

EFFECTS OF REDUCING DIETARY CRUDE PROTEIN WITH AMINO ACIDS SUPPLEMENTATION ON PERFORMANCE OF COMMERCIAL WHITE LEGHORN LAYERS DURING LATE PRODUCTION PERIOD

By

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Abstract: *Several strategies are being developed to minimize nitrogen (N) pollution from poultry waste in areas with high concentrations of commercial poultry operations. This study focus on the effect of feeding reduced protein, (CP) diets on laying hens housed under commercial conditions. The experiment was carried out using 144 Hy-Line W-36 hens which were randomly assigned to one of four dietary treatment groups. Corn-soy based diets varying in dietary crude protein (CP) levels supplemented with commercially amino acids as follows: [1] 17% CP + Met, [2] 16% CP + Met & Lys, [3] 15% CP + Met & Lys, and [4] 14% CP + Met, Lys & Thr, were fed to layers from 53 to 64 weeks of age. Cage was considered the experimental unit (4 hens/cage), and each treatment was replicated 9 times. Using final body weights (BW), feed consumption (FC), protein intake (PI) and feed conversion ratio (FCR), egg production (EP), egg weights (EW), egg mass (EM) and egg components and solids data as growth and productive parameters during the studied period (11 weeks), one slope, broken-line regression models were employed to estimate the break point for each parameter due to reducing CP in Commercial White Leghorn hens diets.*

Mean differences in BW, FC and FCR among dietary CP levels were not significantly different. However, PI data showed that reducing dietary CP levels resulted in a significant ($P \leq 0.001$) PI linear reduction in hens fed the 14% CP consuming the lowest PI 13.10 g/ hen as compared to all other dietary CP treatments. Estimating PI break point, hens fed the 16.78% CP consumed 16.01 g/ hen which was similar ($P \leq 0.001$) to 17% CP and higher than hens fed 14, 15 or 16% CP, respectively. Numerically higher EP was recorded for hens fed on the 17% CP diet (84.74%), and heavier EW was recorded for the 16% CP (63.34 g). Albumen solids were reduced

by lowering CP in dietary treatments from 12.17% (17% CP) to 11.65% (14% CP). Break point significant estimate ($P \leq 0.001$) obtained from the one-slope broken line regression model for albumen solids was 12.12% at 15.53% CP, while no significant effects were noted for FCR, albumen and yolk solids percentages due to dietary treatments.

Based on current results, applying the break point regression model estimates resulted in more accurate data of how much dietary CP could be reduced while maintaining optimum growth and production by amino acids supplementation. Feeding 15 and 16% CP diets supplemented with synthetic amino acids could be suitable for maintaining BW and adequate production of commercial laying hens during late production period.

Key Words: *Laying hens, crude protein, late production, amino acid diets, break point regression model, performance*

INTRODUCTION

Commercial laying hen diets consist of essential amino acids ranging in concentration from 122 to 275% of the requirement. Amino acids in excess of the requirements impair production performance through numerous interactions and result in environmental pollution with nitrogen (Austic, 1981). The most feasible strategy for coping with this problem is the partial replacement of the intact dietary protein with crystalline amino acids. The potential for reducing dietary protein has become a reality because of the commercial availability of Lys, Met, Thr, and Trp in the market (Ishibashi and Yonemochi, 2003). With the current interest in reducing nutrients content of manure as it affects disposal and environmental concerns. Nutrients of greatest concern are usually N and P because of their relative amounts in the manure and compared to that needed for optimum disposal on any given acreage (Leeson and Caston 1996). Dietary energy and protein represent approximately 85% of total feed cost. Dramatic improvements in the productivity of poultry flock in general, and laying hens specifically could be attributed to improvements in feed ration formulations (Yakout *et al.*, 2004). Detailed knowledge of nutrients requirements are indeed of necessity for continuous improvement in productivity (Gunawardana, *et al.*, 2008). Increasing levels of protein (Nahashon *et al.*, 2010, Parsons *et al.*, 1993, Keshavarz and Nakajima, 1995, Leeson, 1989), methionine (Keshavarz, 1995), and lysine (Zimmerman, 1997) have resulted in improvements in production parameters especially in improving EW. Furthermore, several studies have examined the effects of feeding low-protein diets to laying hens. In an early

