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RESPONSE OF LAYERS FED ON DIETS DEFICIENT IN PROTEIN AND SULPHUR AMINO ACIDS SUPPLEMENTED WITH LIPID[1]

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ABSTRACT

An experiment was conducted to investigate the response of brown egg layers to supplemental fats in feeds inadequate in protein and total sulphur amino acids (TSAA). The physical and chemical composition of the egg was also examined. The deficient diets were prepared by diluting a basal diet with a protein-free, isoenergetic dilution diet. The supplemental fats consisting of a mixture of palm oil and olein, were added at levels of 30 and 32 g kg^{-1} respectively. The TSAA content of the first five diets ranged from 340 to 700 mg kg^{-1}. Diets 6-10 were similar to diets 1-5 except they were supplemented with fats. Increasing daily TSAA and protein increased egg weight and egg output significantly. Added fats improved egg weight and egg output significantly. Albumen output increased significantly after the addition of fat, whereas yolk protein output remained unchanged. Total protein output rose by increasing protein and TSAA intakes. Total fat content of the yolk increased due to added fats. The efficiency of protein and TSAA utilization was improved after the addition of fats to the diets, especially when protein and TSAA were inadequate.

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بررسی تاثیرافزایش چربی به‌جیره‌های کم‌پروتئین واسیدهای آمینه‌گوگرددار بر عملکرد مرغهای تخمگذار

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دانشجوی دوره دکترا در دانشکده غرب اسکاتلند و اکنون استادیار پرورش طیور گروه دامپروری دانشگاه صنعتی اصفهان .

خلاصه

به منظور بررسی عملکرد مرغان تخمگذار نسبت به افزایش چربی به جیره‌های کم‌پروتئین و اسیدهای آمینه گوگرددار آزمایشی به اجراء درآمد. همچنین ترکیب شیمیایی و فیزیکی تخم‌مرغها مورد مطالعه قرار گرفت. غذاهای فقیر با رقیق کردن یک جیره هم‌انرژی و فاقد پروتئین تهیه گردیدند. چربیهای اضافه شده شامل مخلوطی از روغن پالم و اولئین بود که به ترتیب به میزان ۳۰ و ۳۲ گرم در کیلوگرم به جیره اضافه شوند. اسیدهای آمینه موجود در پنج جیره اول بین ۳۴۰ تا ۷۰۰ میلیگرم در کیلوگرم در نوسان بود. جیره‌های ۶ تا ۱۰ مشابه با جیره‌های ۱ تا ۵ بودند غیر از اینکه به آنها چربی اضافه شد. افزایش مصرف روزانه اسیدهای آمینه گوگرددار و پروتئین، وزن تخم‌مرغ و تولید تخم‌مرغ را بطور مشخص افزایش داد. افزودن چربی وزن و تولید تخم‌مرغ را بهبود بخشید. با اضافه کردن چربی به جیره تولید سفیده بطور مشخص بالا رفت، درصورتیکه تولید پروتئین زرده تغییری ننمود. کل پروتئین تولیدی پس از افزایش مصرف پروتئین و اسیدهای آمینه گوگرددار زیاد شد. کل چربی زرده با افزایش چربی بالا رفت. راندمان استفاده از پروتئین و اسیدهای آمینه گوگرددار خصوصاً با جیره‌های کم‌پروتئین و اسیدهای آمینه گوگرددار در اثر افزایش چربی به جیره بهبود یافت.

INTRODUCTION

Fat supplementation of laying hens frequently results in an increase in egg weight. This occurred when vegetable oils were included to mitigate a linoleic acid deficiency (4, 23) or increase dietary metabolizable energy (ME) (13).

Some reports showed that the increased egg weight was not attributed to supplemental linoleic acid (39) or increasing of ME (5). Conflicting evidence indicated no effect of added fat on egg weights (2, 14, 37).

The synergistic interaction between fats and other dietary nutrients (36) and enhanced energy utilization from non-lipid dietary constituents of the diet have also been demonstrated (19, 20).

Experiments have shown that apart from fatty acids, no significant changes occurred in the chemical composition of the egg, by supplemental fats (18, 25, 30). On the other hand, increasing fat deposition in the yolk produced by birds kept on a low-fat diet for 12 months from day of hatch by adding fat has been reported (3).

Little information is available regarding changes in the yolk and albumen weights consistent with changes in the whole egg weight.

