## COMPARING THE LOCAL, IMPORTED DL-METHIONINE HYDROXY ANALOGUE – CALCIUM AND DL-METHIONINE FOR EL- SALAM LOCAL LAYING HENS

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#### Abstract

The present study was conducted to compare the biological efficiency of local and imported DL-Methionine hydroxy analogue — calcium (LMHA), imported DL- Methionine hydroxy analogue — calcium (IMHA) and DL-methionine (DLM) in corn- soya diet for local strain layers as a source of methionine .

Two hundred hens (22 weeks old) of developed local strain "EL-Salam" were fed on ten experimental diets for 16 weeks. Each of the three methionine sources DLM, LMHA and IMHA were added to a basal corn-soya diet,deficient in methionine (0.25%), on equimolar basis at levels of 0.04, 0.06 and 0.08 % . At the end of each 4 weeks interval the productive performance and egg quality parameters were measured.

## Results obtained can be summarized as follows:

The best egg production was obtained at 0.33 % total methionine with 0.08% equivalent added from DLM, LMHA or IMHA.

There were a significant increase in shell thickness for all methionine treatments as compared with the basal diet .

DL-methionine was significantly better than LMHA and IMHA on equimolar basis in the egg production parameters .

The relative efficiency of LMHA as compared to DLM was 92.42, 98.57, 91.08 and 86.87% in the term of EN, EW,EM and FC, respectively. Whereas, IMHA is 96.44, 99.35, 95.77 and 93.52%, respectively.

According to the linear regression model (Y = A+CX), the efficiency of LMHA was found to be 55.36, 56.50, 54.65 and 49.65 % for EN, EW, EM and FC, respectively, while, the efficiency of IMHA ( $C_3/C_1$ ) was 79.71, 71.33, 77.37 and 76.24 %, respectively.

## INTRODUCTION

Methionine is generally the first limiting amino acid in practical corn-soya diets. Supplementation of such diets with methionine, provides a means for increase the efficiency of protein utilization (Sell and Johnson, 1974; Schutte and Van weerden, 1978 and Schutte et al., 1983,1984).

For supplementation, two alternative sources of methionine activity have been used for dietary supplementation: DL-methionine (DLM) and methionine hydroxy analogue calcium salt. Both are supplied as dry powders and available commercially with methionine-free acid (MHA-FA the liquid form). Methionine hydroxy analogue calcium was shown by Gordon and Siser (1965) to be readily oxidized metabolically to the corresponding Keto – acid which, in turn, is readily transaminated to produce L- methionine, the naturally occurring form of the amino acid.

The biological activity of MHA either in the powder or liquid form in comparison with L-methionine or DL-methionine has been studied intensively in chicks and brought varying results.

Evaluation of these products in layer diets has received little attention. Schutte et al. (1994) studied the effectiveness of DLM and MHA in layers by using practical type basal diet and found no significant differences in the effectiveness of both methionine sources for laying hen performance.

The present study aimed to compare the biological methionine activity of DLmetionine and the local and imported DL-methionine hydroxy analogue-calcium using corn-soya diet for developed local strain "EL-Salam" layer hens.

#### **MATERIALS AND METHODS**

The present study involved one layer experiment carried out in Seds Research Station, Beny Suif, Animal Production Research Institute, Agric. Res. Centre. Two hundred hens (22 weeks old) of developed local strain "El- Salam" (Nichol X Mamourah) were used. Hens were selected from the standard farm flock layers to be nearly similar in body weight and distributed at random in standard layer cages (4 hens/cage). Ten experimental dietary treatments were formulated to be fed to 10 groups of hens, 5 replicates/group, (4 hens / replicate).

Treatments were based on a corn – soya diet (Table 1) adequate in all the essential nutrients, but low in crude protein14.33%, methionine 0.25% and methionine + cystine 0.49%. Three methionine sources: - DL-methionine (Degussa), local DL- methionine hydroxy analogue – calcium (IBEX International) and imported DL – methionine

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Table 1. Composition and chemical analysis of the basal diet.

