Effect Of Supplementation ARA Oil On Production Performance, Egg Quality And Fatty Acid Composition In Laying Hens

Dian Rizki Purba[1], Xiao-guang Dong[2], Jing Wang[3], Hai-jun Zhang[4]
Guang-hai Qi[5*], Shu-geng Wu[6]

[1,2,3,4,5,6]Risk Assessment Laboratory of Feed Derived Factors to Animal Product Quality Safety (Beijing) of Ministry of Agriculture & Rural Affairs, Institute of Feed Research, Chinese Academy of Agricultural Sciences, Beijing 100081, China
[*] Corresponding Author:
Email: qiguanghai@caas.cn

Abstract.
The effect of supplementation ARA oil on production performance, egg quality, fatty acid composition in laying hens were examined on this study. A total of 450 Roman White laying hens with a similar egg production rate and good body condition at 55 weeks of age were randomly divided into 5 treatments. Each treatment was replicated 6 times with 15 hens per replicate. Hens were fed basal diets with 0, 0.3125%, 0.625%, 1.25% and 2.5% ARA oil addition. The feeding trial lasted for 12 weeks after 1 week of adaption. The supplementation ARA oil was effect to decreased egg production, egg weight, daily feed intake and feed conversion ratio (FCR) ($P<0.05$) during 9-12 week, but on egg quality parameters showed slightly changed during the 1-12 week but not consistency and that change was not statistically significant. Arachidonate acid (ARA) was increased by supplementation ARA oil ($P<0.05$). This effect was detected in directly proportional to the addition of ARA oil on diet. This is opposite effect to DHA, DHA was decreased by supplementation ARA oil ($P<0.05$) and also effect to ratio ARA/DHA was increased ($P<0.05$) by supplementation ARA oil.

Keywords: *ARA, performance. Egg quality, lipid composition*

I. INTRODUCTION

During the first year of life, infants have special nutritional requirements to maintain a healthy body and support rapid growth and development. Human milk is typically the sole source of nutrition that must supply the infant with appropriate amounts of energy and nutrients. DHA and ARA are always present in human milk that play key roles in the structure and function of human tissues, immune function, and brain and retinal development during gestation and infancy [1, 2].

In exclusively breastfed infants, the mean human milk intake at 6 months has been measured to be 854 g/day [3] with the average ARA and DHA intakes are about 169 mg/day and 115 mg/day, respectively [4]. Moreover, many infants continue to receive human milk throughout the first year of life and longer. It is estimated that at 12 months of age the intake of human milk is in the range of 600–900g/day [3]. This amount provides infants with an ARA intake from human milk in the range of 118–178 mg/day and also many infants continue using infant formulas, typically contain levels

of ARA and DHA at 140 mg/day and 100 mg/day, respectfully, based on worldwide averages of ARA and DHA content in human milk [5].

[6] describe that in both developed and developing countries weaning foods contain low amounts of fat, which results in a sharp transition from adequate fat intake during breastfeeding to significantly lower fat intake when children are weaned from the breast. [7] describe that based on these dietary intakes from local and national surveys, it is clear that the diets of young children contain low levels of ARA. Reported mean intakes of ARA at 10 to 18 mg/day in developing and developed countries are only about 10% of the amount of ARA available to infants fed human milk or infant formulas containing DHA and ARA. For these reasons, eggs enriched ARA or ARA: DHA can considered to be one of source ARA and DHA for infant due to vulnerable infants and young children need energy- and nutrient-dense foods to grow and develop both physically and mentally [8].

The hens can convert a proportion of dietary FA to PUFA that can be deposited in the egg yolk, thereby being considered a good source of ω-6 and ω-3 PUFA [9]. Egg yolk approximately contains 10% of phospholipids (on the base of the wet weight), representing 22% of egg yolk total solids. Egg lipids are characterized and distinguished by their high content of PLs, they consisted of 33% PLs, 62% triglycerides, and 5% cholesterol. [10] conclude that a Greek egg yolk would be useful as a supplemental or weaning food just as it has been used for thousands of years. Greek eggs have almost equal amounts of ARA and DHA and therefore are particularly suited to infant feeding. Probiotics can be defined as living microorganisms that can establish colonies in animal intestinal tracts and improve the host micro-ecological balance.