The following experiment was carried out to test the sensitivity of the hens response to supplemental lipids over a wide range of TSAA intakes. The efficiency of utilization of protein and TSAA was also investigated. The effect of supplemental fats added in isoenergetic diets on the physical and chemical composition of the egg was evaluated.

MATERIALS AND METHODS

The experiment was carried out in a windowless house, with a daylength of 17 h and the temperature maintained at 21 to 23°C. Four birds were housed in each three tiers Patchett cage[1].

Six adjacent cages formed an experimental replicate. The birds were fed on a conventional layer diet before the experiment. Daily record of mortality and egg production were kept. Eggs were weighed in bulk on three consecutive days biweekly for each replicate. Seven hundred and twenty Hisex Brown layers at 50 weeks of age were divided at random into 30 plots, 24 birds per plot. Ten experimental isoenergetic diets were fed *at libitum* to three replicates (one replicate per block or tier) of birds from 50 until 60 weeks of age.

The initial body weight was recorded and body weight was then determined in the weeks six and 10 of the experiment per tier. Dietary protein was determined by the method of Spillane (34), and calcium and phosphorus were determined spectrophotometrically (1).

The experimental diets were prepared by mixing appropriate portions of basal and dilution diets and by adding palm oil and olein at the levels of 30 and 32 g kg^{-1}, respectively. Diets one to five contained 3.4, 4.0, 4.6, 6.0 and 7.0 g kg^{-1} TSAA which were 72%, 85%, 98%, 127% and 148% of daily TSAA

1. Ryefield Works, Clayton Heights, Queensbury, BD13 1DS, West Yorkshire, England.

requirement (0.47%) (8), respectively. Diets six to ten were similar to diets one to five accordingly, except they contained palm oil and olein as sources of palmitic and oleic acids respectively. Cellulose was used as an inert compound to keep diets equi-energetic and fat was used *in lieu* of glucose. The composition of the basal, dilution and experimental diets are shown in Tables 1 and 2. Table 3 indicates the fatty acid composition of fats used.

In order to eliminate the effect of loss in body weight the data for the last four weeks of the experiment were taken as the measure of response to the treatment.

At week eight of the experiment, eight eggs were randomly collected from each plot. The eggs were broken and their physical composition was determined as described previously (27).

Samples of egg white and yolk were collected, blended and placed in polyethylene bottles with screw-type polyethylene caps and were frozen for protein determination. Subsamples were saved for fat determination. The fat content of the yolk was determined gravimetrically following extraction in 2:1 V/V chloroform-methanol by a modified procedure (10).

The analyses of variance were performed using Minitab (31) and EDEX (15) computerized programs. Egg component data were subjected to regression analysis.

RESULTS

The results obtained for egg weight, egg output, body weight changes, feed intake and feed conversion are shown in Table 4. Increasing dietary TSAA up to 640 mg/hen/day increased egg weight and egg output significantly ($P<0.01$; Table 1, Fig. 1). Added fats increased egg weight and egg output on all levels of TSAA intake, but the differences were significant at certain dietary TSAA (diet five vs. diet 10 for egg weight and diet three vs. diet eight for egg output). Maximum improvement in egg output due to supplemental fats was

Table 1. Composition of basal and dilution diets.

Ingredients	Basal g kg^{-1}	Dilution[†] g kg^{-1}
Wheat	488.0	-
Maize	100.0	-
Full fat soya	48.0	-
Soybean meal, 44% protein	198.0	-
Fish meal, 65% protein	36.2	-
Corn oil	4.4	26.0
Oat hulls	-	166.2
Maize starch	-	490.0
Glucose	-	193.0
Ground limestone	103.0	103.0
Dicalcium phosphate	13.2	13.2
Salt	3.0	3.0
Vitamin & mineral supplement[‡]	5.0	5.0
Yolk color[§]	0.6	0.6
Tryptophane supplement	0.6	-
Total	1000.0	1000.0

[†] Contained little digestible protein.

[‡] Provided the following vitamins and minerals per kilogram of diet: Vit. A, 16000 IU; Vit. D3, 3000 IU; Vit. E, 16 IU; Vit. K, 4 mg; Riboflavin, 6 mg; Pantothenic acid, 9 mg; Choline, 200 mg; Nicotinic acid, 20 mg; B12, 8 g; Mn, 120 mg; ZN, 120 mg; Fe, 80 mg; Cu, 9 mg; Co, 0.4 mg; Iodine, 1.0 mg; Se, 0.2 mg; Antioxidant, 145 mg.

[§] Carophyll Orange was used as the coloring agent.

Calculated analysis per kg.