Ingredients (19 and 18 and	Donybuk juri si	%
Yellow corn		70.03
Soybean meal (44%)	A construction of the Hill Co.	17.00
Bone meal		6.50
Limestone	ar e saera par surf	5.80
Salt	*	0.30
Vitamin and mineral premix *	, para galaga	0.25
L- Lysine	n mai	0.10
DL- Methionine		0.02
Total	1 Y	100.00
Calculated chemical analysis :**		
Crude protein	%	14.33
Metabolizable energy	( Kcal /Kg )	2802
Crude Fibre	%	1.83
Fat	%	2.80
Calcium	%	4.20
Available phosphorus	%	0.92
Lysine	%	0.74
Methionine	%	0.25
Methionine + Cystine	%	0.49

 $<sup>^\</sup>star$  Each 2.5kg contain: vit. A12000000 IU, D3 2000000 IU, E 4gm, K\_3 4gm, B\_1 3gm, B\_2 6gm, B\_6 4gm, B\_{12} 30gm, Niacin 30gm, Biotin 80mg, Folic acid 1.5gm, Pantothenic acid 12 gm, Cholin chloride 500 gm, Zinc 70 gm, Manganese 80 gm, Iron 40 gm , Copper 10 gm, Iodine 3 gm , Selenium 200mg , Cobalt 100 mg.

<sup>\*\*</sup> By calculation according to NRC, 1994.

hydroxy analogue – calcium (Rhone-Poulene), were added to the basal diet on equimolar basis at levels of 0.04,0,06,and 0.08%. Hens groups were given the 10 experimental diets at random and provided with feed and water ad lib., for 16 hours photoperiod. The experiment extended for 16 weeks in 4 intervals, of 4 weeks each.

At the end of each interval, parameters of egg production: egg number (EN), egg weight, g, (EW), egg mass, g, (EM), feed intake, g. (FI) and feed conversion, FI/EM (FC) were determined. Also, random samples (5 eggs) were taken from each group to estimate egg quality: shell weight % (SW), albumin weight % (AW), yolk weight % (YW) and shell thickness, mm (ST).

Data of the productive performance and egg quality were examined statistically using the computerized analysis of variance and Duncan's multiple range test procedures within the Statistical Analysis System, SAS (1996).

#### **RESULTS AND DISCUSSION**

The effect of supplying the basal diet with each of the three sources of methionine on equimolar basis on the egg production of El-salam hens is shown in Table 2. Results indicated that feed consumption of the basal diet was 103g daily / hen which is equivalent to 258 mg., of dietary methionine. This treatment gave 13.87,45.75 g., 634.1g., 2885.4g. and 4.553 in EN, EW, EM, FI and FC, respectively.

#### Effect of DLM supplementation

Results showed that supplementing the basal diet with 0.04,0.06 or 0.08% DLM did not give significant effect on FI, but it gave significant increase in EN, EW, EM and significant improvement in FC as compared with the basal diet.

According to variation in daily feed intake/hen, the corresponding daily total methionine intake (dietary + supplementary) was calculated to be 304, 321 and 341 mg/hen/day, for the respective levels of supplementation.

Results showed that the increase in the daily intake of methionine was accompanied by significant improvement in egg production. Values of EN, EW and EM increased by 20.19,1.79 and 22.36%, respectively and that of FC was improved by 16.93% rela-

