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EFFECTS OF DIFFERENT LEVELS OF EXCESS METHIONINE AND LYSINE ON SOME ORGANS OF BROILER

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ABSTRACT

This study aimed to investigate the effect of different levels of lysine and methionine on head, brain, lungs, testes, kidneys, notarium, crop, proventriculus and pancreas in male Ross 308 broilers. The experiments included 9 treatments and 3 replications/treatment based on completely randomized design as a 3 × 3 factorial arrangement. All management process, vaccination, ventilation and other environmental conditions, was quite similar for all treatments. Methionine in this experiment consisted of 0, 10 and 20% more than the recommended level by guide catalogue of Ross 308, and lysine levels in this experiment consisted of 0, 10 and 20% more than the recommended level of guide catalogue of Ross 308. The obtained results of statistical analysis showed that the inclusion of lysine and also methionine as much as 10 and 20% more than the guide catalogue of Ross 308 had not significant effect on weights of head, brain, lungs, testes, kidneys, notarium, crop, proventriculus and pancreas. Nine studied treatments had significant difference for lungs weight and also pancreas weight ($P \leq 0.05$), so both lysine and methionine as much as 20% more than recommended level by guide catalogue of Ross 308 had the highest lungs weight significantly (16.057 and 6.043 g respectively). Meanwhile, nine studied treatments had significant difference for notarium weight ($P \leq 0.05$), so lysine as much as recommended level by guide catalogue of Ross 308 and methionine as much as 20% more than recommended level by guide catalogue of Ross 308 had the highest notarium weight significantly (40.090 g).

Key words: Lysine, Methionine, Crop, Chick

INTRODUCTION

Methionine and lysine are important amino acids in broiler nutrition. There are several experiments on methionine role on broiler productivity **[(1, 2, 3, 4]**. Meanwhile there are some reports about role of lysine on broiler productivity **[5, 6, 7, 8]**. However little research exists evaluating body organs of commercial broilers fed diets differing in excess methionine and lysine. The objective of this study was to determine the effect of different excess dietary levels of methionine (0, 10 nd 20% more than Ross 308 guide catalogue) and lysine (0, 10 nd 20% more than Ross 308 guide catalogue). Broiler head, brain, lungs, testes, kidneys, notarium, crop, proventriculus and pancreas was investigated after feeding during a 6-wk period from hatch to 6 wk of age.

MATERIALS AND METHODS

This experiment was conducted in August-September 2014 in the commercial poultry facility. Two hundred and seven male Ross 308 (Aviagen, Newbridge, Scotland, UK 35805) chicks were assigned to one of nine following dietary treatments (each treatment included three replications):

Treatment 1: Increased lysine amount (0%) -Increased methionine amount (0%)

Treatment 2: Increased lysine amount (0%) -Increased methionine amount (10%)

Treatment 3: Increased lysine amount (0%) -Increased methionine amount (20%)

Treatment 4: Increased lysine amount (10%) -Increased methionine amount (0%)

Treatment 5: Increased lysine amount (10%) -Increased methionine amount (10%)

Treatment 6: Increased lysine amount (10%) -Increased methionine amount (20%)

Treatment 7: Increased lysine amount (20%) -Increased methionine amount (0%)

Treatment 8: Increased lysine amount (20%) -Increased methionine amount (10%)

Treatment 9: Increased lysine amount (20%) -Increased methionine amount (20%)

Treatments were randomly assigned and each group (replication) of 10 birds was raised in a 1 m x 1.5 m wire mesh pens within the hall. Facility and equipment were disinfected prior to bird placement.

Composition of used diets and nutrient composition of diets used in this study are shown in the **Tables 1-3** and **Tables 4-6** respectively. Diets were developed to be were iso-energetic and iso-nitrogenous. The diets met or exceeded Ross 308 catalogue recommendations **[9]**.

All birds were given 4 different vaccinations (Avian Influenza: AI; infectious bronchitis virus: IBV; Newcastle disease virus: NDV; Gumboro virus: GV) on varying time intervals. Avian Influenza was provided at d

1. Infectious bronchitis virus (IBV) strain H120 was provided at d 1, and d 16. Newcastle disease virus NDV strain Viscerotropic velogenic was provided at d 8, and d 20. Gumboro virus: GV was provided at d 14, and d 23. Vaccines were prepared per vendor recommendation and were supplied via drinking water after a period of water removal for three hours. On the final day of the experiment, one bird from each replication was slaughtered. Some body organs were removed and weighed. Data were analyzed by analysis of variance using a 3 × 3 factorial arrangement with three methionine levels (0, 10 nd 20% more than Ross 308 guide catalogue), and three lysine levels (0, 10 nd 20% more than Ross 308 guide catalogue), using a two-way ANOVA procedure **[10]** and based on Yijk=μ+Ai+ Bj+ABij+eijk formula. The Duncan post hoc test was used if the initial test result was significant at $P \leq 0.05$. Statements of significance were based on $P \leq 0.05$.