		Summit	Dilution
ME	MJ	11.20	11.2
Protein	g	189.00	-
TSAA	g	6.16	-
Fat	g	22.00	28.0
Linoleic acid	g	13.20	13.2

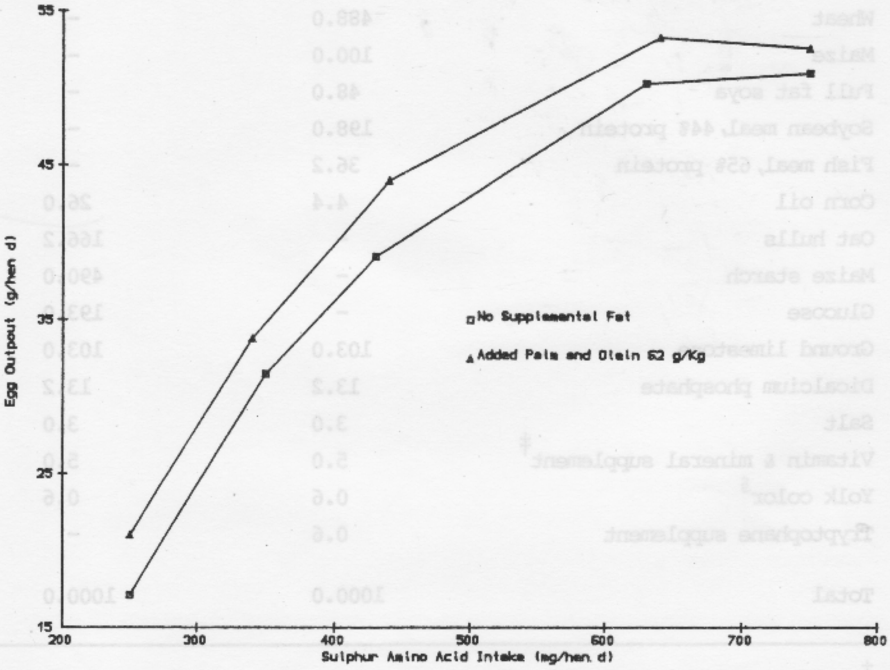

Fig. 1. Egg output response due to increasing SAA intake with and without supplemental fats.

Table 2. Composition of experimental diets.

Diet No.[†]	Basal[‡]	Dilution[‡]	Glucose	Cellulose	Palm oil[§]	Olein[§]	DL-methionine
	Constituents g kg^{-1}						
1	550	300	150	-	-	-	-
2	650	200	150	-	-	-	-
3	750	100	150	-	-	-	-
4	850	-	150	-	-	-	0.75
5	850	-	150	-	-	-	1.70
6	550	300	-	88	30	32	-
7	650	200	-	88	30	32	-
8	750	100	-	88	30	32	-
9	850	-	-	88	30	32	0.75
10	850	-	-	88	30	32	1.70

[†]Diets 1 and 6 contained 104 g kg^{-1} protein and 3.4 g kg^{-1} TSAA.
Diets 2 and 7 contained 123 g kg^{-1} protein and 4.0 g kg^{-1} TSAA.
Diets 3 and 8 contained 142 g kg^{-1} protein and 4.6 g kg^{-1} TSAA.
Diets 4 and 9 contained 160 g kg^{-1} protein and 6.0 g kg^{-1} TSAA.
Diets 5 and 10 contained 160 g kg^{-1} protein and 7.0 g kg^{-1} TSAA.
Diets 1 to 5 contained 23.8 g kg^{-1} total lipid.
Diets 6 to 10 contained 85.8 g kg^{-1} total lipid.
[‡]For composition see Table 1.
[§]For fatty acid composition see Table 3.

Table 3. Fatty acid composition of fats used in layer diets.[†]

Lipid	Fatty acids (%)					
	C12	C14	C16	C18	C18:1	C18:2
Palm oil	-	2	41	5	43	9
Olein	3	3	4	1	71	10

[†]Lipids were supplied by Unichema International Ltd. United Kingdom, Bebington, Wirral, Merseyside L62 4UF.

Table 4. Egg weight, egg output, body weight change, feed intake and feed conversion of laying hens fed on diets different in sulphur amino acids supplemented with fats.

