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(Short Communication)

Effect of Protein and Energy-reduced or Protein-reduced Diet on Mortality and Performance of Broiler Chickens Reared at a High-altitude Area

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Abstract

This study investigated the effect of reducing dietary metabolizable energy (ME) and crude protein (CP and amino acids) or decreasing only dietary CP [and amino acids, except first limiting amino acids (i.e. lysine, methionine+cystine, and threonine)] on mortality and performance of broilers raised in a farm 1,700 m above sea level. Two hundred and fifty-two Ross 308 male broiler chicks were distributed into 3 treatments with 6 replicates per each from 1 to 38 d of age. Dietary treatments were: control) a diet met or exceeded nutritional recommendations; DMC) a diet with the dilution of ME and CP (and amino acids), and DC) a diet with the dilution of CP (and amino acids, except lysine, methionine+cystine, and threonine). Mortality was higher in control compared to that of other treatments from 25 to 38 and 1 to 38 d of age. There was a higher relative weight of the right ventricle in control compared to that in DMC, and DC was being intermediate. During 1 to 38 d of age, control and DC improved average daily gain and adjusted feed conversion ratio compared to those of DMC. But in respect of the unadjusted feed conversion ratio and European poultry efficiency factor, DC improved these parameters compared to those of other treatments. In conclusion, decreasing CP content without any reductions in ME, lysine, methionine+cystine, and threonine concentration reduces mortality and enhances European poultry efficiency factor without any negative effects on feed efficiency or weight gain in broilers reared at a high-altitude area.

Introduction

Modern broiler chickens have more potential to show pulmonary hypertension syndrome (PHS) especially when they are reared in hypoxic condtions. Given that there are some farms with high-altitude conditions in some countries, it is essential to find nutritional and non-nutritional approaches to counteract the development of PHS (Leeson *et al.,* 1996; Khajali *et al.,* 2014). Appropriate nutritional strategies are needed to reduce metabolic activity, oxygen consumption, and PHS development when modern broiler chickens are reared at high altitudes that limit the concentration of atmospheric oxygen (Leeson *et al.,* 1996).

One of the nutritional strategies in such a condition is feeding of reduced-protein diets to broilers. It is hypothesized that the catabolism of dietary protein enhances oxygen consumption and using diets with high CP content may increase mortality due to PHS incidence (Leeson *et al.,* 1996). It is assumed that catabolism of 1 g of protein makes chicken consume approximately 1 L of oxygen (Leeson *et al.,* 1996). However, there are conflicting results regarding the literature. Because Behrooj *et al.* (2012), Buys *et al.* (1998a,b), and Maxwell and Robertson (1998) reported that the mortality rate was increased in broilers fed the reduced-protein diets.

Another nutritional approach for reducing the mortality rate of broilers reared at a high-altitude area is feeding low-energy diets. It is claimed that decreasing dietary energy concentration reduces metabolic rate and subsequently oxygen demand and PHS incidence (Julian *et al.,* 1989).

Concerning the above explanations, we hypothesized that decreasing the dietary nitrogen or energy and nitrogen content from those recommended for current broiler strain may be effective to reduce the mortality of broiler chickens reared in a mild

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Table 1. Ingredient and nutrient composition of dietary treatments[1] (g/kg, as-fed basis, unless otherwise indicated)

	1 to 10 d of age			11 to 24 d of age			25 to 38 d of age		
	Control	DMC	DC	Control	DMC	DC	Control	DMC	DC
Ingredient									
Corn	465.3	570.2	520.6	524.0	623.4	574.3	574.9	665.0	615.5
Soybean meal (44%)	441.4	384.3	392.4	381.8	330.3	337.1	328.2	285.8	292.4
Soybean oil	48.4	0.2	39.2	52.6	4.3	44.2	58.3	10.5	51.5
Dicalcium phosphate	18.0	18.4	18.4	16.1	16.4	16.5	14.5	14.7	14.8
Calcium carbonate	10.2	10.3	10.2	9.3	9.5	9.4	8.5	8.7	8.6
Common Salt	2.6	2.4	2.5	2.6	2.5	2.6	2.6	2.6	2.6
Sodium bicarbonate	3.1	3.3	3.2	3.1	3.2	3.1	3.0	3.1	3.0
DL-Methionine	3.5	3.1	4.0	3.2	2.8	3.6	2.9	2.5	3.1
L-Lysine HCl	1.7	2.0	3.1	1.6	1.9	2.9	1.6	1.7	2.6
L-Threonine	0.8	0.8	1.4	0.7	0.7	1.3	0.5	0.4	0.9
Vitamin premix[2]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Mineral premix[3]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Nutrient composition									
ME (Kcal/kg)	3000	2790	3000	3100	2883	3100	3200	2976	3200
Crude protein	238	222	223	216	202	203	197	186	186
Lysine	14.4	13.4	14.4	12.9	12.0	12.9	11.6	10.8	11.5
Methionine + Cystine	10.8	10.0	10.8	9.9	9.2	9.9	9.1	8.5	9.0
Threonine	9.7	9.0	9.7	8.8	8.2	8.8	7.8	7.2	7.7
Valine	11.0	10.2	10.2	10.0	9.3	9.3	9.1	8.6	8.5
Isoleucine	10.0	9.2	9.2	9.0	8.3	8.3	8.1	7.5	7.5
Calcium	9.6	9.6	9.6	8.7	8.7	8.7	7.8	7.9	7.9
Non-phytate phosphorus	4.8	4.8	4.8	4.3	4.3	4.3	3.95	3.95	3.95
Sodium	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0