RESULTS AND DISCUSSION

Obtained results are summarized in **Tables 7-9**. From obtained results, it is showed that lysine level had not significant effect on head weight (P>0.05), although lysine supplementation as much as 10% more than recommended level by guide catalogue of Ross 308 had the highest head weight numerically (62.113 g). Also, methionine level had not significant effect on head weight (P>0.05), however methionine supplementation as much as 10% more than recommended level by guide catalogue of Ross 308 had the highest head weight numerically (62.984 g). Nine studied treatments had not significant difference for head weight (P>0.05), however lysine and methionine as much as recommended level by guide catalogue of Ross 308 had the highest head weight numerically (67.573 g).

It is showed that lysine level had not significant effect on brain weight (P>0.05), although lysine supplementation as much as recommended level by guide catalogue of Ross 308 had the highest brain weight numerically (2.552 g). Also, methionine level had not significant effect on brain weight (P>0.05), however methionine supplementation as much as 20% more than recommended level by guide catalogue of Ross 308 had the highest brain weight numerically (2.528 g). Nine studied treatments had not significant difference for brain weight (P>0.05), however lysine and methionine as much as recommended level by guide catalogue of Ross 308 had the highest brain weight numerically (2.690 g).

Lysine level had not significant effect on lungs weight (P>0.05), although lysine

supplementation as much as 20% more than recommended level by guide catalogue of Ross 308 had the highest lungs weight numerically (13.532 g). Also, methionine level had not significant effect on lungs weight (P>0.05), however methionine supplementation as much as 20% more than recommended level by guide catalogue of Ross 308 had the highest lungs weight numerically (13.040 g). Nine studied treatments had significant difference for lungs weight (P≤0.05), so both lysine and methionine as much as 20% more than recommended level by guide catalogue of Ross 308 had the highest lungs weight significantly (16.057 g).

From obtained results, it is showed that lysine level had not significant effect on testes weight (P>0.05), although lysine supplementation as much as recommended level by guide catalogue of Ross 308 had the highest testes weight numerically (0.942 g). Also, methionine level had not significant effect on testes weight (P>0.05), however methionine supplementation as much as 10% more than recommended level by guide catalogue of Ross 308 had the highest testes weight numerically (0.849 g). Nine studied treatments had not significant difference for testes weight (P>0.05), however lysine and methionine as much as recommended level by guide catalogue of Ross 308 had the highest testes weight numerically (1.083 g).

It is showed that lysine level had not significant effect on kidneys weight (P>0.05), although lysine supplementation as much as recommended level by guide catalogue of Ross 308 had the highest kidneys weight numerically (17.208 g). Also, methionine level had not significant effect on kidneys weight (P>0.05), however methionine supplementation as much as 20% more than recommended level by guide catalogue of Ross 308 had the highest kidneys weight numerically (17.231 g). Nine studied treatments had not significant difference for kidneys weight (P>0.05), however lysine as much as recommended level by guide catalogue of Ross 308 and methionine as much as 20% more than recommended level by guide catalogue of Ross 308 had the highest kidneys weight numerically (17.830 g).

Lysine level had not significant effect on notarium weight (P>0.05), although lysine supplementation as much as recommended level by guide catalogue of Ross 308 had the highest notarium weight numerically (38.232 g).

Also, methionine level had not significant effect on notarium weight (P>0.05), however methionine supplementation as

much as 10% more than recommended level by guide catalogue of Ross 308 had the highest notarium weight numerically (36.128 g). Nine studied treatments had significant difference for notarium weight (P≤0.05), so lysine as much as recommended level by guide catalogue of Ross 308 and methionine as much as 20% more than recommended level by guide catalogue of Ross 308 had the highest notarium weight significantly (40.090 g).

Lysine level had not significant effect on crop weight (P>0.05), although lysine supplementation as much as 10% more than recommended level by guide catalogue of Ross 308 had the highest crop weight numerically (17.296 g). Also, methionine level had not significant effect on crop weight (P>0.05), however methionine supplementation as much as 20% more than recommended level by guide catalogue of Ross 308 had the highest crop weight numerically (17.097 g). Nine studied treatments had not significant difference for crop weight (P>0.05), however lysine as much as 10% more than recommended level by guide catalogue of Ross 308 and methionine as much as 20% more than recommended level by guide catalogue of Ross 308 had the highest crop weight numerically (35.447 g).