Diets	Added fat g/kg	TSAA intake	Egg weight g	Egg output g $hen^{-1}d$	Body wt change g hen^{-1} d	Feed intake g hen^{-1} d	Feed conversion g feed g^{-1} egg
1	None	250	55.3a	17.1a	-1.6	80.2a	4.76a
2	None	350	59.6b	31.5b	-1.0	96.4b	3.10c
3	None	430	62.3cd	39.1c	-1.3	102.3bc	2.64cd
4	None	630	67.0f	50.3e	-0.7	114.0de	2.27e
5	None	750	65.2e	51.0e	0.0	115.5e	2.26e
6	62 P+O	250	56.4a	21.0a	-1.3	80.2a	3.89b
7	62 P+O	340	61.3bc	33.8b	-1.4	94.6b	2.81cd
8	62 P+O	440	63.0d	44.0d	-0.4	105.6cd	2.41de
9	62 P+O	640	67.7f	53.3e	0.4	117.1e	2.20e
10	62 P+O	750	67.8f	52.6e	0.4	115.0e	2.19e
SE			±0.56	±1.63	±0.7	±3.08	±0.18

Daily TSAA intake were calculated on the basis of determined TSAA content of basal diet.

Values followed by the same letters in each column are not significantly different (P 0.05).

P = Palm oil, O = olein.

obtained with daily TSAA intake of 440 mg hen^{-1}. Egg output response to added fats diminished at higher daily TSAA intake. Feed conversion was improved significantly at the lowest level of daily protein and TSAA intake when diets were supplemented with fats (diet one vs. diet six). Feed conversion was improved slightly with added fats. While daily TSAA and protein intakes increased, rate of improvement in feed conversion diminished. Supplementary lipids had no significant effect on feed intakes and daily body weight changes. Efficiency of protein and TSAA utilization was improved when diets were supplemented with fats (Table 5; Fig. 2). The maximum improvement was obtained when protein and TSAA were most deficient (104 g kg^{-1} protein and 3.4 g kg^{-1} TSAA, diet six). The rate of improvement in protein and TSAA utilization decreased, after increasing protein and TSAA intake.

Table 5. Daily output efficiency of protein and sulphur amino acids as percent of daily intake.

Diet No.	Added fat g/kg	Efficiency (%)	
		Protein	TSAA
1	None	26.7	40.9
2	None	32.8	53.0
3	None	34.7	53.7
4	None	34.6	47.4
5	None	34.5	40.1
6	62 P&O	31.5(4.8)	50.2(9.3)
7	62 P&O	36.0(3.2)	58.0(5.0)
8	62 P&O	36.7(2.0)	58.7(5.0)
9	62 P&O	34.8(0.2)	48.9(1.5)
10	62 P&O	35.5(1.0)	42.4(2.3)

Protein efficiency was calculated on the basis of determined daily protein output. TSAA efficiency was calculated on the basis of 5.91 mg of TSAA content per gram of shell egg, respectively, Stadelman and Cotterill (35).
Figures in parentheses represent percent changes in the protein and TSAA efficiencies of corresponding diets without added lipid.
P = Palm oil; O = olein.

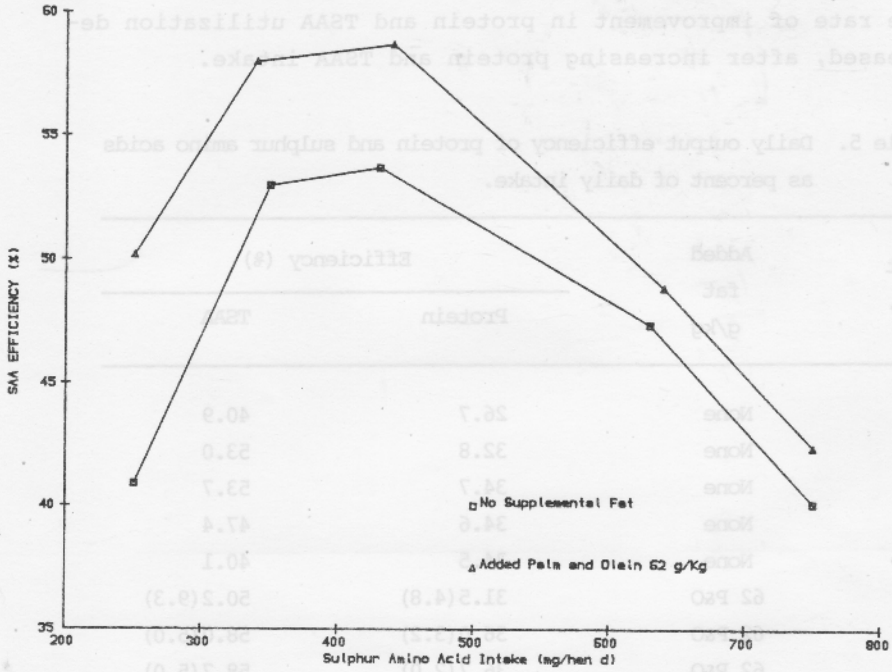

Fig. 2. Efficiency of SAA utilization due to increasing SAA intake with and without supplemental fats.

