Effect of canola oil on mucosal leucine aminopeptidase activity enzymes in small intestine of turkey chicks

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Abstract: Canola is one of the rapeseed varieties and is in temperate and cold climate areas. Contains 94% unsaturated fatty acid and 6% saturated fatty acid, thus, has best fatty acid composition among other oils. Canola oil causes alteration in pancreatic enzymes such as leucine aminopeptidase activity. The aim of this study was assessment of mucosal leucine aminopeptidase activity enzymes subsequently using of canola oil on turkey chicks diet. According to this survey results revealed that using of canola oil in turkey chick's diet causes increasing of leucine aminopeptidase activity (in 5% treatment than control and 2.5% treatments). It seems that use of different amounts of canola oil in turkey chick's diet causes increasing of leucine aminopeptidase activity, because this enzyme play a important role in protein hydrolyzing and this enzyme activity be more subsequently reducing of digesta transient ratio. [Jamshid Ghiasi Ghalehkandi, Ramin Salamat Doust Nobar, Abolfazl ghorbai, Ali Asghar Gharachorlu, Rahim Behesti, Alireza Fani. Effect of canola oil on mucosal leucine aminopeptidase activity enzymes in small intestine of turkey chicks. Journal of American Science 2011;7(6):704-707]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: canola oil, leucine aminopeptidase, intestine, turkey chicks.

1. Introduction

Canola is one of the rapeseed varieties and is in temperate and cold climate areas. Canola is rich of sulfurous amino acids and vitamins. Canola meal has about 40% protein. Its erucic acid is less than 2% of total fatty acids (Pajohan Mehr et al., 2008). Consist of less than 30 micromole glucosinolate per oil free dry matter. Contains 94% unsaturated fatty acid and 6% saturated fatty acid, thus, has best fatty acid composition among other oils. Because of having 61% of oleic acid, considered as full resources of unsaturated fatty acids and from this aspect, occupied in second class after olive oil. Its tocopherol is higher than olive and soya oils, that from this aspect can be attribute high antioxidant effect to it. Canola oil combination is inserted in table 1 (Pajohan mehr et al., 2008, Mohammadi et al., 2007).

Leucine aminopeptidase (LAPs) are enzymes that preferentially catalyze the hydrolysis of leucine residues at the N-terminus of peptides and proteins. Other N-terminal residues can also be cleaved, however. LAPs have been found across super kingdoms (Strater et al., 1999).

Identified LAPs include bovine lens LAP, porcine LAP, E. coli LAP (also known as PepA or XerB), and the solanaceous-specific acidic LAP (LAP-A) in tomato (Gu et al., 2002). Historically, the mechanisms of carboxypeptidase and endoprotease have been much more well-studied and understood by researchers. Work within the past two decades has provided vital knowledge regarding the mechanisms of aminopeptidase. In this mechanism, the bicarbonate ion acts as a general base. For LAP-A, R1 could be the R group of leucine, methionine, or arginine (Kraft et al., 2006). Leucine aminopeptidase is brush border and cytosolic enzyme which hydrolyses small peptides from terminal of long peptide chains. Peptidase enzymes activity elicit to protein digestion in diet (Do¢gan et al., 1999; Talebali and Farzinpour, 2006).

2. Materials and methods

2.1. Animals and diet

This research was performed one 108 Iranian native turkey chicks (from 4th to 20th week of age). In this study, the turkey chicks by chance divided into 3 treatments and each treatment divided into 3 replicates and each replicate was contained 12 turkey chicks and were fed in separate cage with 0, 2.5 and 5 percent of canola oil. The experimental diets formulated isonitrogenouse and isoenergetic and balanced according to 1994 national research council

(NRC, 1994). The birds were given access to water and diets ad-libitum. The composition and calculated nutrient composition of the mixture of treatment is shown in Table 2.