It is showed that lysine level had not significant effect on proventriculus weight (P>0.05), although lysine supplementation as much as recommended level by guide catalogue of Ross 308 had the highest proventriculus weight numerically (11.120 g). Also, methionine level had not significant effect on proventriculus weight (P>0.05), however methionine supplementation as much as 10% more than recommended level by guide catalogue of Ross 308 had the highest proventriculus weight numerically (10.168 g). Nine studied treatments had not significant difference for proventriculus weight (P>0.05), however lysine as much as recommended level by guide catalogue of Ross 308 and methionine as much as 10% more than recommended level by guide catalogue of Ross 308 had the highest proventriculus weight numerically (11.567 g). From obtained results, it is showed that lysine level had not significant effect on pancreas weight (P>0.05), although lysine supplementation as much as recommended level by guide catalogue of Ross 308 had the highest pancreas weight numerically (5.440 g). Also, methionine level had not significant effect on pancreas weight (P>0.05), however methionine supplementation as much as 20% more than recommended level by guide catalogue of

Ross 308 had the highest pancreas weight numerically (5.449 g). Nine studied treatments had significant difference for pancreas weight ($P \leq 0.05$), so lysine and methionine as much as recommended level by guide catalogue of Ross 308 had the highest pancreas weight significantly (6.043 g). Our findings confirmed legible effects of lysine and methionine on some body organs in broilers, however more experiments need to clarifying careful effects of these amino acids on broiler organs. There are little similar experiments about role of amino acids on body organs in broilers **[11, 12]**, other birds and other animals.

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Table 1: Feed ingredients of used diets during the starter (1st-14th days of age) period

Ingredient (%)	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6	Treatment 7	Treatment 8	Treatment 9
Corn	58.94	58.94	58.98	59.23	59.24	59.29	59.52	59.56	59.59
Soybean Meal	34.23	34.18	34.10	33.81	33.76	33.68	33.40	33.33	33.255
Soybean oil	2.33	2.33	2.31	2.26	2.25	2.23	2.19	2.17	2.16
Ca%22P%18	1.94	1.94	1.94	1.95	1.95	1.95	1.95	1.95	1.95
CaCO3	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40
Vitamin premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
NaCl	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28
Sodium bicarbonate (NaHCO3)	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
DL-Methionine	0.14	0.19	0.24	0.14	0.19	0.24	0.145	0.19	0.245
Lysine-Hydro-Chloride	0.14	0.14	0.15	0.33	0.33	0.33	0.515	0.52	0.52
Price (Rials/kg)	15079	15148	15218	15184	15251	15313	15293	15353	15425

Table 2: Feed ingredients of used diets during the grower (15th-28th days of age) period

Ingredient (%)	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6	Treatment 7	Treatment 8	Treatment 9
Corn	59.87	59.87	59.885	60.10	60.14	60.16	60.38	60.40	60.425
Soybean Meal	32.32	32.28	32.22	31.97	31.90	31.85	31.59	31.54	31.49
Soybean oil	4	4	3.99	3.95	3.93	3.92	3.88	3.87	3.85
Ca%22P%18	1.67	1.67	1.68	1.68	1.68	1.68	1.68	1.68	1.68
CaCO3	1.17	1.17	1.17	1.17	1.17	1.17	1.17	1.17	1.17
Vitamin premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
NaCl	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
Sodium bicarbonate (NaHCO3)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
DL-Methionine	0.10	0.14	0.185	0.10	0.145	0.185	0.105	0.145	0.19
Lysine-Hydro-Chloride	0	0	0	0.16	0.165	0.165	0.325	0.325	0.325
Price (Rials/kg)	15079	15134	15194	15170	15230	15282	15266	15318	15375

Table 3: Feed ingredients of used diets during the finisher (29th-42nd days of age) period

Ingredient (%)	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6	Treatment 7	Treatment 8	Treatment 9
Corn	65.09	65.09	65.13	65.30	65.32	65.34	65.56	65.56	65.60
Soybean Meal	27.265	27.225	27.17	26.97	26.92	26.87	26.63	26.59	26.53
Soybean oil	3.99	3.99	3.97	3.94	3.93	3.92	3.87	3.87	3.85
Ca%22P%18	1.58	1.58	1.58	1.57	1.57	1.57	1.57	1.57	1.57
CaCO3	1.12	1.12	1.12	1.13	1.13	1.13	1.13	1.13	1.13
Vitamin premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
NaCl	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
Sodium bicarbonate (NaHCO3)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
DL-Methionine	0.085	0.125	0.16	0.085	0.125	0.165	0.09	0.13	0.165
Lysine-Hydro-Chloride	0	0	0	0.135	0.135	0.135	0.28	0.28	0.285
Price (Rials/kg)	14610	14664	14706	14682	14734	14786	14764	14820	14865

Table 4: Nutrient analysis of used diets during the starter (1st-14th days of age) period