Table 2. Effect of DLM, LMHA and IMHA on laying hen productive performance

					The second secon		1	
, i	Mark	Added	Methionine	ō	Ā	M	ī	2
rearments	Teatments Methionine source	methionine	equivalent intake	í	(a.)	(a·)	(g·)	!
		equivalents%	(mg/hen/day)					
1(basal)		0.00	258	13.87 <sup>f</sup>	45.75 <sup>f</sup>	634.1 h	2885.4 <sup>c</sup>	4.553 a
2	DLM	0.04	304	16.67 °	46.57 de	775.9 de	2931.9 bc	3.782 <sup>d</sup>
8	DLM	0.06	321	17.3.7 b	47.67 b	827.9 <sup>b</sup>	2899.6 <sup>c</sup>	3.508
4	DLM	0.08	341	18.23 a	48.67 a	887.6 a	2891.5 °	3.263
22	LMHA	0.04	309	15.57	46.20 ef	719.1 9	2983.7 ab	4.155 b
9	LMHA	0.06	335	16.07 <sup>d</sup>	47.15 bcd	757.7 ef	3022.6 <sup>a</sup>	3,997 °
7	LMHA	0.08	353	16.67 °	47.53 b	792.3 cd	2998.2 <sup>a</sup>	3.788 <sup>d</sup>
8	IMHA	0.04	311	15.97 <sup>d</sup>	46.72 cde	745.7	3006.8 <sup>a</sup>	4.038 °
6	IMHA	0.06	328	16.87 °	47.37 bc	798.9 <sup>c</sup>	2966.3 ab	3.715 <sup>d</sup>
10	IMHA	0.08	345	17.57 b	47.90 <sup>b</sup>	841.5 <sup>b</sup>	2930.5 bc	3,485 <sup>e</sup>
Methionine e	Methionine effect ( source ) :							
1 ( Basal )				13.87	45.75	634.1	2885.4	4.553
2+3+4(A)				17.42	47.64	830.5	2907.7	3.518
5+6+7(B)				16.10	46.96	756.4	3001.5	3.980
8+9+10 (C)				16.80	47.33	795.4	2967.9	3.746
Relative effice	Relative efficiency (B/A)			92.42	98.57	91.08		86.870
Relative effic	Relative efficiency (C/A)			96.44	99.35	95.77		93.520
Level effect ( dose	( dose ) :							
1 ( Basal )				13.87	45.75	634.1	2885.4	4.553
2+5+8				16.07	46.50	746.9	2974.1	3.992
3+6+9				16.77	47.40	794.8	2962.8	3.740
4+7+10				17.49	48.03	840.5	2940.1	3.512

Means having different superscripts in the same column are significantly different ( P < 0.05 ) .

tive to those of the basal diet when 0.04% DLM was added. The addition of 0,06% DLM caused 25.23,4.20 and 30.56% increase in EN,EW and EM, respectively, and improved FC by 22.95%. Addition of 0.08% DLM increased significantly EN, EW and EM by 31.43,6.38 and 39.98%, respectively, and improved FC by 28.33% as compared to basal diet.

#### Effect of LMHA supplementation

Results of productive performance (Table 2) showed that FI increased significantly by 3.41,4.75 and 3.91% with supplementing the basal diet with 0.04,0.06 and 0.08% LMHA, respectively. The respective methionine intake (dietary methionine + supplementary LMHA) calculated on daily FI / hen was 309, 335 and 353 mg.

A significant progressive increase in egg production was achieved with the increase of LMHA intake. The former level increased EN,EW and EM by 12.26, 0.98 and 13.40%, respectively, and improved FC by 8.74% relative to those of the basal diet. The second level (0.06%) resulted in 15.86 3.06 and 19.49% further impovements in EN,EW and EM, respectively, and improved FC by 12.21% whereas, the third level (0.08%) increased significantly EN, EW and EM by 20.19,3.89 and 24.95 %, respectively, and improved FC by 16.80% as compared to basal diet.

#### Effect of IMHA supplementation

As shown in Table 2, FI also significantly increased by 4.21,2.80 and 1.56% with adding 0.04,0.06 and 0.08% IMHA, respectively. The corresponding daily total methionine intake (dietary methionine + supplementary IMHA) was calculated to be 311,328 and 345 mg / hen.

Significant increase in egg production was obtained with 0.04,0.06 and 0.08% IMHA. The first level caused 15.15,2.12 and 17.60% increase in EN, EW and EM, respectively, and improved FC by 11.31% relative to those of the basal diet. The level of (0.06%) increased EN, EW and EM by 21.36,3.54 and 25.99%, respectively, and improved FC by 18.41%. The third level (0.08%) gave 26.68,4.70 and 32.71% increase in EN, EW and EM, respectively, and improved FC by 23.46%.

The results presented in Table 2 and illustrated in figure 1 showed that the best egg mass (887.6) of El- salam hens was obtained with 0.33% DL-methionine (basal methionine 0.25 + added methionine 0.08%) at 2802 Kcal, ME / Kg diet followed by (841.5) for IMHA and (792.3) for LMHA. These findings indicated that 0.33% total methionine can be used in El-salam layer diets to give the best egg production. However, results showed that this level increased egg number by 31.43,20.19 and 26.68% when either DLM, LMHA or IMHA was used as source of methionine in the layer diets, respectively relative to egg number of the basal diet.

