

## **The effect of substitution of soybean with canola meal in laying hen diets formulated on the basis of total or digestible amino acids on performance and blood parameters**

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**Abstract** An experiment was conducted to study the effect of substitution of soybean meal (SBM) with canola meal (CM) in diets formulated on the basis of total or digestible amino acid (AA) on productive performance, egg quality, and selected organ weight and blood parameters in laying hens from 73<sup>rd</sup> to 83<sup>rd</sup> weeks of age. A total of 128 laying hens were allotted randomly to a completely randomized design arranged as a 2×2 factorial experiment with two protein sources (soybean meal and 30% soybean replaced by canola meal), and two diets formulated for total or digestible amino acids. Four experimental diets were formulated with four replicates of eight birds each. Increased egg weight, egg mass and improved feed conversion ratio (FCR) were recorded for diets containing SBM compared with CM (P<0.05). Egg weight was increased (P<0.05) by SBM formulated on digestible amino acid compared to other dietary treatments. Increased shell thickness (P<0.05) was recorded for CM when the diet was formulated on total amino acid basis. Yolk color and relative weight (RW) of the liver were increased (P<0.05) by canola meal diet formulated on digestible amino acid. Blood parameters were not affected (P>0.05) by treatments. The results of this study showed that soybean meal caused better performance than canola meal, and feed formulation based on digestible AA in SBM diet resulted in better performance in laying hens.

**Keywords:** canola meal, amino acids, feed formulation, performance, laying hens

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### **Introduction**

Soybean meal is used extensively as a plant protein source in poultry nutrition. Canola meal a by-product of canola oil industry, is considered a cost-effective substitute for soybean meal in diets when it costs around 60 to 70% of SBM price (Canola Council of Canada, 2009). Soybean meal is expensive and currently is not produced in sufficient quantities in Iran with a semiarid climate. The use of CM in poultry diets has been limited due to the presence of anti-nutrients (NRC, 1994). Crude fiber (4-6%), non-fiber polysaccharides (13-16%) and phytic acid (4%) contents of canola meal are also higher in CM compared to SBM (Slominski and Campbell, 1990). The high contents of anti-nutritive substances in CM inhibit cell wall digestibility and animal performance (Bell, 1993). Canola meal has a higher content of methionine and cysteine, but less lysine and arginine than SBM; however, lower and more variable amino acid digestibility has been reported for CM compared with SBM (Bell, 1993). Canola meal also contains more fiber than SBM, which is largely responsible for lower total tract digestibility of crude protein and energy

(Khajahi and Slominski, 2012). In monogastrics, the negative effects of glucosinolates are related to dysfunction of the thyroid. Weight of thyroid and the liver were linearly related to the amount of glucosinolate ingested by monogastrics (Bourdon and Aumaitre, 1990). Recently, there has been an increased trend in the application of the standardized ileal amino acid digestibility (SID) to feed formulation for poultry (Adedokun et al., 2008). Soto et al. (2013) reported that feed formulation using near infrared reflectance spectroscopy as the source of information for ingredient amino acid content improved broiler body weight at 21 and 42 d of age without affecting feed conversion ratio (FCR). The term SID is used when apparent ileal digestibility (AID) coefficients are corrected for the endogenous amino acid (AA) losses (Lemme et al., 2004). It is generally assumed that absorption of intact AA in the hindgut of monogastrics is minimal, even though colonocytes do contain AA transporters and have been shown to absorb AA. This assumption is supported by the observation that infusion of AA into the hindgut does not improve

nitrogen balance in monogastrics. In addition, growth performance of monogastrics is more highly correlated with ileal than fecal AA digestibility. It is suggested that ileal digestibility coefficients provide a better estimate of AA bioavailability than fecal digestibility coefficients (Columbus and Lange, 2012). The objective of the present study was to evaluate the influence of substitution of soybean with canola meal in diets formulated on the basis of total or digestible amino acids on productive performance, egg quality, and selected organ weight and blood parameters in laying hens.