II. METHODS

Location and duration

The experiment was conducted at the Zhuozhou Animal Testing Base and Feed Laboratory of Feed Research Institute, Chinese Academy of Agricultural Sciences, and started from May 2019 to December 2019.

Experimantal design, animal and diets

A completely randomized design was employed in this experiment. A total of 450 Roman White laying hens with a similar egg production rate and good body condition at 55 weeks of age were randomly divided into 5 treatments. Each treatment was replicated 6 times with 15 hens per replicate. Hens were fed basal diets with 0, 0.3125%, 0.625%, 1.25% and 2.5% ARA oil addition. ARA-rich oil from *Mortierella apina* fermentation produced from Inner Mongolia Jindawei Pharmaceutical co., Ltd. This oil is a mixture of triglycerides containing polyunsaturated fatty acids (PUFA) in which the predominant fatty acid (≥40%) is ARA.

The ingredient composition contents are shown in table 1.

Table 1. Composition levels of the experiment diets

Ingredients (%)	0% ARA oil	0.3125% ARA oil	0.625% ARA oil	1.25% ARA oil	2.5% ARA oil
玉米 Corn	57.45	57.45	57.45	57.45	57.45
豆粕 Soybean Meal	24.66	24.66	24.66	24.66	24.66
麸皮 Wheat Bran	3.00	3.00	3.00	3.00	3.00
Corn Oil	2.50	2.19	1.88	1.25	0.00
ARA Oil	0.00	0.3125	0.625	1.25	2.5
石粉 Stone Powder	9.50	9.50	9.50	9.50	9.50
磷酸氢钙 CaHPO4	0.80	0.80	0.80	0.80	0.80
食盐NaCl	0.15	0.15	0.15	0.15	0.15
硫酸钠 sodium sulphate	0.40	0.40	0.40	0.40	0.40
DL-Met	0.14	0.14	0.14	0.14	0.14
多矿	0.20	0.20	0.20	0.20	0.20
多维	0.03	0.03	0.03	0.03	0.03
氯化胆碱	0.15	0.15	0.15	0.15	0.15
倍肽德	1.00	1.00	1.00	1.00	1.00
植酸酶	0.02	0.02	0.02	0.02	0.02

[1]The premix provided the diet per kilogram as follows：15,000 IU of Vitamin A, 3,900 IU of Vitamin D3, 30 IU of Vitamin E, 3 mg of Vitamin K3, 12.4 mg Vitamin C, 29 mg of Vitamin C, 4.5 mg of C6, 0.021 mg of C12, 30 mg of pantothenic acid, 45 mg of nicotinamide， 1.2 mg of folic acid and 0.18 mg of biotin, 8 mg of cuprum, 100 mg of manganese, 40 mg of zinc, 80 mg of ferric, 0.35 mg of iodine and 0.15 mg of selenium

Feeding management

The experiment was conducted at Zhuozhou Animal Experiment Station of the Chinese Academy of Agricultural Sciences. The hens will be reared in battery cages. The feed will be offered at the rate of 130 g/day and water ad libitum. Light will be provided for 16 h/day throughout the experiment. Room 0temperature will be maintained between 22 and 26℃, relative humidity 50%-60%, natural ventilation combined with longitudinal negative pressure ventilation. The feeding trial lasted for 12 weeks after 1 week of adaption.

Performance parameters

Eggs will be collected daily at 14.00 pm and the number of eggs and egg weight in each replicate will be recorded. Feed consumption by each replicate will be recorded weekly. Average egg weight (AEW) will be calculated as the mean weight of all eggs from each replicate. The feed conversion ratio (FCR) will be calculated as feed consumption divided by the total egg weight (feed/egg, g/g). Mortality will be recorded daily as it occurs. Daily feed intake (DFI) will be adjusted for mortalities and will be calculated using the following equation: DFI = feed consumption(g)/ (hen number ×d).

