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Assessment of digestible amino acid requirement in White leghorn Layers based on production performance at low protein levels in diet

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Supplementary Table 1. Ingredient and nutrient composition of diets formulated for d. lysine optimization in WL layers (25–44 weeks) (diets in kilograms)

D-lysine (%)	0.50	0.55	0.60	0.65	0.70	0.55	0.60	0.65	0.70	0.70
CP (%)			13.36				15.78			17.00
Group	T1	T2	T3	T4	T5	T7	T8	T9	T10	Control
Maize	681	680	681	678	678	635	634	636	636	627
Maize gluten meal	33.0	30.0	30.0	21.0	15.0	82.0	80.0	75.0	72.0	60.0
Soy bean meal	80.0	80.0	80.0	77.0	75.0	74.0	76.0	81.0	84.0	139
De oiled rice bran	45.0	47.0	45.0	42.0	43.0	47.0	47.0	44.0	42.0	16.0
Rape seed meal	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Ground nut cake	0.00	0.00	0.00	16.0	25.0	0.00	0.00	0.00	0.00	0.00
Sunflower cake	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Guar meal	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Stone grit	109	109	109	109	105	109	109	109	109	104
Salt (Nacl)	3.80	3.80	3.80	3.80	3.80	3.80	3.80	3.80	3.80	3.80
Lysine-Hcl	0.25	0.90	1.60	2.20	2.90	0.70	1.30	1.80	2.40	0.95
DL-methionine	0.10	0.70	1.20	1.80	2.50	0.00	0.45	1.00	1.50	1.30
L-threonine	0.00	0.00	0.20	0.35	0.80	0.00	0.00	0.00	0.00	0.00
L-tryptophan	0.00	0.00	0.00	0.10	0.20	0.00	0.00	0.10	0.15	0.00
Di calcium phosphate	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Premix*	5.80	5.80	5.80	5.80	5.80	5.80	5.80	5.80	5.80	5.80
Total	1000.0	1000	1000	1000	1000	1000	1000	1000	999	1000
Nutrient composition (% calculated)										
ME (kcal/kg)	2700	2698	2699	2696	2702	2698	2699	2699	2696	2697
CP (%)^	13.39	13.34	13.40	13.36	13.38	15.75	15.80	15.79	15.80	17.06
Ca (%)^	3.603	3.603	3.603	3.603	3.451	3.605	3.605	3.606	3.606	3.428
P (%)^	0.457	0.457	0.456	0.455	0.454	0.450	0.450	0.451	0.450	0.453
d. lysine	0.501	0.550	0.604	0.650	0.704	0.553	0.603	0.650	0.701	0.704
d. M + C	0.435	0.481	0.522	0.563	0.617	0.492	0.528	0.570	0.609	0.614
d. threonine	0.408	0.404	0.423	0.432	0.472	0.464	0.464	0.464	0.463	0.516
d. tryptophan	0.114	0.113	0.113	0.125	0.137	0.118	0.119	0.130	0.135	0.144
d. arginine	0.708	0.705	0.703	0.736	0.756	0.748	0.751	0.758	0.761	0.894
d. Iso leucine	0.463	0.458	0.457	0.450	0.444	0.540	0.540	0.538	0.537	0.604
d. valine	0.565	0.559	0.558	0.552	0.548	0.648	0.647	0.645	0.642	0.701

*Provided (/kg diet): Vit A 50 MIU, Vit D3 14 MIU, Vit E 20 g, Vit K3 8 g, Vit B1 3.2 g, B2 32 g, B6 3.6 g, B12 0.024 g, Niacin 28 g, calciumpantothenate 16 g, folic acid 5.6 g, Trace minerals (in each kg): manganese 70 g, zinc 70 g, iron 50 g, cobalt 0.6 g, iodine 0.6 g, copper 10 g, selenium 0.06 g. Vit E, C, probiotic, and phytase at 0.1% each, B complex at 0.4%, sodium bicarbonate, choline chloride at 1% each, toxin binder, trace minerals at 0.5% each, acidifier at 2%. ^ Analysed.