Nutrient analysis	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6	Treatment 7	Treatment 8	Treatment 9
Dry Matter (%)	86.334	86.339	86.332	86.144	86.148	86.150	85.968	85.965	85.970
Metabolizable energy (kcal/kg)	3.025	3.025	3.025	3.025	3.025	3.025	3.025	3.025	3.025
Crude protein (%)	22.001	22.005	22.008	22.002	22.008	22.002	22.006	22.006	22.004
Ether extract (%)	4.912	4.912	4.892	4.849	4.839	4.820	4.786	4.767	4.757
Linoleic Acid (%)	2.622	2.622	2.612	2.591	2.586	2.576	2.560	2.550	2.546
Crude fiber (%)	2.632	2.630	2.627	2.622	2.620	2.618	2.612	2.610	2.608
Calcium (%)	1.050	1.050	1.050	1.051	1.051	1.051	1.050	1.050	1.050
Total Phosphorus (%)	0.740	0.740	0.739	0.740	0.740	0.739	0.738	0.738	0.738
Available Phosphorus (%)	0.504	0.504	0.504	0.505	0.505	0.505	0.504	0.504	0.504
Potassium (%)	0.871	0.870	0.869	0.863	0.862	0.861	0.856	0.855	0.853
Chloride (%)	0.236	0.236	0.238	0.273	0.273	0.273	0.309	0.310	0.310
Mn (mg/kg)	402.686	402.664	402.632	402.550	402.529	402.497	402.388	402.360	402.329
Na (%)	0.160	0.160	0.160	0.160	0.160	0.160	0.160	0.160	0.160
Zn (mg/kg)	323.238	323.215	323.183	323.088	323.066	323.035	322.932	322.905	322.874
Choline (g/kg)	1.520	1.519	1.517	1.511	1.509	1.507	1.501	1.500	1.498
Fulic acid (mg/kg)	2.018	2.016	2.014	2.004	2.002	2.000	1.990	1.988	1.986
Arg (%)	1.415	1.413	1.411	1.402	1.400	1.397	1.388	1.386	1.384
Gly (%)	0.896	0.895	0.894	0.889	0.888	0.886	0.881	0.880	0.878
Cer (%)	1.067	1.066	1.064	1.058	1.056	1.055	1.049	1.047	1.045
Glycine + Cerine (%)	2.305	2.303	2.299	2.284	2.282	2.278	2.264	2.260	2.256
Hys (%)	0.574	0.573	0.572	0.569	0.568	0.567	0.564	0.564	0.563
Iso-Leucine (%)	0.897	0.896	0.894	0.889	0.888	0.886	0.881	0.879	0.878
Leucine (SID) (%)	1.870	1.868	1.865	1.857	1.855	1.853	1.844	1.842	1.840
Lysine (%)	1.271	1.270	1.271	1.399	1.399	1.397	1.527	1.528	1.526
Methionine (%)	0.473	0.519	0.570	0.470	0.519	0.568	0.473	0.517	0.569
Cysteine (%)	0.353	0.352	0.352	0.350	0.350	0.349	0.348	0.347	0.347
Methionine + Cysteine (%)	0.825	0.873	0.922	0.820	0.869	0.917	0.821	0.864	0.917
Phe (%)	1.025	1.024	1.022	1.016	1.015	1.013	1.008	1.006	1.005
Tyr (%)	0.844	0.843	0.842	0.837	0.836	0.835	0.830	0.829	0.827
Phe + Tyr (%)	1.869	1.867	1.864	1.853	1.851	1.848	1.838	1.835	1.832
Threonine (%)	0.811	0.810	0.809	0.804	0.803	0.802	0.797	0.796	0.795
Tryptophan (%)	0.289	0.288	0.288	0.286	0.285	0.285	0.283	0.282	0.282

Table 5: Nutrient analysis of used diets during the grower (15th-28th days of age) period

Nutrient analysis	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6	Treatment 7	Treatment 8	Treatment 9
Dry Matter (%)	87.022	87.026	87.021	86.862	86.860	86.862	86.704	86.707	86.709
Metabolizable energy (kcal/kg)	3.150	3.150	3.150	3.150	3.150	3.150	3.150	3.150	3.150
Crude protein (%)	21.001	21.005	21.003	21.003	21.003	21.004	21.002	21.002	21.006
Ether extract (%)	6.598	6.598	6.588	6.554	6.534	6.525	6.490	6.481	6.461
Linoleic Acid (%)	3.486	3.486	3.481	3.465	3.455	3.450	3.434	3.429	3.419
Crude fiber (%)	2.578	2.576	2.574	2.569	2.567	2.566	2.560	2.559	2.557
Calcium (%)	0.901	0.900	0.902	0.902	0.902	0.901	0.901	0.901	0.901
Total Phosphorus (%)	0.680	0.680	0.682	0.681	0.680	0.680	0.679	0.679	0.679
Available Phosphorus (%)	0.450	0.450	0.451	0.451	0.451	0.451	0.450	0.450	0.450
Potassium (%)	0.835	0.834	0.833	0.828	0.827	0.826	0.822	0.821	0.820
Chloride (%)	0.232	0.232	0.232	0.263	0.264	0.264	0.295	0.295	0.295
Mn (mg/kg)	400.411	400.394	400.399	400.302	400.274	400.254	400.153	400.132	400.112
Na (%)	0.161	0.161	0.161	0.161	0.161	0.161	0.161	0.161	0.161
Zn (mg/kg)	322.201	322.183	322.168	322.077	322.049	322.029	321.934	321.913	321.893
Choline (g/kg)	1.474	1.473	1.471	1.466	1.464	1.463	1.457	1.456	1.455
Fulic acid (mg/kg)	1.953	1.952	1.949	1.941	1.939	1.937	1.929	1.927	1.925
Arg (%)	1.352	1.351	1.349	1.341	1.339	1.337	1.329	1.327	1.325
Gly (%)	0.860	0.859	0.858	0.854	0.852	0.851	0.847	0.846	0.845
Cer (%)	1.023	1.022	1.021	1.015	1.014	1.012	1.007	1.006	1.005
Glycine + Cerine (%)	2.206	2.204	2.201	2.189	2.185	2.182	2.170	2.167	2.164
Hys (%)	0.551	0.551	0.550	0.547	0.547	0.546	0.543	0.543	0.542

