{abcde}	1.386	0.723	0.893	3.351	1.389
	1.25	0.718°	1.391	0.723	0.933	3.333	4.167
	1.30	0.702 ^{abc}	1.364	0.736	0.890	3.393	1.389
	1.35	0.699 ^{abcd}	1.407	0.715	0.899	3.349	2.778
	1.40	0.709ab	1.395	0.721	0.922	3.325	4.167
23	1.10	0.598 ⁱ	1.595	0.668	0.826	2.905	6.944
	1.15	0.611 ^{kl}	1.503	0.649	0.877	2.823	6.944
	1.20	0.675 ^{bcdefgh}	1.427	0.703	0.894	3.055	2.778
	1.25	0.677 bcdefgh	1.383	0.728	0.870	3.167	4.167
	1.30	0.691 ^{abcde}	1.366	0.735	0.879	3.197	4.167
	1.35	0.678 bodefgh	1.405	0.715	0.885	3.109	2.778
	1.40	0.680 ^{abcdefgh}	1.416	0.709	0.894	3.081	2.778
SEM		0.0114	0.0292	0.0139	0.0183	0.0642	2.2284
P-value		0.0002	0.3257	0.3965	0.3590	0.5548	0.9334

^{a,b}Means with different superscripts in the same column are significantly different (P<0.05).

Thus, our results suggest that maximum body weight could be obtained with a 21% low-CP plus EAAs supplementation to the same identical level as that of the chicks fed high protein diet (23% CP). Chickens fed a low-CP diet (19 or 21%) which met the minimum essential amino acids requirements according to NRC (1994) but without additional EAA had similar growth rate as did chicks fed the 23% CP positive control diet. Optimum dietary lysine level for performance was affected by dietary protein level and amino acid balance. Optimum dietary lysine level for broilers are higher than suggested by NRC (1994) during the starter period for maximum weight gain.

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¹Awt18 = average weight at 18 day; FCR = feed conversion ratio; FE = feed efficiency; FC = feed consumption; PER = protein efficiency ratio; Mort = mortality

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