Egg production (EP), DFI, AEW, and FCR will be calculated for wk. 1 to 6, wk. 7 to 12, and wk. 1 to 12 of the experiment.

Egg quality

On the 4th, 8th and 12th weeks of the experiment, 3 eggs will be taken randomly in each replication to determine the egg quality. Egg weight, Haugh unit, concentrate protein height, egg yolk color will be determined using SONOVA egg quality analyzer (Egg Analyzer TM, Orka Technology Ltd.); eggshell thickness will be measured by eggshell thickness meter (PEACOCK P-1, JAPAN); The eggshell strength will be measured by an Egg Force Reader (Orka Technology Ltd.); the egg shape index will be measured by an Egg Index Reader (Fuji Index Reader, Fujibira Industry Co., Ltd.).

Egg composition analysis: Each egg will be weighed individually and then will be broken, and the yolk will be separated from the albumen. The yolk weight will be determined after the chalazae are removed with forceps. Each yolk is rolled on a paper towel to remove adhering albumen. The eggshells are washed, air-dried, and weighed. Albumen weight will be calculated by subtracting the yolk and eggshell weights from the weight of the individual egg. These measurements will be used to determine the albumen proportion (%; albumen weight/egg weight×100) and the yolk proportion (%; yolk weight/egg weight×100).

Fatty acid composition

To analysis fatty acid on egg yolk will using the Fatty acid methyl esters (FAME) analysis.

Three eggs per replicate were collected at random and analysis for determination of fatty acid methyl esters (FAME) after maintenance on the 4th, 8th and 12th weeks of the experiment was done. Eggs were broken and the egg yolk was separated and stored at -20°C until analysis. Fatty acid methyl esters (FAME) were measured according to the method of Shahid et al., (2015). Fatty acids of the egg yolk were quantified by using gas chromatography. Fatty acid methyl esters were separated using a GC-9A gas chromatograph equipped with a flame ionization detector and silica capillary column. Nitrogen was used as the carrier gas with a flow rate of 35 mL/min. The pressure of hydrogen and air was set at 0.5 kg/cm. The temperature of the injector and detector was maintained at 250°C. Fatty acid methyl esters were identified based on comparison of retention times and standards. The fatty acid composition was expressed as the percentage of the total fatty acids. Briefly, 50 mg egg yolk was mixed with 1.5mL of methanolic sodium hydroxide and boiled at 100°C for 5 min. After cooling, 2.5mL boron trifluoride (14%) was added into the tubes to collect the residual lipids (30 min at 80°C). Fatty acid esters were separated in 1mL hexane. Five milliliters of saturated salt (400 g of NaCl/L) was added, and samples were vortexed for 2 min, followed by centrifugation at 800 x g for 5 min. The top hexane layer was transferred into a new gas chromatography (GC) vial. Complete fatty acids methyl esters were separated by adding 1 ml hexane and vortexed for 2 min followed by centrifugation (800 x g for 5 min). The fatty acid layer was transferred into GC vials and analyzed with gas

chromatography-mass spectrometry with a fused silica capillary column with 50 m × 0.250 mm and 0.2 µm film thickness using hydrogen as a carrier gas. One microliter of the sample was injected into the GC with a split ratio of 1:5. The flame ionization detector was set to 280°C. The time-temperature program used started with an initial temperature of 140°C for 4 min, increased by 4°C per minute to a final temperature of 240°C and was held at this temperature for 20 min. The fatty acid methyl esters were identified using external standards, and the fatty acid contents were calculated from the peak area of the corresponding fatty acid in relation to the total area of all peaks.

Statistical analysis

SPSS software (IBM-SPSS Inc., Chicago, IL) was used to analyze the data. An one-way analysis of variance (ANOVA) was used to evaluate the treatmental effects. Tukey HSD was employed for mutiple comarison of means. The values were expressed as. All statement of significance was considered at P <0.05.