work, Keshavarz (1984) observed lower BW at 20 wk and decreased performance during the early phase of the egg production cycle when pullets were given low-protein diets during the rearing period. Hsu *et al.*, (1998) evaluated the layer response to either a low-protein (14%) or control (17%) diet in a 5-wk experimental period and found similar responses to both diets in terms of EP and FCR. Blair *et al.*, (1999) compared a 13.5% CP diet with a control diet (17% CP) on layer performance. They concluded that layer performance could be maintained well on the low-protein diet when diets were properly supplemented with essential amino acids. At the same time, nitrogen excretion was reduced by 30 to 35%. More recently, Junqueira *et al.*, (2006) indicated that the performance of laying hens in the 2nd laying cycle of an 8-wk experiment was comparable between the 16 and 20% CP diets.

With the fact that the liquid egg and breaker egg industry growing dramatically during the last decade, there are very few studies in which the effects of the protein on egg composition and egg solids of Hy-Line W-36 hens have been prevailed. With sharp increases in energy cost, it is important to have a better understanding of how to maximize the use of dietary feed rations at different protein levels to optimize performance and yield especially for the egg breaker industry (Gunawardana, *et al.*, 2008).

In order to gain optimal revenue of research resources, an effective procedure can be used to evaluate nutrient requirements (Lamberson and Firman, 2002). Also, utilization of alternative feedstuffs, formulating diets based on digestible amino acids, applications of biomass, bio-refinery of agriculture by-products, and application of ideal protein concept (Baker and Han, 1994; Kerr and Kidd, 1999) may result in utilizing lower crude protein levels than those recommended by NRC (1994) or currently used by the industry. Applying the broken-line models, as an application that yields an objective estimate of the most suitable dietary level of any nutrient may be considered as a fully adequate to optimize growth performance, known as nutrient “*requirement*” (Robbins, 1986; Robbins *et al.*, 1979). Taken in consideration the simplicity of broken-line models, its use for growth data determination is sometimes preferable since it is often easier to interpret (Robbins, 1986).

The current trial was carried out to determine how much dietary crude protein could be reduced, while supplementing diets with synthetic amino acids would serve for better growth and/ or production parameter of commercial white leghorn laying hens during late production phase from 53 to 64 weeks of age.

MATERIALS AND METHODS

Experimental birds and management:

This study was conducted at a private sector farm in Behaira¹ district. One hundred and forty four White Commercial laying hens² at 53 weeks of age were randomized into layer cages and were fed one of four dietary treatment diets (Table 1). Corn-soy based diets varying in dietary protein supplemented with commercially available amino acids as follows: [1] 17% CP + Met, [2] 16% CP + Met & Lys, [3] 15% CP + Met & Lys, and [4] 14% CP + Met, Lys & Thr were fed from 53 to 64 weeks of age. Treatment diets were formulated on a digestible amino acid basis according to NRC (1994) and Hy-Line W-36 commercial management guide³ recommendations for laying hen nutrients requirements. Cage was considered the experimental unit (4 hens/ cage) with each treatment replicated 9 times.

Feed and water were provided *ad-libitum* all over the experimental period (11 weeks) from 53 to 64 weeks of age. Starting the 53rd week, hens were individually weighed, wing-banded and were allocated into treatments based on almost similar BW.

All hens were individually weighed once every 4 weeks and at the end of the experiment, while FC was recorded on daily basis. Growth and productive performance criterions were evaluated as of BW, FC, PI, FCR, EP and EM while a weekly, 1- day egg production was used for measuring EW and wet egg components percentages and solids which were conducted every 2 weeks.

Statistical analysis:

A one way analysis was utilized in which all data generated was analyzed by general linear models (GLM) procedures of SAS[®] software (SAS, 2003). In addition, one slope, broken-line regression models (Robbins, 1986; Knowles *et al.*, 1997) were used to estimate how much reduction could be utilized with crude protein for laying hens during the studied period. The following model was used to determine differences $Y_{ij} = \mu + a_i + e_{ij}$; where

¹Al-Ashraf for Animal Production farm, Behaira - Egypt

²White Commercial Leghorn hens (WCL) Hy-Line W36

³Hy-Line W036 commercial management guide 2009-2011, 1755 West Lakes Parkway, West Des Moines, Iowa 50266 U.S.A