According to NRC, 1994, methionine requirement for Leghorn laying hens at 2900 Kcal, ME / Kg diet, was 0.33% to obtain maximum egg production.

Some studies were carried out to determine the requirements of sulpher amino acids for egg production. El-Moghazy (1993) reported that the highest egg number and mass were recorded by feeding dietary levels of 0.49% methionine for Hisex White layers. Much of variation in the reported requirement is accounted for by differences in feed intake when expressed as percentage of diet ( Pilbrow and Morris, 1974 ). Variety of methods which are used to derive requirement from a set of data is also another factor (Morris and Blackburn, 1982).

The results of egg production parameters showed that DLM was significantly the best on equimolar basis, whereas, IMHA was better than LMHA. Van Weerden et al. (1984) reported that, on a composite basis, feed conversion efficiency of the DL- methionine fed birds was slightly but significantly superior to that of the MHA fed birds. Also, they found that MHA fed hens consumed slightly more feed than the DLM fed hens to produce the same amount of egg mass. This was explained by a slightly lower molar effectiveness of MHA. Birds compensate this by eating more. These findings were in agreement with the present results.

### Effect of methionine supplementation on egg quality

The effect of methionine supplementation of the basal diet with DLM, LMHA or IMHA on the egg quality parameters is shown in Table 3. Values of AW, YW, SW and ST of the basal diet were 50.08, 36.95, 12.97 and 0.343, respectively.

Table 3. Effect of DLM, LMHA and IMHA on egg quality parameters.

Treatments	Methionine	Added methionine	Methionine equivalent	AW	W	SW	ST	AW+YW	AW/YW
	source	equivalent %	intake ( mg /hen / day )	%	%	%	(mm)	%	
1(basal)	;	0	258	50.08 ab	36.95 <sup>ab</sup>	12.97 <sup>ab</sup>	0.343 <sup>h</sup>	87.03	1.36
2	DLM	0.04	304	50.70 ab	37.09 ab	12.21 <sup>b</sup>	0.370 <sup>ef</sup>	87.78	1.37
က	DLM	90.0	321	50.73 ab	36.70 ab	12.57 <sup>ab</sup>	<sub>opo</sub> 086.0	87.43	1.38
4	DLM	0.08	341	51.28 a		13.00 <sup>ab</sup>	0.402 a	87	1.44
വ	LMHA	0.04	309	50.76 ab	35.70 <sup>b</sup>	13.54 a	<sub>6</sub> 858.0	86.46	1.42
9	LMHA	90.0	335	50.79 ab	36.55 <sup>ab</sup>	12.66 <sup>ab</sup>	0.375 def	87.34	1.39
7	LMHA	0.08	353	50.98 ab	36.34 ab	12.68 ab	0.388 <sup>bc</sup>	87.32	1.4
80	IMHA	0.04	311	49.43 <sup>b</sup>	37.36 <sup>a</sup>	13.21 <sup>ab</sup>	0.365 <sup>fg</sup>	86.79	1.32
6	IMHA	90.0	328	50.55 <sup>ab</sup>	37.04 ab	12.41 <sup>b</sup>	0.385 bod	87.59	1.36
10	IMHA	0.08	345	51.21 <sup>a</sup>	36.42 <sup>ab</sup>	12.38 <sup>b</sup>	0.395 <sup>ab</sup>	87.63	1.41
Methionine ef	Methionine effect (source): -								
1 (Basal)				50.08	36.95	12.97	0.343	87.03	1.36
2+3+4				50.90	36.50	12.59	0.384	87.40	1.39
2+9+2				50.84	36.18	12.96	0.374	87.02	1.41
8+9+10				50.40	36.94	12.67	0.382	87.34	1.36
Level effect (dose):	(dose):								
1 (Basal)				50.58	36.95	12.97	0.343	87.03	1.36
2+5+8				50.30	36.72	12.99	0.364	87.02	1.37
3+6+9				50.69	36.76	12.55	0.380	87.45	1.38
4+7+10				51.16	36.16	12.69	0.395	87.32	1.41

Means having different superscripts in the same column are significantly different (P<0.05).