III. RESULT AND DISCUSSION

Performance parameters

The effects of supplementation ARA oil on performance parameter are presented in Table 2. The data showed that the production performance was decreased during the whole week maintenance in all parameters among all group experiment.

Table 2. effects of supplementation ARA oil on performance parameter

Parameters	Week	Arachidonate oi ratio in diet					SEM	P-Value
		0 %	0.3125	0.625%	1.25%	2.5%		
Egg Production	1-4	80.35	80.27	80.67	80.75	78.01	0.817	0.840
	5-8	71.90	77.69	80.31	77.81	71.90	1.419	0.216
	9-12	61.93^{c}	73.08^{ab}	77.24^{a}	74.32^{ab}	65.59^{bc}	1.768	0.020
	1-12	71.51	77.06	79.43	77.67	71.91	1.200	0.119
Egg Weight	1-4	59.88	60.78	60.01	60.36	59.99	0.204	0.857
	5-8	61.17	62.03	61.65	61.76	60.40	0.213	0.117
	9-12	62.68^{ab}	63.84^{a}	62.95^{ab}	62.55^{ab}	61.55^{b}	0.242	0.042
	1-12	61.10	62.05	61.50	61.51	60.60	1.855	0.141
Egg Mass	1-4	48.13	48.59	48.38	48.72	46.82	0.505	0.791
	5-8	43.98	48.20	49.53	48.08	43.41	0.919	0.117
	9-12	38.76^{c}	46.67^{ab}	48.65^{a}	46.45^{ab}	40.38^{bc}	1.136	0.010
	1-12	43.68^{b}	47.83^{ab}	48.86^{a}	47.77^{ab}	43.58^{b}	0.769	0.059
FCR (Feed/Egg)	1-4	2.28	2.28	2.33	2.27	2.35	0.026	0.810
	5-8	2.49	2.37	2.28	2.28	2.58	0.045	0.154
	9-12	2.57^{ab}	2.34^{a-c}	2.20^{a}	2.30^{ab}	2.68^{b}	0.058	0.040
	1-12	2.44	2.33	2.27	2.28	2.54	0.038	0.115
Daily Feed Intake	1-4	109.67	110.19	112.19	110.40	109.49	0.438	0.319
	5-8	109.14^{a}	113.37^{b}	112.53^{b}	108.89^{a}	110.06^{a}	0.462	0.001
	9-12	99.04^{a}	107.65^{b}	105.88^{b}	106.82^{b}	105.73^{b}	0.747	<0.001
	1-12	105.95^{a}	110.40^{b}	110.20^{b}	108.71^{b}	108.43^{b}	0.409	0.001

During 1-4 week and 5-8-week maintenance, the production parameters among all group experiment was decreased by supplementation ARA oil but was not statistically significant. At the end period of maintenance (9-12 week), the data showed that the egg production, egg weight, egg mass, average daily feed intake and FCR were showed significant decreased ($P<0.05$). Ratio ARA oil on diet (group 2,3,4,5) showed that when ratio ARA oil was reduced, the production performance slightly increased, but not statistically different.

Egg quality

The effects of supplementation ARA oil on egg quality are displayed in Table 3. Overall, no significant change observed in egg quality parameters. Eggshell strength, eggshell thickness, Haugh unit, albumen height, egg yolk color and egg yolk rate showed slightly changed during the maintenance but not consistency and change was not statistically significant.