[1]Dietary treatments were: control) a diet met or exceeded nutritional recommendations; DMC) a diet with dilution of metabolizable energy and crude protein (and amino acids); and DC) a diet with dilution of crude protein (and amino acids, except lysine, methionine+cystine, and threonine).

[2]Provided the following per kilogram of diet: vitamin A, 9,000 IU (retinyl acetate); cholecalciferol, 2,000 IU; vitamin E, 36 IU (dl-α-tocopheryl acetate); vitamin B_{12}, 0.015 mg; menadione, 2 mg; riboflavin, 6.6 mg; thiamine, 1.8 mg; pantothenic calcium, 10 mg; niacin, 30 mg; folic acid, 1 mg; biotin, 0.1 mg; pyridoxine, 3 mg.

[3]Provided the following per kilogram of diet: manganese ($MnSO_4 \cdot H_2O$), 100 mg; zinc (ZnO), 85 mg; iron ($FeSO_4 \cdot 7H_2O$), 20 mg; copper ($CuSO_4 \cdot 5H_2O$), 10 mg; selenium (Na_2SeO_3), 0.2 mg; iodine ($Ca(IO_3)_2$), 1 mg; choline (choline chloride), 250 mg.

hypoxic condition. The present study aimed to investigate the reduction of CP (and amino acids) and ME simultaneously in one scenario or just CP (and amino acids, except lysine, methionine+cystine, and threonine) in another scenario to reduce mortality of broiler chickens raised in a farm 1,700 m above sea level. Therefore, growth performance, mortality, European poultry efficiency factor (EPEF), relative weight (RW) of carcass, and right ventricle (RV) were measured in broiler chickens reared in 1,700 m above sea level. The RW of RV was determined as an index of PHS development in different treatment's birds.

Materials and Methods

Two hundred and fifty-two one-day-old male Ross 308 broiler chicks were divided into 18 groups. This research had 3 dietary treatments with 6 replicates of 14 broilers per each. The pen was the experimental unit and assigned to each replicate. Chicks were reared in an environmentally controlled house on the litter-floor pen. The lightning program was a 22:2 L:D cycle. The temperature of the rearing house was 31°C during 1 to 3 d of age, it was reduced until reaching 21°C at 24 d of age. This temperature was kept from 25 to 38 d of age. The chicks had ad libitum access to water and feed. The rearing farm was 1,700 m above sea level.

Corn-soybean meal basal diets were used from 1 to 10, 11 to 24, and 25 to 38 d of age (Table 1). Three dietary treatments were: control) a diet met or exceeded nutritional recommendations of Aviagen (2019); DMC) a diet with the dilution of ME and CP (and amino acids); and DC) a diet with the dilution of CP [and amino acids, except first limiting amino acids (i.e. lysine, methionine+cystine, and threonine)]. The ME content was diluted by 7% in the DMC treatment. In both DMC and DC treatments, because of some limitations in ration formulation, the dilution of relevant amino acids content was around 5-7%, and the CP was diluted by 6.7, 6.5, and 5.6%, respectively, for 1 to 10, 11 to 24 and 25 to 38 d of age.

The mortality rate was recorded from 1 to 24, 25 to 38, and 1 to 38 d of age. Average daily feed intake (ADFI) and average daily gain (ADG) were determined from 1 to 24, 25 to 38, and 1 to 38 d of age. Feed intake was adjusted for mortality, and their weight gain was included in the calculation of the adjusted feed conversion ratio (FCR). Also, without adjusting for ADFI of mortalities, the unadjusted FCR was calculated. The mortality rate was determined in all phases. The EPEF of 24 and 38 d of age was calculated as follows: [(livability in percentage × live body weight in kg)/(age in day × FCR)] × 100.

At 38 d of age, 2 chicks per pen were randomly selected, weighed individually, and killed to measure the RW (g/100 g body weight) of carcass, breast, thigh+drumstick, liver, pancreas, spleen, and RV.