The results for physical components and percentage of yolk fat of eggs are presented in Table 6. Table 7 shows the regression coefficients of egg components. A significant increase in yolk and albumen output was obtained due to increased protein and TSAA intake. Although added fats improved daily yolk and albumen output only the difference in yolk output for one level of TSAA was significant (diet three vs. diet eight).

At all TSAA levels yolk fat was numerically increased by the addition of the dietary fats and this effect was significant ($P<0.01$) at the highest level of protein and TSAA intakes (diet five vs. diet ten. Those hens fed on high TSAA and

Table 6. Yolk and albumen percentages and outputs of hens fed diets with different sulphur amino acid content supplemented with fats.

Diet No.	Added fat g kg^{-1}	TSAA intake mg $hen^{-1} d^{-1}$	Yolk (%)	Albumen (%)	Yolk g $hen^{-1} d^{-1}$	Albumen g $hen^{-1} d^{-1}$	Yolk fat (%)
1	None	250	28.8	61.0	4.9a	10.4a	33.1abc
2	None	350	28.6	60.8	9.0b	19.1b	29.5a
3	None	430	27.9	61.3	10.9c	24.0c	30.2a
4	None	630	27.6	62.7	13.3de	31.5d	32.4ab
5	None	750	26.8	63.0	13.6de	32.1d	33.0ab
6	62 P+O	250	28.3	61.3	5.7a	13.2a	33.4abc
7	62 P+O	340	28.2	61.0	9.5bc	20.6b	31.0ab
8	62 P+O	440	28.8	61.0	12.7d	26.7c	33.5abc
9	62 P+O	640	26.8	62.7	14.4de	33.4d	34.4bc
10	62 P+O	750	27.8	62.0	14.5e	32.6d	36.1c
SE			±0.5	±0.58	±0.57	±0.96	±0.75

Not significant.

Values followed by the same letters in each column are not significantly different ($P>0.05$).

P = Palm oil; O = olein.

Table 7. Regression coefficients of physical components of the egg.

Independent variable	Dependent variable		
	Albumen	Yolk	Shell
Total egg	0.731±0.014	0.200±0.013	0.073±0.005
Yolk	1.220±0.158	--	--

protein diets produced eggs which contained higher yolk fat when their diets were supplemented with fats. Results for yolk, albumen and total protein outputs are presented in Table 8. There were no significant differences between percentage of yolk and albumen protein. Daily yolk, albumen and total protein outputs increased significantly ($P<0.01$) due to increasing daily protein and TSAA intakes. Only protein output from albumen was increased significantly by the addition of lipid. Total protein output was increased significantly ($P<0.01$) only at the lowest level of dietary protein and TSAA (diet one vs. diet six). Generally, the rate of increase in albumen and total protein output diminished as the protein and TSAA intakes increased.

DISCUSSION

The data obtained indicate an important effect of unsaturated fats on egg weight as reported previously (39). Results showed that about 630 mg hen^{-1} d^{-1} of TSAA was enough for optimum egg output (50.3 g $hen^{-1}d^{-1}$ without supplemental lipids. When fat was included a daily TSAA intake of 640 mg hen^{-1} was enough to obtain an egg output response of 53.3 g hen^{-1} d^{-1}. This can be attributed to the effect of fats on improving efficiency of dietary nutrients and increasing the percentage of yolk fat which resulted in an increase in both yolk and albumen output.

Table 8. Yolk, albumen and total protein outputs of hens fed diets with different sulphur amino acid content supplemented with fats.

Diet No.	Added fat g kg^{-1}	TSAA intake mg $hen^{-1} d^{-1}$	Protein output g $hen^{-1} d^{-1}$		
			Yolk	Albumen	Total
1	None	250	1.15a	1.07a	2.22a
2	None	350	1.89b	2.02c	3.91c
3	None	430	2.53c	2.51e	5.04e
4	None	630	2.84d	3.50g	6.34f
5	None	750	2.95e	3.45g	6.40f
6	62 P+O	250	1.26a	1.56b	2.62b
7	62 P+O	340	1.86b	2.34d	4.20c
8	62 P+O	440	2.53c	2.98f	5.51e
9	62 P+O	640	2.85d	3.57g	6.42f
10	62 P+O	750	2.99e	3.54g	6.53f
SE			±0.09	±0.06	±0.13

Values followed by the same letter in each column are not significantly different ($P>0.05$).
P = Palm oil; O = olein.