**Materials and Methods**

*Birds and experimental design*

A total of 128 Hy-Line W-36 laying hens, with an initial age of 73 weeks were used in a completely randomized design (CRD) arranged as a 2 × 2 factorial experiment with 4 treatments containing two sources of protein (soybean, or 30% soybean replaced by canola meal), and two types of amino acid recommendations (total or digestible), with 4 replicates and 8 hens per replicate. The experimental diets were formulated to supply the requirements of total or digestible amino acid, as recommended by Hy-Line International (2012). The iso-nutritive basal diet was based on corn-soybean meal. The crude protein and amino acid contents in feed ingredients (corn, soybean meal and canola meal) were measured by near-infrared reflectance spectroscopy (NIRS) using an NIR System (model 5000, FOSS, Sweden). The standardized ileal AA digestibility values were calculated using the SID coefficient by Evonik Degussa Company, GmbH, Germany (Table 1). Canola meal was

replaced with 30% soybean meal protein in the diet. Dietary ingredients and composition are presented in Table 2.

The experimental diets were fed from week 73 to 83. Light was provided for 16 h daily and temperature was maintained at 23±3 °C throughout the experiment. Feed and water were provided *ad libitum*.

*Productive performance and egg quality traits*

Body weight of the hens was determined at the beginning and the end of the experiment. Egg production and egg weight were determined daily and feed intake was recorded weekly. This information was used to calculate average daily feed intake (ADFI), egg mass and FCR. Egg quality traits were measured on a weekly basis. The eggs (3 eggs per replicate were taken after feeding the diets) were individually weighed and the egg external (shape index, shell weight, shell thickness) and internal quality (yolk color, Haugh unit) traits were measured. The shell was separated from the yolk and albumen, and then weighed after drying overnight at 60°C (Grobass et al., 2001). Egg shell thickness was measured using a digital micrometer (Echometer 1061, Robotmation Company, Tokyo, Japan). The Haugh units (HU) was calculated by using the egg weight (EW/g) and albumen height (AH/mm) (Haugh, 1937).  $HU = 100 \text{ Log} (AH + 7.57 - 1.7 \text{ EW}^{0.37})$ .

*Blood parameters and organ weight*

At the end of the experiment (83<sup>rd</sup> wk), two birds per replicate (n=4) were randomly selected and blood samples collected via wing vein puncture. Serum samples,

**Table 1.** Amino acid contents of corn, soybean meal and canola meal

Amino acid	Total amino acid content <sup>1</sup> (% as is)			Standardized digestibility of amino acids SID coefficient <sup>2</sup> (%)			Digestible amino acid content (% as is)		
	Corn	SBM <sup>3</sup>	CM <sup>4</sup>	Corn	SBM	CM	Corn	SBM	CM
Arginine	0.357	3.096	2.030	93	93	87	0.332	2.879	1.766
Cystine	0.175	0.661	0.839	87	82	77	0.152	0.542	0.646
Histidine	0.223	1.156	0.909	95	92	85	0.211	1.063	0.772
Isoleucine	0.269	1.948	1.391	95	89	79	0.256	1.734	1.099
Leucine	0.938	3.267	2.451	94	89	82	0.882	2.908	2.010
Lysine	0.237	2.363	1.716	92	90	80	0.218	2.372	1.373
Methionine	0.156	0.592	0.699	94	91	84	0.147	0.539	0.587
M+C <sup>5</sup>	0.333	1.250	1.540	90	86	80	0.299	1.075	1.232
Phenylalanine	0.381	2.168	1.385	94	89	83	0.358	1.929	1.149
Threonine	0.283	1.710	1.542	85	85	73	0.241	1.453	1.126
Tryptophan	0.060	0.592	0.469	81	89	80	0.049	0.527	0.375
Valine	0.369	2.064	1.788	92	88	79	0.340	1.816	1.412

<sup>1</sup>Total amino acid measured by NIRS.