Table 3. Effects of supplementation ARA oil on egg quality of laying hens

Parameters	Week	Arachidonate oil ratio in diet					SEM	P-Value
		0%	0.3125%	0.625%	1.25%	2.5%		
Eggshell Strength	4	29.71	31.41	28.82	27.6	31.09	0.840	0.612
	8	41.8	38.80	42.86	36.22	43.87	1.032	0.102
	12	33.80	30.56	30.09	27.96	32.18	0.844	0.248
Eggshell Thickness	4	39.34	41.09	41.55	41.22	41.31	0.327	0.206
	8	46.38	44.57	45.53	44.70	46.07	0.310	0.252
	12	41.09	40.42	41.55	39.88	40.72	0.250	0.281
Haugh Unit	4	78.51^{b}	83.05^{ab}	82.61^{ab}	82.81^{ab}	84.85^{a}	0.753	0.092
	8	84.39	81.43	82.24	84.87	85.23	0.744	0.408
	12	85.36	80.36	82.38	82.64	86.98	0.868	0.112
Albumen Height	4	6.43	7.09	7.07	6.9	7.17	0.097	0.108
	8	7.44^{ab}	7.07^{b}	7.15^{ab}	7.48^{ab}	7.53^{a}	0.121	0.628
	12	7.53	6.84	7.08	7.04	7.71	0.110	0.054
Egg Yolk Color	4	4.66	4.83	4.72	4.97	4.55	0.085	0.628
	8	5.44	5.44	4.78	6.33	6.80	0.086	0.264
	12	4.25	4.22	4.38	4.25	4.72	0.082	0.532
Egg Yolk Rate (%)	4	27.21	28.29	27.70	27.82	26.67	0.216	0.156
	8	27.41	26.54	27.06	26.89	26.60	0.280	0.290
	12	26.99	27.90	27.77	26.19	27.83	0.349	0.488

Fatty acid composition

The effects of supplementation ARA oil on fatty acid composition are displayed in Table 4 and Table 5 shown the effects of supplementation ARA oil on ARA/DHA contain in egg.

Table 4. Effect of supplementation ARA oil on ARA contain in egg

FA (mg/g)	Arachidonate oil ratio on diet				
	0%	0.3125%	0.625%	1.25%	2.50%
C12:0	0.03716	0.034668	0.034771	0	0.047953
C14:0	2.168169	2.306825	2.169591	2.125336	3.00493
C14:1	0.381355	0.432247	0.504627	0.266938	0.447502
C15:0	0.322709	0.281837	0.347994	0.328827	0.500664
C16:0	108.3567	109.9737	102.2558	99.15466	116.2379
C16:1	4.539323	2.090221	1.630512	2.720837	3.208599
C17:0	1.686809	1.472835	1.406212	1.424247	2.037889
C18:0	94.4704	61.55806	73.92502	72.8826	94.36323
C18:1n9	171.4651	178.3798	167.7419	141.972	162.7737
C18:2n6 LA	51.25203	52.05938	69.72265	70.24992	85.5477
C18:3n6	0.118653	0.10606	1.01143	1.035069	0.076544
C18:3n3 ALA	2.679736	1.608685	1.671865	2.31165	3.568544
C20:0	0.687562	0.605062	0.500174	0.524046	0.707195
C20:1	0.4101	0.280174	0.33725	0.283381	0.307521
C20:3n6	0.960661	0.559084	2.102985	0.377026	0.541696
C20:4n6 ARA	28.94207	29.63131	30.12238	34.90048	59.25198
C20:3n9	0.575464	0.443196	0.270815	0.192829	0.192679
C22:0	0.653459	0.502819	0.42965	0.438799	0.587606
C24:0	0.580593	0.552769	0.501954	0.534404	0.885627
C24:1	0.942812	0.523138	0.495707	0.484097	0.492748
C22:6n3 DHA	8.241096	6.143199	5.749498	5.345941	7.501813
	479.4719	449.5451	462.9328	437.5531	542.284
SFA	208.9636	177.2886	181.5712	177.4129	218.373
MUFA	6.27359	3.32578	2.968095	3.755254	4.456369
PUFA	264.2348	268.9307	278.3936	256.3849	319.4546
n3	10.92083	7.751884	7.421363	7.657592	11.07036
n6	81.27341	82.35583	102.9594	106.5625	145.4179
n6/n3	7.442054	10.62398	13.87339	13.91593	13.13579

The data showed that supplementation ARA oil on diet significant ($P<0.05$) increased the ARA content on egg yolk and also influenced the DHA content ($P<0.05$) slightly decreased when the ARA ratio on diet increased, its caused ARA and DHA have opposite metabolism, and also affect to ratio between ARA:DHA showed significant ($P<0.05$).