The pen was the experimental unit for all parameters. The study was conducted as a completely randomized design with 3 treatments (diets) and analyzed using the GLM procedure of SAS (SAS, 2001). Transformation of mortality data to their arcsine square root did not show any further significant effects; therefore, it was not used. Differences among treatments were considered significant at $P \leq 0.05$. Significant differences between means (three diets) were separated by Fisher's Least Significant Difference.

Results

The effect of dietary treatments on the growth performance of broiler chickens is presented in Table 2. The ADFI was not affected ($P > 0.05$) by the treatments. Control and DC improved (at least, $P \leq 0.05$) ADG and adjusted FCR compared to those of DMC. Unadjusted FCR was not affected ($P > 0.05$) by the treatments from 25 to 38 d of age. However, control and DC had the lower ($P \leq 0.001$) unadjusted FCR compared to that of DMC from 1 to 24 d of age. During the whole period, DC improved ($P \leq 0.05$) the unadjusted FCR compared to that of other treatments.

Table 2. Effect of dietary treatments on growth performance of broiler chickens

	ADFI[1] (g)			ADG[2] (g)			Adjusted FCR[3]			Unadjusted FCR		
	1 to 24 d	25 to 38 d	1 to 38 d	1 to 24 d	25 to 38 d	1 to 38 d	1 to 24 d	25 to 38 d	1 to 38 d	1 to 24 d	25 to 38 d	1 to 38 d
Treatments[4]												
Control	52.0	160	91.8	40.9^{a}	85.1^{a}	57.2^{a}	1.27^{b}	1.88^{b}	1.60^{b}	1.31^{b}	2.25	1.79^{a}
DMC	53.8	162	93.7	36.6^{b}	81.4^{b}	53.1^{b}	1.47^{a}	1.99^{a}	1.76^{a}	1.48^{a}	2.08	1.81^{a}
DC	53.4	161	93.1	42.9^{a}	84.8^{a}	58.3^{a}	1.24^{b}	1.90^{b}	1.60^{b}	1.26^{b}	2.10	1.69^{b}
SEM[5] (n=6)	0.84	2.8	0.99	0.65	1.03	0.46	0.019	0.028	0.017	0.023	0.055	0.029
P-value	0.31	0.87	0.39	<0.0001	0.03	<0.0001	<0.0001	0.03	<0.0001	<0.0001	0.10	0.03

[a,b]Within a column, means without a common superscript differ ($P \leq 0.05$).
[1]ADFI = average daily feed intake.
[2]ADG = average daily gain.
[3]FCR = feed conversion ratio.
[4]Dietary treatments were: control) a diet met or exceeded nutritional recommendations; DMC) a diet with the dilution of metabolizable energy and crude protein (and amino acids), and DC) a diet with the dilution of crude protein (and amino acids, except lysine, methionine+cystine, and threonine).
[5]*SEM* = standard error of the mean.

The effect of dietary treatments on mortality rate and EPEF is presented in Table 3. During 1 to 24 d of age, the mortality rate was not affected ($P > 0.05$) by the treatments. From 25 to 38 d of age ($P \leq 0.001$) and 1 to 38 d of age ($P \leq 0.01$), control showed an increased mortality rate than that of other treatments. On d 24, EPEF was greater ($P \leq 0.001$) in DC than that in control, and on the other hand, control showed a greater EPEF than that of DMC. On d 38, DC showed the increased ($P \leq 0.01$) EPEF compared to that of other treatments.

The effect of dietary treatments on the RW of carcass, organs, and RV in broiler chickens is presented in Table 4. The RW of carcass, thigh+drumstick, liver, pancreas, and spleen was not affected ($P > 0.05$) by the treatments. However, control and DC had a greater ($P \leq 0.05$) RW of the breast than that of DMC. There was the greater ($P \leq 0.05$) RW of RV in control than that in DMC, and DC was being intermediate.

Table 3. Effect of dietary treatments on mortality rate (%) and European poultry efficiency factor (EPEF[1]) of broiler chickens

	Mortality			EPEF	
	1 to 24 d	25 to 38 d	1 to 38 d	24 d	38 d
Treatments[2]					
Control	4.4	13.3[a]	17.7[a]	320[b]	298[b]
DMC	2.2	2.2[b]	4.4[b]	255[c]	294[b]
DC	2.2	6.7[b]	8.9[b]	350[a]	339[a]
SEM[3] (n = 6)	1.72	1.62	2.22	8.8	9.3
P-value	0.58	0.0008	0.002	<0.0001	0.005

[a-c]Within a column, means without a common superscript differ ($P \leq 0.05$).
[1]European production efficiency factor = [(livability in percentage × body weight in kg)/(period length in days × feed conversion ratio)] × 100.
[2]Dietary treatments were: control) a diet met or exceeded nutritional recommendations; DMC) a diet with the dilution of metabolizable energy and crude protein (and amino acids), and DC) a diet with the dilution of crude protein (and amino acids, except lysine, methionine+cystine, and threonine).
[3]*SEM* = standard error of the mean.