Y_{ij} variable measured; μ overall mean; a_i = effect of the i^{th} level and e_{ij} = error component. Significance of difference was based on the probability of a type I error set at ($P \leq 0.05$). The differences among means were tested utilizing Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

No significant effects were found due to reducing dietary CP to 14%; however a 2.20% decrease in BW was obtained as a result of dietary protein reduction to 14% with amino acids supplementation (Table 2). These results of BW were in agreement with data reported by Sohail *et al.*, (2003) and Keshavarz and Nakajima (1995), who reported no significant effect of reducing dietary protein levels on BW. Feeding the strain guide recommendation which is used by the industry CP (16% + Met & Lys) resulted in slightly heavier hens BW (1560 g/ hen) as compared to the NRC recommended group (15%; 1486 g/ hen). Average final BW for the whole trial was around 1526 g/ hen, which is slightly below the strain guide recommendations at similar age. It seems that body protein reserves were not depleted in birds fed reduced-CP diets at late production period. Tested diets may have provided hens with all needed essential amino acids that were needed to maintain their BW, or due to the availability and balanced amino acids provided through tested diets. Results obtained suggest that BW reduction was due to lowering dietary CP (*although it was non-significant*), and indicating the availability of synthetic amino acids (especially Lysine) and good utilization of feed rations used which resulted in better performance as shown for layers BW at upper dietary levels (16 and 17% CP).

Feeding low protein diets supplemented with amino acids non-significantly ($P \geq 0.001$) reduced FC from 94.15 (diet 1) to 93.50 g/ hen/ day (diet 4) with hens fed diet 4 consuming the lowest amount of feed (93.50 g/ h/ d) as compared to all other dietary CP treatments. Research results with laying hens reported by (Novak *et al.*, 2007, Novak *et al.*, 2008) drew similar conclusions. In the present study, PI was significantly reduced ($P \leq 0.001$) with dietary CP reductions, as hens fed the 17% diet consumed (5.06%) more protein as compared to the strain guide recommendation which is used by the industry (16% CP), 11.93% more PI when compared to the NRC (1994), and 18.18% when compared to the lowest CP diet (14%), respectively. Zou *et al.*, (2005) reported that reducing dietary PI from 16.3 to 15.3 g/ hen per day reduced EP by 3.2%, while Keshavarz (1995) indicated that EP was only decreased by 1.9% with dietary protein intake reduction from 21.4 to 17.4 g/hen per day. Hens fed the lowest CP level (diet 4) numerically presented the

poorest FCR values (1.92 g. feed/ g. egg), while hens fed the other three CP levels had an average of 1.84 g. feed/ g. egg.

Hen-day egg production was lower in the low protein group as reducing dietary protein levels from 17 to 14% numerically reduced EP by 4.89% (compared to the 16% diet; industry) and 6.77% (compared to 14% CP; Table 2), respectively. With respect to EP, results of this experiment were in agreement with those reported by Novak *et al.* (2006), who suggested that the effects of the reduced-CP diet were more dramatic during the late stage production (43 to 63 weeks of age). Similarly, Liu *et al.*, (2005) and Wu *et al.*, (2005) reported that reducing dietary protein reduced EP. Despite the satisfactory results of feeding a reduced-CP diet for short periods Keshavarz and Austic (2004), concluded that feeding reduced-CP diets for a longer period can result in poor egg production.

The heaviest EW was recorded for the 16% CP (63.34 g; Table 3), while the lowest EW (61.91 g) was recorded for hens fed the 14% CP (diet 4), although it maintained hen needs but this lower EW may be attributed to an inadequate level of total N (Leeson and Caston, 1996). However, comparable EW between reduced-CP diets and Hy-line W-36 guide recommendations used by the industry diet (16% CP) in the present study suggest that the reduced-CP diet was well fortified with essential amino acids and had an adequate level of total N. The improvement in EW in the other three CP diets might be; as mentioned earlier in BW data; related to the availability and balanced amino acids provided through tested diets.