Iso-Leucine (%)	**0.859**	**0.858**	**0.857**	**0.852**	**0.851**	**0.850**	**0.845**	**0.844**	**0.843**
Leucine (SID) (%)	**1.807**	**1.806**	**1.804**	**1.797**	**1.794**	**1.793**	**1.785**	**1.784**	**1.782**
Lysine (%)	**1.112**	**1.111**	**1.109**	**1.222**	**1.223**	**1.222**	**1.334**	**1.332**	**1.331**
Methionine (%)	**0.422**	**0.462**	**0.505**	**0.420**	**0.464**	**0.503**	**0.423**	**0.462**	**0.506**
Cysteine (%)	**0.340**	**0.340**	**0.340**	**0.338**	**0.338**	**0.338**	**0.336**	**0.336**	**0.335**
Methionine + Cysteine (%)	**0.763**	**0.801**	**0.845**	**0.759**	**0.802**	**0.841**	**0.759**	**0.798**	**0.841**
Phe (%)	**0.984**	**0.983**	**0.982**	**0.976**	**0.975**	**0.974**	**0.969**	**0.968**	**0.966**
Tyr (%)	**0.810**	**0.809**	**0.808**	**0.804**	**0.802**	**0.802**	**0.797**	**0.796**	**0.795**
Phe + Tyr (%)	**1.794**	**1.792**	**1.789**	**1.780**	**1.777**	**1.775**	**1.766**	**1.764**	**1.762**
Threonine (%)	**0.778**	**0.777**	**0.776**	**0.772**	**0.771**	**0.770**	**0.766**	**0.765**	**0.764**
Tryptophan (%)	**0.275**	**0.275**	**0.274**	**0.273**	**0.272**	**0.272**	**0.270**	**0.270**	**0.269**
Valine (%)	**0.957**	**0.956**	**0.955**	**0.950**	**0.949**	**0.948**	**0.943**	**0.942**	**0.941**

Table 6: Nutrient analysis of used diets during the finisher (29th-42nd days of age) period