Table 4. Effect of dietary treatments on relative weight (g/100 g body weight) of carcass and organs in broiler chickens (on d 38)

	Carcass[1]	Breast	Thigh+Drumstick	Liver	Pancreas	Spleen	Right ventricle
Treatments[2]							
Control	63.1	25.1[a]	20.6	2.39	0.23	0.11	0.138[a]
DMC	61.7	23.5[b]	20.6	2.50	0.24	0.12	0.107[b]
DC	62.6	25.1[a]	20.3	2.41	0.22	0.11	0.126[ab]
SEM[3] (n = 6)	0.63	0.50	0.17	0.106	0.008	0.009	0.0076
P-value	0.69	0.04	0.32	0.67	0.13	0.65	0.03

[a,b]Within a column, means without a common superscript differ ($P \leq 0.05$).
[1]Without skin, leg, neck, and others.
[2]Dietary treatments were: control) a diet met or exceeded nutritional recommendations; DMC) a diet with the dilution of metabolizable energy and crude protein (and amino acids), and DC) a diet with the dilution of crude protein (and amino acids, except lysine, methionine+cystine, and threonine).
[3]*SEM* = standard error of the mean.

Discussion

In the current study, two scenarios were used to reduce the PHS and mortality rate of broiler chickens reared at the high-altitude. In both scenarios, CP was reduced to a level that lysine, methionine+cystine, threonine, valine, and isoleucine were diluted by 7% of nutritional requirements. Other essential amino acids were also diluted by 5 to 7% of nutritional requirements. Practical formulation of diets does not allow reducing all essential amino acids by a fixed ratio; therefore, according to the "ideal protein" concept, it was assumed that 7%-reduction of one or several essential amino acids is enough for our objective. In the first scenario, ME was also diluted by 7%. In the second scenario, the level of lysine, methionine+cystine, and threonine was enhanced to 100% of nutritional recommendations to prevent significant growth depression due to CP reduction.

The high mortality rate of control may be related to the higher dietary nutrient content and metabolic rate of this treatment. The birds of this group received the recommended concentration of ME, CP, and amino acids, which may be detrimental to the survivability of current broiler strains in a high-altitude area. Although the reduction of just CP (and amino acids, except lysine, methionine+cystine, and threonine) of diet did not affect growth performance negatively, it decreased the mortality rate of broiler chickens at the high-altitude area. The lower mortality due to the reduction of dietary CP in the

present study is in agreement with the statement of Leeson *et al.* (1996) who claimed that decreasing dietary protein reduces oxygen demand which may account for a lower incidence of PHS. However, the result of the present research does not agree with those studies reported that the incidence of mortality is reduced by feeding a high-protein diet (Behrooj *et al.,* 2012; Buys *et al.,* 1998a,b; Maxwell and Robertson, 1998). It is claimed that feeding high-protein diets produces an elevated level of uric acid which has antioxidant activity against reactive oxygen species (Machin *et al.,* 2004). Therefore, the high concentration of uric acid can have protective effects against oxidative stress in broiler chickens rearing at a high-altitude area (Khajali and Wideman, 2016). However, we did not observe a protective effect of a high-protein diet (i.e. control) against PHS development. The RW of RV is an index of PHS development, and control could not reduce this indicator compared to that of other treatments.

The lower mortality rate and the RW of RV in DMC may be related to the lower metabolic rate and oxygen demand. Because this treatment showed depressed growth performance as a result of receiving the diluted diet of ME, CP, and amino acids. The lower RW of RV in DMC (compared to that of control) means that simultaneous reduction of ME and CP (and amino acids) represents more potential to reduce the development of PHS in comparison with the reduction of just CP (and amino acids, except lysine, methionine+cystine, and threonine). The effect of dietary energy concentration on PHS incidence has been reported in the literature. Julian *et al.* (1989) showed a higher incidence of ascites when broiler chickens received a high-energy diet in comparison with the low-energy diet. In addition, a high-energy diet needs to include high concentration of fat in the diet to increase energy concentration, and it has been claimed that fat metabolism needs more oxygen than that of carbohydrate metabolism. Therefore, a high-energy diet may enhance the development of PHS through this mechanism (Khajali and Wideman, 2016).

Conclusion

In conclusion, decreasing dietary crude protein without any reductions in metabolizable energy, lysine, methionine+cystine, and threonine concentration could decrease mortality and enhance the European poultry efficiency factor without any negative effects on feed efficiency or weight gain in broilers reared at a high-altitude area.

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