Table 5. Effect of supplementation ARA oil on ARA contain in egg

FA (mg/g)	Arachidonate oil ratio on diet					SEM	P-Value
	0%	0.3125%	0.63%	1.25%	2.50%		

C20:4n6 ARA	28.94207[b]	29.63131[b]	30.12238[b]	34.90048[b]	59.25198[a]	0.510	0.001
C22:6n3 DHA	8.241096[a]	6.143199[c]	5.749498[bc]	5.345941[bc]	7.501813[ab]	0.403	0.035
ARA/DHA	3.51192	4.823433	5.239132	6.528406	7.898355	0.501	<0.001

Supplementation ARA oil on diet also effect to C18:2n6 LA and C18:3n3 ALA content on egg yolk. LA content was increased while LA content was decreased caused by supplementation ARA oil on diet. LA and ALA content were effect to ARA and DHA metabolism also, LA are converted into ARA and ALA are converted to EPA, EPA will be converted to DHA. [11] describe that ALA and LA were play a role in metabolic pathway for both ω-3 and ω-6 fatty acid. LA and ALA are converted into ARA and EPA respectively by a series of desaturation and elongation reactions. The EPA to DHA conversion involves elongation of EPA to 24:6(n-3) occurring sequentially, followed by a single β-oxidation process, to yield DHA.

Growth and production performance of poultry are improved by supplementation of fatty acids or their sources. The supplementation of fats and oils (as an omega source) in limited amounts leads to better utilization of feed and energy, with subsequent improvement in growth and performance [12]. Based on data, the supplementation ARA oil was effect to decreased egg production, egg weight, egg mass, the daily feed intake and FCR ($P<0.05$) during 9-12 week, but on egg quality parameters showed slightly changed during the 1-12 week but not consistency and that change was not statistically significant. The contain of arachidonate acid (ARA) was increased by supplementation ARA oil ($P<0.05$) among all group experiment. This effect was detected in directly proportional to the addition of ARA oil on diet. This is opposite effect to DHA, DHA was decreased by supplementation ARA oil ($P<0.05$) and also effect to ratio ARA/DHA was increased ($P<0.05$) by supplementation ARA oil. [13] evaluated the effect of dietary supplementation on two levels (15g/kg and 30g/kg diet) of SO, rapeseed oil, and LO for 12 weeks in laying hens. They concluded that egg production, egg weight, feed intake, FCR, and live weight were not significantly affected by the treatments. However, hens receiving SO produced less intensively colored egg yolks than those receiving other oils in their diet ($p < 0.05$). Moreover, the composition of fatty acid in egg yolks was significantly ($p < 0.05$) affected by the treatment, whereas the cholesterol content was not influenced. There was a significant ($p < 0.05$) interaction between fat source and the level of inclusion in the diet, and LNA content was increased when hens were fed diets with linseed and rapeseed oil (30 g/kg diet). In contrast, [14], studied the effects of the dietary inclusion (for 12 weeks) of different fat sources (cottonseed oil, soybean oil, lard, SO, or canola oil) on egg quality, and egg yolk lipid profiles.

The different fat sources did not affect eggshell quality; however, the lipid profile of the egg yolk changed based on dietary fat sources. Optimal changes were considered to be lower levels of SFA and LA, and higher levels of ALA and DHA.

Such changes were promoted by the addition of different fat sources, particularly canola oil; however, it did not enhance the egg content of PUFA.