Nutrient analysis	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6	Treatment 7	Treatment 8	Treatment 9
Dry Matter (%)	**87.094**	**87.098**	**87.099**	**86.965**	**86.968**	**86.971**	**86.825**	**86.829**	**86.826**
Metabolizable energy (kcal/kg)	**3.200**	**3.200**	**3.200**	**3.200**	**3.200**	**3.200**	**3.200**	**3.200**	**3.200**
Crude protein (%)	**19.000**	**19.004**	**19.001**	**19.003**	**19.004**	**19.004**	**19.001**	**19.005**	**19.004**
Ether extract (%)	**6.736**	**6.736**	**6.717**	**6.691**	**6.681**	**6.672**	**6.628**	**6.627**	**6.608**
Linoleic Acid (%)	**3.576**	**3.576**	**3.566**	**3.554**	**3.549**	**3.544**	**3.523**	**3.522**	**3.513**
Crude fiber (%)	**2.495**	**2.494**	**2.492**	**2.488**	**2.487**	**2.485**	**2.481**	**2.479**	**2.478**
Calcium (%)	**0.850**	**0.850**	**0.850**	**0.851**	**0.851**	**0.850**	**0.850**	**0.850**	**0.850**
Total Phosphorus (%)	**0.647**	**0.647**	**0.646**	**0.644**	**0.643**	**0.643**	**0.642**	**0.642**	**0.642**
Available Phosphorus (%)	**0.426**	**0.426**	**0.426**	**0.424**	**0.424**	**0.423**	**0.423**	**0.423**	**0.423**
Potassium (%)	**0.748**	**0.747**	**0.746**	**0.743**	**0.742**	**0.741**	**0.737**	**0.736**	**0.735**
Chloride (%)	**0.232**	**0.232**	**0.232**	**0.258**	**0.258**	**0.258**	**0.286**	**0.286**	**0.287**
Mn (mg/kg)	**398.078**	**398.061**	**398.040**	**397.962**	**397.942**	**397.921**	**397.829**	**397.812**	**397.788**
Na (%)	**0.161**	**0.161**	**0.161**	**0.161**	**0.161**	**0.161**	**0.161**	**0.161**	**0.161**
Zn (mg/kg)	**320.358**	**320.340**	**320.320**	**320.237**	**320.216**	**320.196**	**320.110**	**320.092**	**320.069**
Choline (g/kg)	**1.368**	**1.367**	**1.366**	**1.361**	**1.360**	**1.359**	**1.354**	**1.353**	**1.351**
Fulic acid (mg/kg)	**1.792**	**1.790**	**1.789**	**1.782**	**1.780**	**1.779**	**1.771**	**1.769**	**1.767**
Arg (%)	**1.196**	**1.195**	**1.193**	**1.187**	**1.185**	**1.183**	**1.176**	**1.174**	**1.173**
Gly (%)	**0.774**	**0.773**	**0.772**	**0.768**	**0.767**	**0.766**	**0.762**	**0.761**	**0.760**
Cer (%)	**0.917**	**0.916**	**0.915**	**0.910**	**0.909**	**0.908**	**0.903**	**0.902**	**0.901**
Glycine + Cerine (%)	**1.963**	**1.961**	**1.958**	**1.949**	**1.946**	**1.943**	**1.932**	**1.929**	**1.926**
Hys (%)	**0.499**	**0.498**	**0.498**	**0.495**	**0.495**	**0.494**	**0.492**	**0.491**	**0.490**
Iso-Leucine (%)	**0.767**	**0.766**	**0.765**	**0.761**	**0.760**	**0.759**	**0.755**	**0.754**	**0.753**
Leucine (SID) (%)	**1.671**	**1.669**	**1.667**	**1.662**	**1.660**	**1.658**	**1.652**	**1.650**	**1.648**
Lysine (%)	**0.976**	**0.975**	**0.974**	**1.069**	**1.067**	**1.066**	**1.167**	**1.166**	**1.168**
Methionine (%)	**0.383**	**0.422**	**0.456**	**0.382**	**0.420**	**0.459**	**0.385**	**0.424**	**0.458**
Cysteine (%)	**0.313**	**0.313**	**0.313**	**0.312**	**0.311**	**0.311**	**0.310**	**0.309**	**0.309**
Methionine + Cysteine (%)	**0.697**	**0.735**	**0.769**	**0.693**	**0.732**	**0.770**	**0.694**	**0.733**	**0.767**
Phe (%)	**0.885**	**0.884**	**0.883**	**0.879**	**0.878**	**0.877**	**0.872**	**0.871**	**0.870**
Tyr (%)	**0.727**	**0.726**	**0.725**	**0.722**	**0.721**	**0.720**	**0.716**	**0.715**	**0.714**
Phe + Tyr (%)	**1.612**	**1.611**	**1.608**	**1.601**	**1.599**	**1.597**	**1.588**	**1.587**	**1.584**
Threonine (%)	**0.699**	**0.698**	**0.697**	**0.694**	**0.693**	**0.692**	**0.688**	**0.687**	**0.686**
Tryptophan (%)	**0.241**	**0.241**	**0.240**	**0.239**	**0.238**	**0.238**	**0.236**	**0.236**	**0.236**
Valine (%)	**0.866**	**0.865**	**0.864**	**0.860**	**0.859**	**0.858**	**0.853**	**0.853**	**0.851**

Table 7: Mean (±SEM) of invaluable body parts at 42nd days of age in Ross 308 broilers affected by the three different amounts of lysine and methionine*

Treatment	Trait	Head weight (gr)	Relative weight of head (%)	Brain weight (gr)	Relative weight of brain (%)	Lungs weight (gr)	Relative weight of lungs (%)
Lysine amount increasment relative standard requirements (%)	**0**	**61.690[a]**	**2.598[a]**	**2.552[a]**	**0.107[a]**	**12.267[a]**	**0.516[a]**
	10	**62.113[a]**	**2.697[a]**	**2.429[a]**	**0.105[a]**	**11.761[a]**	**0.510[a]**
	20	**59.949[a]**	**2.489[a]**	**2.479[a]**	**0.103[a]**	**13.532[a]**	**0.558[a]**
P		**0.657**	**0.262**	**0.772**	**0.879**	**0.203**	**0.250**
SEM (Standard Error of Mean)		**1.713**	**0.087**	**0.093**	**0.004**	**0.692**	**0.022**
Methionine amount increasment relative standard	**0**	**58.393[a]**	**2.494[a]**	**2.430[a]**	**0.102[a]**	**12.253[a]**	**0.520[a]**
	10	**62.984[a]**	**2.691[a]**	**2.461[a]**	**0.105[a]**	**12.262[a]**	**0.521[a]**