Egg mass data presented in (Table 3) showed that diets 2 & 3 (16 & 15% CP) maintained EM at 51.07 and 50.38 g, while reducing CP to 14% (diet 4) resulted in a significantly the lowest EM ($P \leq 0.001$; 48.86 g.) as compared to diet 1 (17% CP). The broken line-slope estimated better EM of 51.33 g at 16.15% CP, which is slightly higher than the strain guide recommendation of 16%. Hens fed the reduced-CP diet produced fewer eggs and, as a result, had lower egg mass compared with other dietary treatments. This discrepancy with the present findings may be due to a difference in essential amino acid balance between treatments. Sohail *et al.*, (2003) demonstrated that essential amino acids had a significant influence on egg weight so that removing an indispensable amino acid resulted in reducing EW within 2 weeks. It is concluded, based on these results of the present study and a review of the relevant literature, that the response in EP was more sensitive to reduced CP diets than was egg size.

Yolk percentages (Table 3) confirm EW data as reducing dietary CP resulted in significantly ($P \leq 0.01$) higher yolk percent which was especially noted for hens fed on 14% CP diet having higher yolk (29.98%) as compared to those fed the upper CP (17%; 29.02% yolk). Confirming EW data and showing that with older hens less number of eggs is produced and EW is heavier which may affect egg components by increasing yolk percent. No significant effects ($P \geq 0.05$) due to dietary treatments were noted for albumen percentages. Dietary treatments had no significant effects on yolk solids (Table 3). However, albumen solids were reduced by lowering CP in dietary treatments from 12.17% (17% CP) to 11.65% (14% CP). Significant estimate ($P \leq 0.001$) obtained from the one-slope broken line regression model was for albumen solids of 12.12% at the break point of 15.53% CP.

Current research results indicate that CP requirements (15%) of laying hens recommended by NRC (1994) and the strain guide recommendations (16%) is adequate to maintain BW, FC, EP, FCR, EW and egg components (albumen). However, 16.150% is needed to maintain better EM, Yolk% (14.90%), 15.53% for albumen solids, and 16.78% for PI. Applying the one-slope broken Line regression model estimates resulted in obtaining accurate data of which dietary CP would serve better for optimum growth and productivity. Feeding 15 or 16% CP diet supplemented with synthetic amino acids could be suitable for adequate growth and production in order to ensure that dietary CP will not be in excess for commercial laying hens during late production period (53 to 64 weeks of age).

Table (1): Composition and calculated analysis of experimental diets

Ingredients, %	Diet (%)			
	1	2	3	4
Corn yellow	60.00	55.00	52.00	54.00
Soybean Meal (48%)	23.90	22.00	20.00	16.00
Di-Calcium P.	2.00	2.02	2.04	2.03
Lime Stone	10.34	10.34	10.34	10.35
Salt	0.40	0.40	0.40	0.40
Wheat bran	3.00	5.00	6.30	11.10
Oil	0.0022	0.0043	0.0058	0.0054
Meth.	0.07	0.10	0.13	0.15
Lys.	--	0.06	0.11	0.20
Thr.	--	--	--	0.01
Vit. & Min.premix ¹	0.30	0.30	0.30	0.30
Sand	--	4.78	8.37	5.46
Total	100.00	100.00	100.00	100.00
Calculated Analysis, %				
Crude Protein	17.04	16.02	15.01	14.01
ME, (kcal/ kg)	2840.00	2840.00	2840.00	2840.00
Lysine	0.88	0.88	0.88	0.88
Methionine	0.34	0.36	0.37	0.38
TSAA	0.63	0.63	0.63	0.63
Threonine	0.64	0.60	0.56	0.52
Tryptophan	0.22	0.21	0.19	0.18
Ca.	4.45	4.45	4.45	4.45
Avail P.	0.48	0.48	0.48	0.48
Total P.	0.72	0.73	0.72	0.76

¹Vitamin and Minerals premix provides by kg: Vit A. 8900 IU; Vit E, 16 IU; Vit D3. 3500 IU; riboflavin, 6mg; Ca pantothenate, 7mg; niacin, 30mg; choline chloride, 110mg; vitamin B₁₂, 22.1mg; vitamin B₆, 3.3mg; thiamine (as thiamine mononitrate), 2.2 mg; folic acid, 0.65 mg; d-biotin, 60mg. Trace mineral (mg /kg diet): Mn, 88; Zn, 90; Fe, 65; Cu, 5.5; Se, 0.3

Table 2: Growth and productive performance of commercial laying hens fed different dietary CP levels from 53 to 64 wks of age.