<sup>2</sup>Standardized Digestibility of Amino Acids SID Content and SID Coefficient (Evonik Degussa, Germany)

<sup>3</sup>Soybean meal (SBM); <sup>4</sup>Canola meal (CM); <sup>5</sup>Methionine+cystine

## Feed formulation based on amino acid type for laying hens

**Table 2.** Ingredient composition and calculated and chemical analyses of the experimental diets (% , as-fed basis)

Ingredient	Soybean meal diet		Canola meal diet	
	Total amino acid	Digestible amino acid	Total amino acid	Digestible amino acid
Corn	61.82	62.82	59.68	59.79
Soybean meal	22.77	21.93	17.52	17.40
Canola meal	-	-	6.83	6.83
Soybean oil	2.64	2.45	3.27	3.25
Dicalcium phosphate	1.37	1.38	1.35	1.35
Oyster shell	10.43	10.43	10.36	10.36
NaCl	0.34	0.34	0.35	0.35
Vitamin premix <sup>1</sup>	0.25	0.25	0.25	0.25
Mineral premix <sup>2</sup>	0.25	0.25	0.25	0.25
Methionine	0.13	0.13	0.11	0.10
Lysine	-	0.02	0.03	0.04
Calculated analysis				
AME <sub>n</sub> (kcal/kg)	2800	2800	2800	2800
CP (%)	14.6	14.3	14.6	14.5
Calcium (%)	4.37	4.37	4.37	4.37
Available phosphorus (%)	0.39	0.39	0.39	0.39
Na (%)	0.17	0.17	0.17	0.17
Cl (%)	0.24	0.25	0.25	0.25
Arginine (%)	0.92	0.84	0.89	0.82
Isoleucine (%)	0.61	0.54	0.59	0.53
Lysine (%)	0.75	0.67	0.74	0.67
Methionine (%)	0.36	0.34	0.35	0.32
Methionine+ cystine (%)	0.62	0.55	0.63	0.55
Threonine (%)	0.56	0.47	0.57	0.47
Tryptophan (%)	0.17	0.15	0.17	0.15
Valine (%)	0.69	0.61	0.70	0.61
Determined analysis				
Dry matter (%)	92.45	92.15	91.55	92.35
Ash (%)	10.80	10.30	11.30	10.50
Crude protein (%)	14.67	15.67	15.52	15.19

<sup>1</sup>Provided per kg diet: 6,000 IU vitamin A, 2,000 IU vitamin D3, 35 mg vitamin E, 2.0 mg vitamin K3, 1.0 mg vitamin B1, 3.0 mg vitamin B2, 30 mg niacin, 7.5 mg calcium D-pantothenate, 2.0 mg vitamin B6, 10 µg vitamin B12, 0.75 mg folic acid, 0.075 mg D-biotin, 0.63 mg endox D dry.

<sup>2</sup>Provided per kg diet: 100 mg Mn, 80 mg Fe, 80 mg Zn, 8 mg Cu, 0.2 mg Co, 0.15 mg Se, 300 mg choline chloride, 1.0 mg I.

after centrifugation at 2,000 × g for 10 min and at 4°C were stored at -20°C until analysis. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) concentrations in the serum were measured by using commercial kits (Biosystem Company, France) by Fully Automatic Biochemistry Analyzer. Serum triiodothyronine (T3) and tetraiodothyronine (T4) concentrations were determined by using Gamma Coat M Total T3 (Autobio, France) and T4 kit (Autobio, France), by Elisa Reader. The relative weights of the liver and heart were also calculated.

### Statistical analysis

The data were analyzed using the GLM procedure of SAS (SAS, 2004) according to the following model:

$$Y_{ijkl} = \mu + S_i + A_j + W_k + SA_{ij} + SW_{ik} + AW_{jk} + SAW_{ijk} + Ea_{ijl} + Eb_{ijkl}$$

in which,  $\mu$  is the overall mean;  $S_i$  is the  $i^{\text{th}}$  source of protein;  $A_j$  is the  $j^{\text{th}}$  types of amino acid in feed formulation;  $W_k$  is the  $k^{\text{th}}$  week of the experiment;  $SA_{ij}$ ,  $SW_{ik}$ ,  $AW_{jk}$  and  $SAW_{ijk}$  are interactions and  $Ea_{ijl}$  and  $Eb_{ijkl}$  are whole unit and subunit errors, respectively. Normality distribution of all data was evaluated using the Shapiro-Wilk test. All traits showed normal distribution and needed no transformation. Treatment means were compared using the Duncan's multiple range test (Duncan, 1995).