requirements (%)	**20**	**61.276[a]**	**2.564[a]**	**2.528[a]**	**0.106[a]**	**13.040[a]**	**0.543[a]**
P		**0.271**	**0.337**	**0.852**	**0.922**	**0.630**	**0.647**
SEM (Standard Error of Mean)		**1.713**	**0.087**	**0.093**	**0.004**	**0.692**	**0.022**
Increased lysine amount (0%) -Increased methionine amount (0%)		**67.573[a]**	**2.613[a]**	**2.690[a]**	**0.104[a]**	**14.477[a b]**	**0.559[ab]**
Increased lysine amount (0%) -Increased methionine amount (10%)		**62.483[a]**	**2.686[a]**	**2.540[a]**	**0.109[a]**	**13.190[a b]**	**0.564[ab]**
Increased lysine amount (0%) -Increased methionine amount (20%)		**60.897[a]**	**2.509[a]**	**2.563[a]**	**0.106[a]**	**11.343[b]**	**0.468[b]**
Increased lysine amount (10%) - Increased methionine amount (0%)		**59.827[a]**	**2.647[a]**	**2.197[a]**	**0.096[a]**	**11.453[b]**	**0.505[b]**
Increased lysine amount (10%) - Increased methionine amount (10%)		**65.850[a]**	**2.788[a]**	**2.513[a]**	**0.107[a]**	**12.110[b]**	**0.512[b]**
Increased lysine amount (10%) - Increased methionine amount (20%)		**60.663[a]**	**2.654[a]**	**2.577[a]**	**0.114[a]**	**11.720[b]**	**0.511[b]**
Increased lysine amount (20%) - Increased methionine amount (0%)		**56.960[a]**	**2.341[a]**	**2.663[a]**	**0.109[a]**	**13.053[a b]**	**0.534[ab]**
Increased lysine amount (20%) - Increased methionine amount (10%)		**60.620[a]**	**2.599[a]**	**2.330[a]**	**0.101[a]**	**11.487[b]**	**0.488[b]**
Increased lysine amount (20%) - Increased methionine amount (20%)		**62.267[a]**	**2.528[a]**	**2.443[a]**	**0.099[a]**	**16.057[a]**	**0.650[a]**
P		**0.541**	**0.711**	**0.416**	**.782**	**0.140**	**0.081**
SEM (Standard Error of Mean)		**3.362**	**0.155**	**0.151**	**.007**	**1.188**	**0.036**

[*] Means (± standard errorof means) within each column of dietary treatments with no common superscript differ significantly at P<0.05.

Table 8: Mean (±SEM) of other invaluable body parts at 42nd days of age in Ross 308 broilers affected by the three different amounts of lysine and methionine[*]

Treatment	**Trait**	**Testes weight (gr)**	**Relative weight of testes (%)**	**Kidneys weight (gr)**	**Relative weight of kidneys (%)**	**Back thoracic vertebrae (notarium) weight (gr)**	**Relative weigh of back thoraci vertebrae (notarium) (%**
Lysine amount increasment relative standard requirements (%)	**0**	**0.942[a]**	**0.039[a]**	**17.208[a]**	**0.723[a]**	**38.232[a]**	**1.600[a]**
	10	**0.714[a]**	**0.031[a]**	**16.628[a]**	**0.716[a]**	**33.799[a]**	**1.461[a]**
	20	**0.772[a]**	**0.032[a]**	**16.447[a]**	**0.679[a]**	**36.812[a]**	**1.531[a]**
P		**0.594**	**0.598**	**0.902**	**0.603**	**0.121**	**0.278**
SEM (Standard Error of Mean)		**0.143**	**0.007**	**1.013**	**0.032**	**1.353**	**0.054**
Methionine amount increasment relative standard requirements (%)	**0**	**0.775[a]**	**0.033[a]**	**16.527[a]**	**0.704[a]**	**35.820[a]**	**1.516[a]**
	10	**0.849[a]**	**0.036[a]**	**16.298[a]**	**0.692[a]**	**36.128[a]**	**1.544[a]**
	20	**0.749[a]**	**0.031[a]**	**17.231[a]**	**0.716[a]**	**36.091[a]**	**1.504[a]**
P		**0.864**	**0.810**	**0.811**	**0.858**	**0.930**	**0.813**
SEM (Standard Error of Mean)		**0.143**	**0.006**	**1.013**	**0.032**	**1.353**	**0.054**
Increased lysine amount (0%) -Increased methionine amount (0%)		**1.083[a]**	**0.042[a]**	**16.210[a]**	**0.626[a]**	**37.837[ab]**	**1.464[a]**
Increased lysine amount (0%) -Increased methionine amount (10%)		**1.030[a]**	**0.044[a]**	**16.587[a]**	**0.711[a]**	**36.373[ab]**	**1.551[a]**
Increased lysine amount (0%) -Increased methionine amount (20%)		**0.853[a]**	**0.035[a]**	**17.830[a]**	**0.736[a]**	**40.090[a]**	**1.648[a]**
Increased lysine amount (10%) -Increased methionine amount (0%)		**0.743[a]**	**0.033[a]**	**17.500[a]**	**0.770[a]**	**34.523[ab]**	**1.513[a]**
Increased lysine amount (10%) -Increased methionine amount (10%)		**0.827[a]**	**0.035[a]**	**16.000[a]**	**0.677[a]**	**35.800[ab]**	**1.518[a]**
Increased lysine amount (10%) -Increased methionine amount (20%)		**0.573[a]**	**0.025[a]**	**16.383[a]**	**0.703[a]**	**31.073[b]**	**1.351[a]**
Increased lysine amount (20%) -Increased methionine amount (0%)		**0.807[a]**	**0.032[a]**	**15.553[a]**	**0.638[a]**	**37.117[ab]**	**1.520[a]**
Increased lysine amount (20%) -Increased methionine amount (10%)		**0.690[a]**	**0.029[a]**	**16.307[a]**	**0.689[a]**	**36.210[ab]**	**1.563[a]**
Increased lysine amount (20%) -Increased methionine amount (20%)		**0.820[a]**	**0.033[a]**	**17.480[a]**	**0.709[a]**	**37.110[ab]**	**1.511[a]**
P		**0.875**	**0.916**	**0.989**	**0.776**	**0.382**	**0.603**
SEM (Standard Error of Mean)		**0.236**	**0.010**	**1.789**	**0.059**	**2.298**	**0.088**