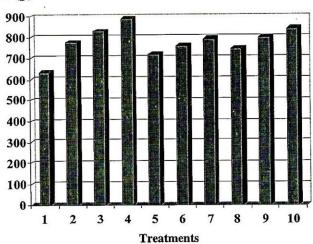


Figure 1. Effect of DLM, LMHA and IMHA on egg mass

There was insignificant effect on AW, YW and SW due to supplementation with DLM, LMHA and IMHA as compared with the basal diet.EL- Moghazy (1993) found that, increasing the level of methionine in layer diet slightly decreased these parameters, however, the effect was insignificant.

Results showed that AW/YW ratio of the basal diet is 1.36, whereas, those of DLM, LMHA and IMHA treatments were 1.39, 1.41 and 1.36, respectively. No significant differences could be found between those of the basal diet and those of IMHA methionine treatments. There were numerical differences between the three sources of methionine, whereas, LMHA gave the highest ratio of AW/YW.

Shafer *et al.* (1998) reported that albumin component yield increased significantly on a mass basis at 507 and 556 mg methionine / hen / day compared to 413 mg methionine / hen / day , also yolk mass yield was significantly increased at 556 mg methionine / hen / day compared to 413 mg methionine / hen / day .

Concerning shell thickness, results showed that there were significant differences between the basal diet and all treatments. It can be noticed that the shell thickness was improved by increasing methionine level of the three sources of methionine. Regarding DL-methionine supplementation, Petersen et al. (1983) reported that, reduced methionine intake significantly decreased egg weight and improved shell quality as measured by specific gravity.

#### The relative efficiency of DLM,LMHA and IMHA in layer diets

Irrespective to methionine supplemental levels, as an effect of methionine source, the results presented in Table 2 showed that DLM is better than LMHA and IMHA. However, the IMHA is better than LMHA in egg production of hens. The relative efficiency of LMHA as compared to DLM as an effect of methionine source was calculated to be 92.42,98.57,91.08 and 86.87% for EN, EW, EM and FC, respectively. While, the relative efficiency of IMHA was calculated to be 96.44, 99.35, 95.77 and 93.52% for EN, EW, EM and FC, respectively.

Several studies using chickens indicated substantially lower methionine activity for DL-Methionine hydroxy analogue calcium (MHA) than for DL-methionine (DLM) as reviewed by Saunderson (1985). However, Yanming Han et al. (1990) reported that

MHA is well absorbed by chickens and that any difference in bioavilability between DLM and MHA must be related to the in vivo conversion of MHA into L-methionine.

Analysis of data presented in Table 4 according to linear regression for the three levels of daily methionine intake (mg/hen) virsus the productive performance parameters, applied to estimate the relative efficiency, showed that the linear model fitting the data is Y = A + C X, where, Y is EN, EW, EM or FC, A is intercept, C is the regression coefficient and X is the daily methionine intake.

The parameters estimates for the linear model fitting DLM ( $Y_1=A_1+C_1X_1$ ), LMHA ( $Y_2=A_2+C_2X_2$ ) and IMHA ( $Y_3=A_3+C_3X_3$ ) are presented in Table 4.

From the slope calculations, the relative efficiency of LMHA ( $C_2/C_1$ ) was 55.36, 56.50, 54.65 and 49.65% for EN, EW, EM and FC, repectively (mean:54.04), while, the relative efficiency of IMHA ( $C_3/C_1$ ) was 79.71, 71.33, 77.37 and 76.24% for EN, EW, EM and FC, respectively (mean: 76.16).

Fisher *et al.* (1973) found that egg output increased linearly with incremental additions of methionine up to approximately 43g.,egg mass/ hen / day, with a methionine intake of about 235 mg / hen / day, above this level the increase in egg output was curvilinear, increasing levels of methionine brought about a gradual increase in egg production which plateaued at approximately 52g.egg output / hen / day, with a methionine level of 340 mg / hen / day.

Table 4. Parameters estimates of the linear regression (Y = A + CX).