## Results

### Productive performance

Feed intake and egg production were not affected by treatments but increased egg weight and egg mass and

**Table 3.** Effect of soybean or canola meal and types of amino acid (total or digestible) on performance in laying hens from 73 to 83 weeks of age

Effect/index	Feed intake (g/d)	Egg production (%)	Egg weight (g)	Egg mass (g/d)	FCR <sup>5</sup>
Protein source					
SBM <sup>1</sup>	101.5	82.4	53.1 <sup>a</sup>	44.2 <sup>a</sup>	2.399 <sup>a</sup>
CM <sup>2</sup>	101.9	79.4	49.9 <sup>b</sup>	40.2 <sup>b</sup>	2.765 <sup>b</sup>
Type of AA					
Total AA <sup>3</sup>	101.5	80.2	50.4	41.0	2.651
Digestible AA	101.9	81.7	52.6	43.5	2.512
SEM <sup>4</sup>	0.005	0.02	0.01	0.02	0.001
Protein source					
		<u>Type of AA</u>			
SBM	101.5	Total	51.2 <sup>b</sup>	41.9 <sup>ab</sup>	2.531 <sup>ab</sup>
SBM	101.5	Digestible	54.9 <sup>a</sup>	46.6 <sup>a</sup>	2.267 <sup>b</sup>
CM	101.2	Total	49.6 <sup>b</sup>	40.0 <sup>b</sup>	2.772 <sup>a</sup>
CM	102.5	Digestible	50.2 <sup>b</sup>	40.4 <sup>b</sup>	2.757 <sup>a</sup>
SEM <sup>4</sup>	0.01		0.03	0.05	0.003
P-value					
Source of protein	0.54	0.14	0.02	0.05	0.02
Type of AA	0.49	0.45	0.08	0.20	0.35
Source of protein × Type of AA	0.57	0.33	0.21	0.26	0.40
Week	<0.0001	0.02	0.02	0.02	0.11
Week × Source of protein	0.09	0.06	0.35	0.21	0.33
Treatment	0.75	0.68	0.03	0.09	0.09
Treatment × Week	0.23	0.95	0.086	0.79	0.86

<sup>a,b</sup>Means within a column with common superscripts are not different ( $P>0.05$ ).

<sup>1</sup>Soybean meal (SBM), <sup>2</sup>Canola meal (CM), <sup>3</sup>Amino acid (AA)

<sup>4</sup>SEM: Standard error of the means (n=4 per treatment).

<sup>5</sup>Feed conversion ratio

improved FCR ( $P<0.05$ ) were found by soybean than canola meal over the entire of experimental period. Performance parameters were not affected by type of amino acids (total or digestible) formulation of the diet. Increased egg weight ( $P<0.05$ ), egg mass and improved FCR ( $P=0.09$ ) were found by SBM diet formulated on digestible AA basis (Table 3).

### Egg quality traits

Egg quality traits were not affected by protein sources except the yolk color which was improved ( $P<0.05$ ) by canola meal. Increased ( $P<0.05$ ) egg shell thickness was observed by feed formulated on total than digestible AA basis. Thicker shell thickness was shown by CM formulated based on total AA than other treatments ( $P<0.05$ ). Significant improvement ( $P<0.05$ ) in yolk color was recorded by CM formulated on digestible amino acid than other treatments (Table 4).

### Blood parameters and organ weights

Blood parameters including AST, ALT, ALP, LDH, T3 and T4 were not affected by treatments (data not tabulated).

The relative weight (RW) of the liver increased ( $P<0.05$ ) by canola than soybean meal, it was also increased by CM diet formulated on digestible AA ( $P<0.05$ ) but heart RW was not influenced by treatments in this respect (Table 5).