[*] Means (± standard errorof means) within each column of dietary treatments with no common superscript differ significantly at P<0.05.

Table 9: Mean (±SEM) of cranial gut segments at 42nd days of age in Ross 308 broilers affected by the three different amounts of lysine and methionine*

Trait / Treatment		Crop weight (gr)	Relative weight of crop (%)	Proventriculus weight (gr)	Relative weight of proventriculus (%)	Pancreas weight (gr)	Relative weight of pancreas (%)
Lysine amount increasment relative standard requirements (%)	0	8.260[a]	0.346[a]	11.120[a]	0.470[a]	5.440[a]	0.228[a]
	10	17.296[a]	0.760[a]	9.818[a]	0.426[a]	4.909[a]	0.212[a]
	20	8.764[a]	0.361[a]	9.453[a]	0.391[a]	5.368[a]	0.222[a]
P		0.434	0.423	0.155	0.105	0.299	0.389
SEM (Standard Error of Mean)		5.711	0.259	0.577	0.024	0.218	0.006
Methionine amount increasment relative standard requirements (%)	0	8.658[a]	0.361[a]	10.157[a]	0.434[a]	4.960[a]	0.209[a]
	10	8.698[a]	0.371[a]	10.168[a]	0.436[a]	5.148[a]	0.219[a]
	20	17.097[a]	0.738[a]	9.746[a]	0.405[a]	5.449[a]	0.227[a]
P		0.455	0.471	0.593	0.403	0.465	0.369
SEM (Standard Error of Mean)		5.711	0.259	0.577	0.024	0.218	0.006
Increased lysine amount (0%) -Increased methionine amount (0%)		11.633[a]	0.452[a]	10.757[a]	0.417[a]	6.043[a]	0.234[a]
Increased lysine amount (0%) -Increased methionine amount (10%)		9.417[a]	0.406[a]	11.567[a]	0.500[a]	5.247[ab]	0.225[a]
Increased lysine amount (0%) -Increased methionine amount (20%)		7.103[a]	0.286[a]	10.673[a]	0.439[a]	5.633[ab]	0.232[a]
Increased lysine amount (10%) -Increased methionine amount (0%)		7.190[a]	0.312[a]	10.497[a]	0.466[a]	4.663[b]	0.203[a]
Increased lysine amount (10%) -Increased methionine amount (10%)		9.250[a]	0.391[a]	9.940[a]	0.422[a]	4.943[ab]	0.209[a]
Increased lysine amount (10%) -Increased methionine amount (20%)		35.447[a]	1.576[a]	9.017[a]	0.390[a]	5.120[ab]	0.222[a]
Increased lysine amount (20%) -Increased methionine amount (0%)		10.127[a]	0.410[a]	9.817[a]	0.401[a]	5.257[ab]	0.216[a]
Increased lysine amount (20%) -Increased methionine amount (10%)		7.427[a]	0.317[a]	8.997[a]	0.385[a]	5.253[ab]	0.224[a]
Increased lysine amount (20%) -Increased methionine amount (20%)		8.740[a]	0.354[a]	9.547[a]	0.388[a]	5.593[ab]	0.227[a]
P		0.524	0.527	0.744	0.648	0.414	0.628
SEM (Standard Error of Mean)		9.360	0.425	1.082	0.045	0.388	0.011

* Means (± standard errorof means) within each column of dietary treatments with no common superscript differ significantly at P<0.05.