Dietary Protein, %	BW ¹	FC ²	PI ^{3,8}	FCR ⁴	EP ⁵
	(g./ h)	(g./ h./ d.)	(g./ h./ d.)	g. feed/ g. egg	(%)
17	1546± 35.17	94.15 ± 2.17	16.01 ^a ± 0.36	1.79± 0.07	84.74 ± 2.18
16	1560± 43.88	95.02 ± 1.02	15.20 ^b ± 0.16	1.87± 0.03	80.60 ± 1.70
15	1486± 41.13	93.84 ± 1.06	14.10 ^c ± 0.16	1.86± 0.06	81.11 ± 1.34
14	1512± 39.88	93.50 ± 0.87	13.10 ^d ± 0.12	1.92± 0.06	79.00 ± 2.22
Protein estimate ^{6,7}	--	--	16.01 ± 0.22	--	--

¹Protein effect (linear $P = 0.561$; NS: *non-significant*)

²Protein effect (linear $P = 0.87$; NS: *non-significant*)

³Protein effect (linear $P \leq 0.001$)

⁴Protein effect (linear $P = 0.34$; NS: *non-significant*)

⁵Protein effect (linear $P 0.19$; NS: *non-significant*)

⁶Protein estimates were obtained from one-slope broken line regression models and are expressed as a percentage of the diet.

⁷ $Y=L+U(R-X_{LR})$; where **L**: the ordinate of the broken line in the curve; **R**: the abscissa of the broken line in the curve (the estimate); **X_{LR}**: a value of x less than R and **U**: the slope of the line for X less than R.

⁸**PI**, $Y=16.01-1.056(16.78-X_{LR})$.

Table 3: Egg production and components of commercial laying hens fed different dietary CP levels from 53 to 64 wks of age.

Dietary Protein, %	EW ¹	EM ^{2,10}	Albumen ³	Yolk ^{4,11}	Shell ^{5,12}	Alb. Solids ^{6,13}	Yolk solids ⁷
	(g.)	(g.)	(%)				
17	62.32 ± 0.65	52.77 ^a ± 1.27	57.30 ± 0.27	29.02 ^b ± 0.33	13.05 ^b ± 0.15	12.17 ^a ± 0.05	50.48 ± 0.10
16	63.34 ± 0.50	51.07 ^{ab} ± 0.98	57.01 ± 0.26	28.96 ^b ± 0.36	13.57 ^a ± 0.22	12.08 ^a ± 0.08	50.03 ± 0.15
15	62.11 ± 0.77	50.38 ^{ab} ± 1.07	57.30 ± 0.29	28.98 ^b ± 0.44	13.32 ^{ab} ± 0.14	11.96 ^a ± 0.06	50.60 ± 0.29
14	61.91 ± 0.83	48.86 ^b ± 1.31	56.50 ± 0.34	29.98 ^a ± 0.27	13.07 ^b ± 0.22	11.65 ^b ± 0.10	50.54 ± 0.49
Protein estimate ^{8,9}	--	51.33 ± 1.15	--	28.99 ± 0.14	13.30 ± 0.10	12.12 ± 0.05	--

¹Protein effect (linear $P = 0.49$; NS: non-significant)²Protein effect (linear $P = 0.07$)³Protein effect (linear $P = 0.21$; NS: non-significant)⁴Protein effect (linear $P \leq 0.03$)⁵Protein effect (linear $P \leq 0.08$)⁶Protein effect (linear $P \leq 0.002$)⁷Protein effect (linear $P = 0.18$; NS: non-significant)⁸Protein estimates were obtained from one-slope broken line regression models and are expressed as a percentage of the diet.⁹ $Y = L + U(R - X_{LR})$; where L: the ordinate of the broken line in the curve; R: the abscissa of the broken line in the curve (the estimate); X_{LR} : a value of x less than R and U: the slope of the line for X less than R.¹⁰EM, $Y = 51.33 - 1.08(16.15 - X_{LR})$.¹¹Yolk, $Y = 28.99 - 1.10(14.90 - X_{LR})$.¹²Shell, $Y = 13.30 - 0.35(14.67 - X_{LR})$.¹³Alb. solids, $Y = 12.12 - 0.31(15.53 - X_{LR})$