2.2. Sample collection

In the Rearing period, all conditions such as temperature, humidity, light, ventilation and management were appropriate and similar for all broilers and 20th week of age of end the rearing period, after 5 hours of starvation, 2 broilers from every group (totally 18 chickens of sampling) which weighed nearly equal to the average weight of each replicate have been chosen and slaughtered. The abdominal cavity was opened, and the entire gastrointestinal tract was removed. The small intestine was isolated, and the length of intestine was determined by a graduate ruler. The positions at 1, 10, 30, 50, 70 and 90 % of the length of small intestine for analyzing the Enzymes activity were separated with specific scissors (an 8-cm sample was taken). The samples for enzymes determination were cut open lengthwise, rinsed carefully with phosphate buffer saline (pH=7), blotted dry, then samples envelop in vacuum packed and stored at −80°C until enzymes analysis (Teshfam, 1984).

Table 1: fatty acids contents of canola oil

Fatty acid	Percent
C12:0	0
C14:0	0
C16:0	4,73
C16:1	0,13
C18:0	2,31
C18:1cis9	61,1
C18:1cis11	0
C18:1trans9	0
C18:2cis	19,73
C18:2trans	1,78
C18:3cis	7,35
C18:3trans	0,71
C20:0	0,53
C20:1	1,18
C20:4	0
C22:0	0,25
C22:1	0,21

Table 2: Percentage composition of experimental diets in four periods

	4 -8 week			**8-12 week**			**12-16 week**			**16-20 week**		
	T_1	**T_2**	**T_3**	**T_1**	**T_2**	**T_3**	**T_1**	**T_2**	**T_3**	**T_1**	**T_2**	**T_3**
Corn	42.50	38.00	36.00	45.60	43.00	35.00	56.64	48.50	40.00	64.41	58.00	48.00
SBM	34.40	36.00	31.15	28.25	27.30	28.24	26.00	27.00	27.50	21.00	21.00	21.00
Oil	0.00	1.25	2.50	0.00	2.50	5.00	0.00	2.50	5.00	0.00	2.50	5.00
Fish	4.80	3.70	6.60	8.00	8.00	8.00	2.64	1.82	1.50	0.65	0.70	0.67
Starch	3.10	3.22	1.56	7.46	3.32	3.37	6.57	6.51	6.50	7.10	5.56	6.71
Alfalfa	3.47	5.00	6.00	3.00	5.00	6.00	1.50	4.00	6.00	1.00	3.80	6.00
DCP	1.38	1.52	1.11	0.63	0.61	0.62	1.03	1.15	1.18	1.17	1.15	1.15
Met	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Lys	1.50	1.50	1.50	1.50	1.50	1.50	1.40	1.50	1.50	1.50	1.50	1.50
Oyster	1.02	1.02	0.86	0.73	0.67	0.62	0.92	0.87	0.82	0.90	0.81	0.73
wheat bran	2.00	3.00	6.00	2.50	5.00	6.00	1.00	3.00	6.00	0.00	1.70	5.00
Vit supp1	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Min supp2	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Sand	3.58	3.54	4.47	0.08	0.85	3.40	0.05	0.90	1.75	0.02	1.03	1.99
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated nutrient content												
ME kcal/kg	2755	2755	2755	2850	2850	2850	2945	2945	2945	3040	3040	3040
Crude protein (%)	24.7	24.7	24.7	20.9	20.9	20.9	18.1	18.2	18.1	15.7	15.7	15.7
Calcium (%)	0.95	0.95	0.95	0.81	0.81	0.81	0.71	0.71	0.71	0.62	0.62	0.62
Available P (%)	0.48	0.48	0.48	0.40	0.40	0.40	0.36	0.36	0.36	0.31	0.31	0.31
ME/CP	112	112	112	136	136	136	163	162	163	194	194	194
Ca/P	2	2	2	2	2	2	2	2	2	2	2	2

1Vitamin content of diets provided per kilogram of diet: vitamin A,D, E and K.

2 Composition of mineral premix provided as follows per kilogram of premix: Mn, 120,000mg; Zn, 80,000 mg; Fe, 90,000 mg; Cu, 15,000 mg; I, 1,600 mg; Se, 500 mg; Co, 600 mg

2.3 Enzyme assay

After thawing, all of vacuum packed were opened and then using a sensitive scale, 0.05 gram of the mucosal small intestine was weighed and along with 10 ml liter phosphate buffer saline (pH=7) was formed into a homogenized solution using sonic Vibracell Sonics (VCX 130 TE USA) device (Teshfam, 1984). Enzymes activity of leucine aminopeptidase was measured according to the procedure Nigel et al., (1964) method was used. For detection of enzymes activity it was needed to measure total protein which Pirogallol (calorimetric) method was used (Watanaba, et al., 1986). The level of activity of enzymes of each sample is divided into the amount of its total protein so the activity level of the enzyme is calculated according to the IU in liter/gram protein (Teshfam, 1984).