### Discussion

The present study showed that canola meal did not affect the feed intake and egg production, while it decreased egg mass and impaired FCR. The results are in accord with previous findings (Bell, 1993; Kermanshahi and Abbasi-pour, 2006; Jia et al., 2008) that showed the performance decreased in broilers and layers receiving high levels of canola meal in their diets. This decrease is attributed to the presence of erusic acid, glucosinolate, phytic acid and various phenolic compounds in the canola meal. Decreased performance may occur in layers and broilers when a great portion of soybean meal is replaced with canola meal. Inclusion of canola meal in the diet decreased the feed consumption at all inclusion levels (Sarıçiçek et al., 2005), which appears to be related to the presence of anti-nutritional factors in canola meal (Newkirk and Classen, 2002). Also, Bourdon and Aumaitre. (1990) reported that glucosinolates in ca-

**Feed formulation based on amino acid type for laying hens**

**Table 4.** Effect of soybean or canola meal and types of amino acid (total or digestible) on egg quality traits in laying hens from 73 to 83 weeks of age

Effect/index		Shape index (%)	Shell weight (g)	Shell thickness (mm)	Yolk color	Haugh unit	
Protein source							
	SBM <sup>1</sup>	76.35	5.68	0.323	6.16 <sup>b</sup>	85.27	
	CM <sup>2</sup>	77.07	5.69	0.325	6.34 <sup>a</sup>	84.43	
Type of AA							
	Total AA <sup>3</sup>	76.65	5.74	0.331 <sup>a</sup>	6.22	84.76	
	Digestible AA	76.77	5.62	0.317 <sup>b</sup>	6.27	84.94	
	SEM <sup>4</sup>	0.30	0.08	0.003	0.05	0.78	
Protein source	<u>Type of AA</u>						
	SBM	Total	76.23	5.64	0.329 <sup>ab</sup>	6.25 <sup>b</sup>	85.59
	SBM	Digestible	76.47	5.72	0.317 <sup>b</sup>	6.07 <sup>b</sup>	84.94
	CM	Total	77.06	5.85	0.333 <sup>a</sup>	6.20 <sup>b</sup>	83.92
	CM	Digestible	77.08	5.52	0.329 <sup>ab</sup>	6.47 <sup>a</sup>	84.95
	SEM <sup>4</sup>		0.43	0.11	0.003	0.07	1.11
P-value							
	Source of protein		0.12	0.96	0.55	0.03	0.46
	Type of AA		0.77	0.31	0.003	0.50	0.87
	Source of protein × Type of AA		0.80	0.09	0.60	0.009	0.46
	Week		0.50	<0.0001	<0.0001	<0.0001	<0.0001
	Week × Source of protein		0.32	0.0001	0.01	0.35	0.94
	Treatment		0.43	0.27	0.02	0.01	0.76
	Treatment × Week		0.05	0.0005	0.13	0.64	0.76

<sup>a,b</sup>Means within a column with common superscripts are not different ( $P>0.05$ ).

<sup>1</sup>Soybean meal (SBM); <sup>2</sup>Canola meal (CM); <sup>3</sup>Amino acid (AA)

<sup>4</sup>SEM: Standard error of the means (n=4 per treatment).

**Table 5.** Effect of soybean or canola meal and type of amino acid (total or digestible) on the relative weight (%BW) of the liver and heart in laying hens at wk 83 of age

Effect/index		Liver	Heart	
Protein source				
	SBM <sup>1</sup>	2.40 <sup>b</sup>	0.36	
	CM <sup>2</sup>	2.82 <sup>a</sup>	0.35	
Type of AA				
	Total AA <sup>3</sup>	2.59	0.36	
	Digestible AA	2.63	0.35	
	SEM <sup>4</sup>	0.05	0.01	
Protein source	<u>Type of AA</u>			
	SBM	Total	2.42 <sup>b</sup>	0.36
	SBM	Digestible	2.38 <sup>b</sup>	0.35
	CM	Total	2.77 <sup>ab</sup>	0.37
	CM	Digestible	2.88 <sup>a</sup>	0.34
	SEM <sup>4</sup>		0.13	0.02
P-value				
	Source of protein		0.006	0.89
	Type of AA		0.79	0.41
	Source of protein × Type of AA Diet		0.56	0.44
	Treatment		0.04	0.72

<sup>a,b</sup>Means within a column with common superscripts are not different ( $P>0.05$ ).