The nutraceutical value and health benefits of eggs can be enhanced by adapting appropriate feeding strategies in poultry as well as by developing designer eggs [15]. These improve the quality and quantity of eggs. Adding n-3 and n-6 fatty acids to the diet has become more important recently [16]. For at least the past three decades, studies on the beneficial activities of long-chain PUFA (LC-PUFA) in biological processes have been conducted. Dietary intervention with n-3 may influence chicken immunity and lead to the production of poultry products with health benefits for the consumer.

Using PUFA in poultry diets significantly reduces the cholesterol and total lipid content in the blood and egg yolk. Dietary n-3 PUFA can reduce TG synthesis and chylomicron secretion from intestinal cell and suppress hepatic fatty acid synthesis on TG production [17]. Diet enrich in linoleic acid and oleic acid also suppress LDL concentration, but n-3 PUFA appear to be more effective [18]. Dietary PUFA may promote lipoprotein metabolism by altering the activity of certain lipolysis and transfer enzymes function in the plasma. Dietary PUFA of vegetable oils, containing mostly linoleic acid, are effective in counter-acting the effects of dietary saturated fatty acids [19]. [20] evaluated the effect of dietary supplementation four different rapeseed oil sources [high erucic acid of Mianyang city (MH); high erucic acid of Deyang city (DH); low erucic acid of Mianyang (ML); low erucic acid of Deyang (DL)] at two levels (2% and 4%) for 12 wk. They concluded that dietary rapeseed oil supplementation decreased total triglyceride (TG; $P<0.01$) and increased high-density lipoprotein cholesterol ($P = 0.02$). Regardless of rapeseed oil levels, layers fed MH had higher TG ($P < 0.01$), TC ($P < 0.05$), low-density lipoprotein cholesterol ($P <0.05$), alanine transaminase ($P < 0.01$) than those fed other sources. If taken together, the addition of rapeseed oil reduced TC and TG in the serum of laying hens. [21] also reported that the cholesterol content of eggs was decreased when birds were fed a diet supplemented with n-3 fatty acids. Moreover, increasing dietary levels of FO and milled flaxseed improved the concentration of linoleic acid (LA), EPA, and DHA in the yolk, and the fatty acid deposition from FO was found to be two times greater than that from milled flaxseed when fed at the same dietary levels.

IV. CONCLUSION

In conclusion, this result shown that supplementation ARA oil significant increase the ARA contain on egg. This effect was detected in directly proportional to the addition of ARA oil on diet. The more ARA was supplementation to the feed then the ARA contain on egg increased but the production performance slightly reduced, but not statistically different. Supplementation ARA oil 0.625% and 1.25% would be considered, the performance not significantly reduced while ARA contain increased on

egg. Egg enriched ARA would be useful as a supplemental or weaning food to meet infant nutrition requirements, but both ARA and DHA were key roles of infant nutrition requirement, therefore in further study need to meet ARA/DHA balance ratio.

V. ACKNOWLEDGMENTS

This study was financially supported by the China Agriculture Research System (CARS-41-K12). Thankful to the staff of Laboratory of Feed Biotechnology of Agricultural Ministry, Feed Research Institute, GSCAAS, Beijing, for the technical support and assistance.

REFERENCES

[1] Martinez, M., Tissue levels of polyunsaturated fatty acids during early human development. ***J. Pediatr***. 1992,120, S129–S138.

[2] Koletzko, B., Carlson, S.E., Van Goudoever, J.B., Should infant formula provide both omega-3 DHA and omega-6 arachidonic acid? Ann. Nutr. Metab. 2015, 66, 137–138.

[3] World Health Organization (WHO). Nutrient Adequacy of Exclusive Breastfeeding for the Term Infant during the First Six Months of Life, 2002. Available online: http://www.who.int/nutrition/publications/ infant feeding/9241562110/en/.

[4] U.S. Department of Agriculture, Agricultural Research Service. USDA National Nutrient Database for Standard Reference, Release 27. Nutrient Data Laboratory Home Page. 2014. Available online: http://www.ars.usda.gov/nutrientdata.