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الملخص العربي

تأثير خفض البروتين الخام مع إضافة الأحماض الأمينية على الأداء الإنتاجي
للدجاج البياض التجارى خلال فترة الإنتاج المتأخر

هيثم محمد ياقوت

قسم إنتاج الدواجن، كلية الزراعة - ٢١٥٤٥ جامعة الإسكندرية، جمهورية مصر العربية

يتم استخدام العديد من الاستراتيجيات للحد من التلوث بالنيتروجين من مخلفات الدواجن في المناطق التي توجد فيها تركيزات عالية من مزارع الدواجن التجارية. تبحث هذه الدراسة تأثير استخدام البروتين المنخفض في أعلاف الدجاج البياض المربي في ظروف تجارية. أجريت هذه الدراسة على ١٤٤ دجاجة Hy-Line W-36 حيث تم تقسيمها عشوائيا إلى أربعة أعلاف تجريبية. تكونت الأعلاف من الذرة الصفراء و كسب فول الصويا و اختلفت في محتواها من البروتين الخام حيث اضيف اليها احماض امينية مصنعة كالتالى: [1] 17% CP + Met, [2] 16% CP + Met & Lys, [3] 15% CP + Met & Lys, [4] 14% CP + Met, Lys & Thr و تم التغذية عليها في خلال الفترة من ٥٣ إلى ٦٤ أسبوع من العمر. أعتبر القصد هو الوحدة التجريبية (٤ دجاجات / قفص) وكررت كل معاملة ٩ مرات. تم قياس صفات وزن الجسم النهائى، استهلاك العلف، استهلاك البروتين، كفاءة التحويل الغذائى، إنتاج البيض، وزن البيض و مكونات البيض كميانات للنمو و الإنتاجية خلال فترة الدراسة (١١ أسبوعا)، و تم تطبيق معادلات الانحدار لتقدير أنماط النمو و تحديد نقطة التغير في احتياجات الدجاج البياض التجارية من البروتين الخام لكل صفة على حدة كنتيجة لتقليل البروتين المقدم في العلف.

متوسطات كلا من وزن الجسم النهائى، استهلاك العلف و الكفاءة الغذائية لم تتأثر معنويا كنتيجة للتغذية على مستويات البروتين المختلفة. نتائج استهلاك البروتين اظهرت ان خفض نسبة البروتين في العلف ادى الى نقص معنوى لإستهلاك البروتين وخاصة للدجاجات المغذاه على ١٤% بروتين (١٣.١٠ جم/ دجاجة) مقارنة بالمعاملات الأخرى. نقطة التغير في استهلاك البروتين ١٦.٠١ جم/ دجاجة تم الحصول عليها عند نسبة بروتين ١٦.٧٨% و كانت اعلى من تلك للدجاجات المغذاه على ١٤، ١٥ او ١٦% بروتين. أعلى نسبة إنتاج بيض (غير معنوية) تم الحصول عليها من الدجاجات المغذاه على ١٧% بروتين (٨٤.٧٤%) و انقل وزن بيض كانت للدجاجات المغذاه على ١٦% بروتين (٦٣.٣٤ جم). انخفضت نسبة المواد الصلبة بالبيض من ١٢.١٧ الى ١١.٦٥%. و نقطة التغير كانت عند ١٥.٥٣% بروتين (١٢.١٢% مواد صلبة بالبيض) في حين انه لم يلاحظ اية تأثيرات معنوية للمعاملات الغذائية على كلا من الكفاءة الغذائية، النسبة المتوية لبيض البيض، المواد الصلبة لصفار البيض او نسبة القشرة.

إستنادا على النتائج الحالية، وبتطبيق نموذج الانحدار لنقطة التغير ادى الى تقديرات أدق لتحديد الى اى مدى يمكن خفض البروتين الخام مع الحفاظ على تحقيق النمو الأمثل والإنتاجية المناسبة. إتضح ان التغذية على أعلاف تحتوى على ١٥% او ١٦% بروتين خام المضاف اليها أحماض أمينية مصنعة، يمكن أن تكون مناسبة لنمو جيد و إنتاجية اعلى للدجاج البياض التجارى خلال الفترة المتأخرة للإنتاج.