2.4 Statistical analyses

The results of the research have been statistically analyzed using the linear model of SAS software (SAS, 2001).

Analysis of variance according to the model,

$$xij = \mu + Tj + eij$$

Where,

xij = All dependent variable

μ = Overall mean

Ti = The fixed effect of RRO levels (i = 1, 2, 3)

eij = The effect of experimental error

Values of different parameters were expressed as the mean ± standard deviation (X±SD). When significant difference among means was found, means were separated using Duncan's multiple range tests.

Table 3: comparison of average leucine aminopeptidase activity between treatments in different periods and segments of small intestine in broiler chicks (IU/g protein)

Intestine length / Groups)	1%	10%	30 %
0 % canola oil	1314.2±121.1	1053.8^{b}±92.9	1567.6^{b}±18.4
2.5 % canola oil	1547.2±261.5	1469.6^{ab}±377.9	1537.3^{b} ±350
5 % canola oil	1504.6±181.1	1811.1^{a}±360.9	1559.3^{a}±414.7

a, b,.. Means in the same column with different superscripts differ significantly X ± SD (P<0.05).

3. Results

According to table 3 and 4, adding different levels of canola oil to turkey chick's diet have different effects on leucine aminopeptidase activity on several regions of small intestine. in part of 1% of small intestine there is a significant increase in 2.5 and 5% treatments than control group.

Leucine aminopeptidase activity in parts of 10 and 30% of small intestine in 5% treatment has significant increase than control and 2.5% treatments whereas; in part of 50% of small intestine in 2.5 and 5% treatments have significant increasing than control group. Also in parts of 70 and 90% of small intestine there is a significant increase in 5% treatment than 2.5% and in 2.5% than to control group (p<0.05).

Table 4: comparison of average leucine aminopeptidase activity between treatments in different periods and segments of small intestine in broiler chicks (IU/g protein)

Intestine Length / Groups	50%	70 %	90%
0 % canola oil	1848 ^{b}± 401.6	2106.9±547.1	2643.2^{c}±295
2.5% canola oil	2821.7^{a}±517.2	2763.7±1011.6	3006.3 ^{b} ±420
5 % canola oil	3060.2^{a}±634.8	2933±692.1	3902.5^{a}±398

a, b,..Means in the same column with different superscripts differ significantly X ± SD (P<0.05).

4. Discussions

In current study obtained that adding different levels of canola oil to turkey chick's diet have different effects on leucine aminopeptidase activity on several regions of small intestine, there are many studies that confirm this result.

In one research were done by Turek et al. (1991), demonstrated that Resident linseed oil had a 15% increase in LAP fluorescence compared to corn oil. In one other study were done by Chambersa et al.(1978), revealed that Saudi Arabian crude oil caused decreases in the specific activities of alkaline phosphatase, γ-glutamyl transpeptidase and leucine aminopeptidase, and increases in the activities of glutamic pyruvic transaminase, glutamic-oxaloacetic transaminase, lactic dehydrogenase and α-hydroxybutyric dehydrogenase. In other study were done by Manuel et al. (1983), observed that rapeseed oil supplementation causes increasing in β-N-Acetylglucosaminidase, βn-d-glucosidase, β-d-glucuronidase, α-l-fucosidase and leucine aminopeptidase activities when compared with control group, that is compatible with our research results. In one other study were done by Gonzalez et al. (2001), revealed that Supplementation of casein with olive oil tended to decrease the intestinal and hepatic enzyme activity. It seems that increasing diet fat reduced transient ratio. Thus digesta more exposures to acid, pepsin and proteolytic enzymes. Therefore, Leucine aminopeptidase activity enhanced

and proteins convert to components such as amino acids. Hence, use of different amounts of canola oil in turkey chick's diet causes increasing of leucine amino peptidase activity.

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