[5] Brenna, J.T., Varamini, B., Jensen, R.G., Diersen-schade, D.A., Boettcher, J.A., Arterburn, L.M., Docosahexaenoic and arachidonic acid concentrations in human milk worldwide. Am. J. Clin. Nutr. 2007, 85, 1457–1464.

[6] Michaelsen, K.F., Dewey, K.G., perez-exposito, A.B., Food sources and intake of n-6 and n-3 fatty acids in low-income countries with emphasis on infants, young children (6–24 months), and pregnant and lactating women. Mater. Child Nutr. 2011, 7 (Suppl. S2), 124–140.

[7] Hadley, Kevin B., Alan S. Ryan, Stewart Forsyth, Sheila Gautier, & Norman Salem JR. ***The Essentiality of Arachidonic Acid in Development. Nutrients Journal,*** 2016, 8,216.

[8] Agostoni, C., Docosahexaenoic acid (DHA): From the maternal-foetal dyad to the complementary feeding period. Early Hum. Dev. 2010, 86 (Suppl. S1), 3–6.

[9] Surai, P. F., & Sparks, N. H. C., Designer eggs: From improvement of egg composition to functional food. Trends in Food Science and Technology, 2001, 12,7-16.

[10] Simopoulos, AP & Salem, N., JR., Egg yolk as a source of long-chain polyunsaturated fatty acids in infant feeding. Am J Clinic Nutr 1992:55:411-4.

[11] Innis S., Dietary ω-3 fatty acids and the developing brain. Brain Res, 2008, 1237, 35-43.

[12] Shang, X.G., Wang, F. L., LI, D. F., Yin, J. D., LI, J.Y., Effects of dietary conjugated linoleic acid on the productivity of laying hens and egg quality during refrigerated storage. Poult. Sci. 2004, 83, 1688–1695.

[13] Schneiderová, D., Zelenka, J., Mrkvicová, E., Poultry meat production as a functional food with a voluntary n-6 and n-3 polyunsaturated fatty acids ratio. Czech J. Anim. Sci. 2007, 52, 203–213.

[14] Ahmad, S., Yousaf, M., Kamran, Z., Sohail, M.U., Production of n-3 Pufa enriched eggs by feeding various dietary ratios of n-6 to n-3 fatty acids and vitamin a level to the laying hens in hot climate. J. Poult. Sci, 2014, 51, 213–219.

[15] Dhama, K., Tiwari, R., Khan, R.U., Chakraborty, S., Gopi, M., Karthik, K., Saminathan, M., Desingu, P.A., Sunkara, L.T., Growth promoters and novel feed additives improving poultry production and health, bioactive principles and beneficial applications: The trends and advances—A review. Int. J. Pharmacol. 2014, 10,129–159.

[16] Lee, S.A., Whenham, N., Bedford, M.R., Review on docosahexaenoic acid in poultry and swine nutrition: Consequence of enriched animal products on performance and health characteristics. Anim. Nutr. 2019, 5,11–21.

[17] Harris, W. S. Fish oils and plasma lipid and lipoprotein metabolism in humans: a Critical Review. ***J. LIPID RES***. 1989, 30:785.

[18] Nestel, P. J., W. E. Connor, M. F. Reardon, S. Connor, S. Wong, and R. Boston, Suppression by diets rich in fish oil of very low-density lipoprotein production in man. ***J. Clin. Invest***. 1984, 74:82–89.

[19] Grundy, S. M., Monounsaturated fatty acids, plasma cholesterol, and coronary heart disease. Am***. J. Clin. Nutr***. 1987, 45:1168–1175.

[20] Yuan, N., J. P. Wang., X. M. Ding, Effects of supplementation with different rapeseed oil sources and levels on production performance, egg quality, and serum parameters in laying hens. Poultry Science, 2019, 98:1697–1705.

[21] Ahmad, S.A., Yousaf, M., Sabri, M.A., Kamran, Z., Response of laying hens to omega-3 fatty acids for performance and egg quality. Avian Biol. Res., 2012, 5, 1–10.