Added fats improved egg weight, egg output and feed efficiency and such improvements have also been demonstrated by other investigators (9, 12, 22, 28, 29, 33).

The response curve (Fig. 1) obtained indicates that laying hens responded to supplemental fats at any level of dietary TSAA but the rate of response diminished as dietary TSAA increased. Similar results were obtained by Reid (29) who indicated a significant increase in egg production and egg output with 14% protein and SAA-restricted diets. However, Waible (38) and March and Biely (17) working with growing chickens reported that when dietary protein was suboptimal, added tallow did not increase growth.

Inconsistency in the observation of Waible (38) with the present result could be due firstly to the age of the birds as they used young chicks in their experiments and secondly to the type of fat used. Response of hens fed suboptimal protein and TSAA can be affected by the nature of the fat (40), rate of inclusion (24) and the hen's age. As indicated by Whitehead (39) unsaturated fats are utilized better, because of better digestibility and absorbability, therefore they exert a better synergistic effect on non-lipid dietary constituents. This shows that lower specification diets can be used when supplemental unsaturated lipids are used in laying hen diets. By adding lipids, lower daily TSAA is required to produce equal egg weight and egg mass output as compared to unsupplemented diets.

Although the egg output differences due to supplemental fats were not statistically significant, the differences are important from an economic point of view. At optimum egg output (diet four vs. nine) adjusted TSAA and protein intakes required for production of 50 g egg d^{-1} were 20 mg and 0.6 g hen^{-1} d^{-1} less, when diet was supplemented with fats. Also about 3.5 g $hen^{-1}d^{-1}$ less feed was required for such egg production which would save about 1.3 kg feed hen^{-1} during the production year. This together with lower specification diets which, can be used in the presence of fats, will reduce the production cost considerably.

The data obtained indicated that supplemental fat did not reduce the total protein output and in fact the albumen output was raised by the addition of fat which suggests that fat caused an increase in albumen weight by producing larger yolks and probably a stimulation of the oviduct to produce more albumen. The regression analysis of egg components data (Table 7) indicated that for an average increase in egg weight of one gram, there was an average increase in yolk weight of 0.2 g and in albumen weight of 0.731 g. For an average increase in yolk weight of 0.2 g there was an increase in albumen weight of 0.244 g which shows that of 0.731 g increase in albumen weight about 0.487 g was due to the direct stimulatory effect of fat on the oviduct. Therefore the total egg protein output remains almost constant and fat is not deposited in the yolk at the expense of the total egg protein. Contribution of both yolk and albumen weights in increasing egg weight due to added fat conforms the results obtained by Griffin *et al.* However Sell *et al.* (32) indicated that fat supplementation increased only yolk weight at the earlier stages of production. This inconsistency may be due to age and strain differences. Supplemental fats improved the efficiency of protein and TSAA and a maximum improvement was obtained at the lowest level of dietary TSAA and protein (see also 26). This indicates a synergistic effect between fats and other key nutrients. Fats slow down the food transport in the gut (21) and/or supply more available metabolizable energy which consequently improves the utilization of dietary nutrients (20).

Added fats caused an increase in percent fat of the yolk, which was proportional to the daily protein and TSAA intake. Balnave (3) indicated a significant increase in yolk size following the addition of fat to the diet of layers. This was attributed to the fat deposition in the yolk which is in agreement with the present results. It is evident that the levels of dietary protein and TSAA affect fat utilization by the hens and suggests fat may be better incorporated into the yolk at higher dietary protein levels (6, 7, 36). However other reports (16, 18) found no changes in the fat content of the yolk by supplementary fat.

It is concluded that a mixture of palm oil and olein could give a good response when added at a level of 62 g kg^{-1} and that oleic acid increased both egg weight and egg output. Also laying hens can be fed with lower specification diets, if fat is included in the diet. Supplemental fats improve utilization of protein and TSAA and less feed is needed during the production period. Percentages of yolk, albumen, yolk protein and albumen protein did not change after the addition of fats, whereas yolk fat content increased. Both yolk and albumen contributed to increasing egg weight. Increasing dietary fat did not reduce yolk protein, albumen protein and total protein outputs and fat was not deposited in the yolk *in lieu* of protein.

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