Productive			DLM					LMHA					IMHA	11.5	angi
performance		;			2		7	•	Č	2		-	C <		81
parameters	mean	<u>-</u> د	mean C.V A	<u>.</u>		mean C.V A.Z	<u>-</u> د	AZ	7.		mean C.v A.s	<u>.</u>	2	53	na.
Egg number	16.53	4.25	1.38	16.53 4.25 1.38 51.305 0.849 15.54 4.03 6.93 28.405 0.746 16.07 3.7 3.79 40.893 0.851	0.849	15.54	4.03	6.93	28.405	0.746	16.07	3.7	3.79	40.893	0.851
Egg weight	47.16	1.68	37.19	47.16 1.68 37.19 33.748 0.657 46.66 1.39 40.88 19.068 0.552 46.93 1.29 39.71 24.073 0.655	0.657	46.66	1.39	40.88	19.068	0.552	46.93	1.29	39.71	24.073	0.655
Egg mass	781.4	4.29	-93.8	781.4 4.29 -93.8 2962.5 0.892 725.8 3.85 235.2 1618.9 0.827 755 3.6 66.98 2292.1 0.896	0.892	725.8	3.85	235.2	1618.9	0.827	755	3.6	86.99	2292.1	0.896
Feed conversion 3.777 5.13 8.21 -15.01 0.864 4.123 4.31 6.38 -7.452 0.715 3.948 4.73 7.38 -11.44 0.820	3.777	5.13	8.21	-15.01	0.864	4.123	4.31	6.38	-7.452	0.715	3.948	4.73	7.38	-11.44	0.820

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# مقارنة دل مثيونين هيدروكسى أنا لوج المحتوى على الكالسيوم المنتج محليا والمستورد ودل مثيونين باستخدام الدجاج البياض المحلى من سلالة السلام

#### مصطفى يوسف عطية

معهد بحوث الإنتاج الحيواني - مركز البحوث الزراعية - وزارة الزراعة - الدقى - جيزة - مصر

يهدف البحث إلى مقارنة الفاعلية الحيوية لكل من المثيونين هيدروكسى أنا لوج المحتوى على الكالسيوم المنتج محليا والمستورد ود.ل مثيونين باستخدام عليقة الذرة - كسب فول الصويا ناقصة في المثيونين (٢٠٥٠٪) للدجاج البياض المحلى .

استخدم في البحث عدد ٢٠٠ دجاجة من "سلالة السلام" عمر ٢٢ أسبوعاً تم تغذيتها على ١٠ معاملات تجريبية لمدة ١٦ أسبوعاً . تم إضافة كل من مصادر المثيونين الثلاثة إلى العليقة الأساسية المنخفضة في البروتين و الناقصة في المثيونين بمستويات ٤٠٠٠،٠٠٠،٠٠٠ ٪ على أساس التساوى في الوزن الوزن الجزيئي وفي نهاية كل فترة ٤ أسابيع يتم اخذ قياسات الأداء الإنتاجي ومواصفات البيض.

#### أهم النتائج المتحصل عليها :

- تم الحصول على افضل إنتاج للبيض عند مستوى 77... مثيونين كلى سواء بإضافة ما يعادل 1... د.ل مثيونين أو د.ل مثيونين هيدروكسى أنا لوج 1...
- لوحظ وجود زيادة معنوية في سمك قشرة البيض في جميع المعاملات بالمقارنة مع العليقة الأساسية .
- أوضحت المعاملات التي تحتوى على دال مثيونين إنتاج بيض افضل من المحتوية على دال مثيونين
  هيدروكسى أنا لوج كالسيوم المطى أو المستورد .
- الكفاءة النسبية للمثيونين هيدروكسى أنا لوج كالسيوم المحلى كانت ٩٢,٤٢ ، ٩٨,٥٧ ، ٩٨,٨٧ ، ٨٦,٨٧ ، ٨٦,٨٧ ٪ لكل من عدد البيض ووزن البيض وكتلة البيض ومعامل التحويل الغذائي على التوالي. وكانت للمثيونين هيدروكسى أنا لوج كالسيوم المستورد ٩٦,٤٤ ، ٩٩,٣٥ ، ٧٧,٥٧ ، ٢٥,٧٧ ٪ على التوالي .