Supplementary Table 2. Ingredient and nutrient composition of diets fed to WL layers (25-44weeks) for d. threonine optimization

	Basal Diet- II		Control
d.Thr % (as % Lysine)	60	60	66
d.Lysine (%)	0.65	0.60	0.70
	D1-D6	D7-12	D13 (Control)
Maize	412	362	364
Soy bean meal	70.0	27.0	108
Deoiled rice bran	126	192	150
Pearl Millet (Bajra)	190	120	98.0
Ground nut cake	41.0	103	95.0
Cotton seed meal	15.0	50.0	40.0
Stone grit	120	120	120
Salt (Nacl)	4.00	4.00	4.00
Lysine Hcl	2.15	2.30	1.45
DL Methionine	1.45	1.29	1.80
L Threonine	0.00	0.00	0.00
Dicalcium Phosphate	12.0	12.0	12.0
Premix*	3.2	3.2	3.2
Trace Minerals**	0.50	0.50	0.50
Lactomax gold***	0.10	0.10	0.10
Acidifier	2.00	2.00	2.00
Vegetable Oil	0.28	0.42	0.41
Total	999.8	999.9	999.8
Nutrient Composition (%) (calculated)			
ME (kcal/Kg)	2704	2702	2706
CP^(%)	13.46	15.56	17.05
Calcium^ (%)	4.350	4.350	4.350
Av. Phosphorus (%)	0.430	0.430	0.440
d.Lys	0.650	0.604	0.701
d.M+C	0.563	0.530	0.630
d.Thr	0.391	0.360	0.470
d.Trp	0.137	0.140	0.170
d.Arg	0.840	1.070	1.200
d.Ile	0.470	0.430	0.540
d.Val	0.550	0.550	0.650

*Provided (/kg diet): Vit A 50 MIU,Vit D3 14 miu, Vit E 20 g, Vit K3 8 g, Vit B1 3.2 g, B2 32 g, B6 3.6 g, B12 0.024 g, Niacin 28 g, Calcium pantothenate 16 g, Folic acid 5.6 g. **Trace minerals (in each kg): Manganese-70 g, Zinc-70 g, Iron-50 g, Cobalt-0.6 g, Iodine-0.6 g, Copper- 10 g, Selenium-0.06 g. *** *Saccharomyces* and *Lactobacillus* species. ^ Analysed values.

Supplementary Table 3. Amino acids composition of ingredients utilized for the study (Exp-I) Amino Degussa Evonik industries

Ingredient / AA	Maize			Maize gluten meal			Soybean meal			DORB			Rapeseed meal			Groundnut cake			Sunflower cake			Gaur meal		
Particular	TAA	Co	DAA	TAA	Co	DAA	TAA	Co	DAA	TAA	Co	DAA	TAA	Co	DAA	TAA	Co	DAA	TAA	Co	DAA	TAA	Co	DAA
Lysine	0.27	92	0.24	1.1	76	0.87	2.91	90	2.6	0.72	74	0.53	1.84	80	1.47	1.39	76	1.06	0.93	87	0.81	3.6	67	2.41
M+C	0.36	90	0.32	2.1	83	1.71	1.24	86	1.0	0.64	72	0.46	1.69	80	1.35	0.90	82	0.74	1.02	87	0.89	2.06	69	1.43
Thr	0.35	85	0.25	1.9	79	1.56	1.82	85	1.5	0.59	69	0.41	1.53	73	1.12	1.05	85	0.89	0.96	82	0.79	2.7	58	1.57
Trp	0.07	81	0.06	0.3	66	0.20	0.61	89	0.54	0.21	79	0.16	0.52	80	0.41	0.48	87	0.42	0.35	87	0.30	1.38	46	0.64
Arg	0.43	93	0.39	1.8	86	1.64	3.61	93	3.35	1.31	86	1.12	2.39	87	2.08	4.22	91	3.84	2.14	93	1.99	1.06	71	7.52
Ile	0.28	95	0.27	2.4	86	2.02	2.15	89	1.90	0.55	75	0.41	1.45	79	1.15	1.27	89	1.13	1.06	89	0.94	2.79	61	1.69
Val	0.46	92	0.37	2.6	85	2.21	2.28	88	1.94	0.91	75	0.68	1.84	79	1.45	1.58	89	1.41	1.29	87	1.12	3.21	63	2.01

TAA: Total amino acid; Co: Co efficient; DAA: Digestible amino acid; M+C: Methionine +Cystine; Thr : Threonine; Trp: Tryptophan; Arg: Arginine; Ile: Isoleucine; Val: Valine.