<sup>1</sup>Soybean meal (SBM); <sup>2</sup>Canola meal (CM); <sup>3</sup>Amino acid (AA)

<sup>4</sup>SEM: Standard error of the means (n=4 per treatment).

nola meal had deleterious effects on feed intake and liver enlargement. In addition, decreased egg production may be due to decrease in feed intake or more specifically, energy intake (Hickling, 2001). Egg production was adversely affected by inclusion of rapeseed meal in layer diets (Richter et al., 1996). In contrast, Thomas et al. (1978) did not observe any reduction in egg production by rapeseed meal inclusion. In the current experiment, shape index, shell weight, shell thickness and Hugh unit were not affected by soybean or canola meal but yolk color increased by canola meal. Similar results were reported by Blair et al. (1975). Increased yolk color may be due to the present of pigments in canola meal (Chaparzadeh and Zarandi, 2011). Richter et al. (1996) reported that rapeseed meal reduced egg Haugh unit. In the current study, shell thickness decreased by digestible than total amino acid. This could be related to the foundation of the shell (protein matrix) because it was suggested that limiting amino acids for the development of this protein matrix may negatively influence shell quality (Fraser et al., 1998). These results are in agreement by Casartelli et al. (2005). They reported that total or digestible amino acid recommendations did not affected egg quality parameters. In the present study, blood parameters were not affected by treatments but RW of the liver was increased by canola meal diet.

## **Conclusions**

Soybean meal improved laying hen performance parameters more than canola meal did, but feed formulation on the basis of total or digestible amino acids did not affect the layer performance in this study. Canola meal diet which was formulated on the basis of total and digestible amino acids decreased the egg weight and egg mass and adversely affected the FCR. Increased yolk color by canola meal and improved egg shell thickness by total amino acid feed formulation were also observed but no changes in blood parameters were recorded.

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## اثر جایگزینی کنجاله سویا با کنجاله کانولا در جیره مرغ‌های تخم‌گذار فرموله شده بر پایه آمینواسید

### کل یا گوارش پذیر بر عملکرد و پارامترهای خونی

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**چکیده** اثر جایگزینی کنجاله سویا با کنجاله کانولا و فرمولاسیون جیره بر اساس اسیدهای آمینه کل یا گوارش پذیر بر عملکرد، صفات کیفی تخم‌مرغ، وزن اندام‌ها و فاکتورهای خونی مرغ‌های تخم‌گذار از سن ۷۳ تا ۸۳ هفتگی بررسی شد. روی هم رفته، ۱۲۸ مرغ تخم‌گذار در قالب طرح کاملاً تصادفی و به شیوه فاکتوریل ۲×۲ با دو منبع پروتئین (کنجاله سویا و ۳۰ درصد جایگزینی کنجاله سویا با کنجاله کانولا) و فرمولاسیون جیره بر پایه آمینواسید کل یا گوارش پذیر توزیع شدند. چهار جیره آزمایشی با چهار تکرار و هشت قطعه پرند در هر تکرار به کار برده شد. کاربرد کنجاله سویا در جیره، وزن تخم‌مرغ و توده تخم‌مرغ را افزایش و ضریب تبدیل خوراک را نسبت به کنجاله کانولا بهبود بخشید ( $P<0.05$ ). جیره کنجاله سویا فرموله شده بر پایه آمینواسید گوارش پذیر موجب افزایش وزن تخم‌مرغ در مقایسه با دیگر تیمارها شد ( $P<0.05$ ). کنجاله کانولا فرموله شده بر پایه آمینواسید کل، ضخامت پوسته را افزایش داد ( $P<0.05$ ). رنگ زرده و وزن نسبی کبد در جیره کنجاله کانولا فرموله شده بر پایه آمینواسید گوارش پذیر افزایش یافتند ( $P<0.05$ ). فاکتورهای خونی تحت تاثیر تیمارهای آزمایشی قرار نگرفتند ( $P>0.05$ ). یافته‌های این پژوهش نشان داد که کنجاله سویا، عملکرد را نسبت به کنجاله کانولا بهبود داد. همچنین جیره کنجاله سویا فرموله شده بر پایه آمینواسید گوارش پذیر، اثر بهتری را بر صفات عملکردی نسبت به پایه آمینواسید کل نشان داد.