Supplementary Table 4. Amino acids composition of ingredients utilized for the study (Exp-II) Amino Degussa Evonik industries

Ingredient /AA	Maize			Soybean meal			DORB			Pearl millet (Bajra)			Groundnut cake			Cotton seed cake		
Particular	TAA	Co	DAA	TAA	Co	DAA	TAA	Co	DAA	TAA	Co	DAA	TAA	Co	DAA	TAA	Co	DAA
Lysine	0.27	92.00	0.25	2.89	90	2.60	0.72	74	0.53	0.30	80	0.24	1.39	76	1.06	1.40	67	0.93
M+C	0.36	90.00	0.32	1.22	86	1.05	0.64	72	0.46	0.40	80	0.32	0.90	82	0.74	1.18	73	0.86
Thr	0.30	85.00	0.26	1.81	85	1.54	0.59	69	0.41	0.41	73	0.30	1.05	85	0.89	1.25	68	0.85
Trp	0.07	81.00	0.06	0.61	89	0.54	0.20	79	0.16	0.17	80	0.14	0.48	87	0.42	0.48	79	0.38
Arg	0.43	93.00	0.40	3.60	93	3.35	1.30	86	1.12	0.43	87	0.38	4.22	91	3.84	4.11	88	3.61
Ile	0.28	95.00	0.27	2.13	89	1.90	0.55	75	0.41	0.41	79	0.32	1.27	89	1.13	1.21	71	0.86
Val	0.37	92.00	0.34	2.20	88	1.94	0.91	75	0.68	0.52	79	0.41	1.58	89	1.41	1.70	74	1.26

TAA: Total amino acid; Co: Co efficient; DAA: Digestible amino acid; M+C: Methionine +Cystine; Thr : Threonine; Trp: Tryptophan; Arg: Arginine; Ile: Isoleucine; Val: Valine.

Supplementary Table 5. Formulas adopted for calculation of performance in the birds

S.No	Indicator	Reference value (Desirable)	Abbreviations
1	$\text{FE/kg egg mass} = \frac{\text{kg. of feed consumed}}{\text{kg. of egg produced}}$	≤2.2	FE= Feed efficiency
2	$\text{H.D.E.P/ Day} = \frac{\text{No. of eggs produced}}{\text{Total no. of live hens on that day}} \times 100$	85% or 310 eggs per year	HDEP= Hen day egg production
3	$\text{NFEI} = \frac{\text{EM +BW}}{\text{FC}} \times 100$	≥45	NFEI= Net Feed Efficiency Index EM= Mean Egg mass in grams. i.e. mean egg weight in grams × Average number of egg/layer during a specific period. BW= Mean body weight gain or loss in grams per layer during a particular period. FC= Average feed consumption per layer in grams during a particular period.
4	$\text{PEI} = \frac{30\,(\text{EW})^2\,\text{P}}{\text{BW} \times \text{F}}$		PEI= Performance Efficiency Index. EW=Average egg weight in grams. BW=Average body weight in grams P=Percentage hen-day egg production. F=Average feed consumed / day in grams.
5	Egg mass / Hen housed It is the kilograms of egg produced per hen housed. This will take into account the rate of egg production, mortality, as well as the egg weight.		
6	$\text{NEM} = \frac{\text{\% HDEP} \times \text{Average egg weight in gm.}}{100}$		NEM= North's Egg Mass.
7	$\text{EFPR} = \frac{\text{Total value of egg produced}}{\text{Total value of feed consumed}}$	≥1.4	EFPR= Egg, Feed Price Ratio
8	$\text{Income: Expenditure} = \frac{\text{EN} \times \text{EP}}{\text{FI} \times \text{FC}} \times 1.176$	≥1.4	EN= Total number of eggs produced per day by the existing flock. EP= Sale price per egg in rupees. FI= Daily feed intake by the flock in kilograms FC= Cost per kg of feed in rupees.

Source: Prof.Asha Rajini. 2013. *Simply poultry science,* Alfa Publications, New Delhi, India. Layer Farm Production Indices.

Supplementary Table 6. Range of mean micro-climatic variables prevailed during study period

Variable	Lysine optimization experiment	Threonine optimization experiment
T_{Max} (°C)	34.22-37.21	35.02-36.36
T_{Min} (°C)	30.04-31.75	20.45-22.49
RH_{Max} (°C)	71.75-78.55	91.71-91.75
RH_{Min} (°C)	38.44-42.64	42.25-42.57

T_{Max}: Maximum temperature in a day; T_{Min}: Minimum temperature in a day; RH: relative humidity.