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Egg Internal Quality and n-3 Fatty Acids of Native Chicken Fed on Skipjack Fish (*Katsuwonus pelamis. l*) Industrial Waste Containing Feed

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Abstract: The objective of this study was to assess the effect of the use of skipjack fish industrial waste to native hen diets on egg internal quality. A total of 180 native laying hens 27-34 weeks of age were used in this experiment and randomly distributed into 180 units of battery cage. There were three types of skipjack waste used as main treatment diets, namely Heads and Entrails (HE) (S1), the rest of filleting (bones) (S2) and company sorting residuals (arachon) (S3). The treatment diets have five levels of skipjack waste each, hence the 15 treatment diets combination which consisted of one diet without skipjack waste (control) and the others four containing 5, 10, 15 and 20% skipjack wastes each. The experiment was carried out based on a nested completely randomized design, comprising three main treatments (A factors), five levels as sub-plot (B factors) with three replicates containing four native hens each. Data were analyzed using and the differences between treatments were tested Duncan's multiple range test. The variables measured were egg weight, Hen Day Production (HDP), egg weight, yolk color and n-3 FA. The results revealed that the main treatments HE, bones and arachon were not significantly effects (P>0.05) on HDP, egg weight and yolk color. Meanwhile, increasing the level of HE, Bones and Arachon did not affect significantly (P>0.05) against HDP, egg weight, yolk color, Furthermore, the treatments (HE, Bones, Arachon) were not significantly effects (P>0.05) on SAFA, MUFA, Σn-3, Σn-6, Ratio of n-3:n-6. Similarly, increasing the level of up to 20% of HE, Bones and Arachon to hen diets did not affect significantly (P>0.05) against SAFA, MUFA, Σn-3, Σn-6 and ratio of n-3: n-6. Therefore, the skipjack industrial waste, as Head and Entrails (HE), bones and arachon could be used to the ration for optimizing productivity of native hen. It was recommended, the level of using the skipjack waste of up to 15% in ration.

Key words: Internal quality, n-3 FA, skipjack fish industry waste

INTRODUCTION

Native chicken is one among animal resources which generally become a mainstay, as well as an economic buffer for the rural communities. This proved that the rural communities now prefer to keep native chicken, because it has special specifications on both the taste and aroma of its eggs and meat and the efficacy of native hen egg and meat can not be replaced by any of race chicken products. Various studies have been conducted on the characteristics and quality of chicken products especially egg and meat.

Native hen eggs is one of livestock product which has a high nutritional value that can be accepted by all consumers. Public awareness of the native hen eggs which is a conventional food and medicine due to its bioactive components form of functional food which able to prevent the disease. This gets a particular interest in order to meet consumer demand and the quality of native chicken eggs, mainly on the content of amino acids and n-3 fatty acids. This can be done by feeding trials on chickens. Others function of omega-3 fatty acids (PUFA)-enriched eggs are decreases the risk of heart disease, inhibits the growth of prostate and breast cancer and is required for normal fetal brain and visual development (Lewis *et al*., 2000).

Skipjack fish industrial waste has potential as an alternative feed source in Indonesia Skipjack industrial waste is a byproduct of making wooden fish and canning fish. North Sulawesi Utara is known as one of the region's potential skipjack fishing ground in Indonesia. The production of skipjack in Sulawesi Utara in 2010 amounted to 60.168.1 tons/per year. PT Nichindo is one of the wooden fish export company in Sulawesi Utara. Skipjack waste is by-product from the process of making skipjack wooden fish, where one ton of wet skipjack waste can produce 250 kg of dry waste with 10 moisture content. Skipjack fish industrial waste is classified as Heads and Entrails (HE), the rest of filleting (Bones), company sorting residuals (Arachon). To get standardized as animal feed, it is necessary to test the materials trough laboratory proximate analysis and biological experiments, so it suitable to be used as poultry feed in improving product quality, both eggs and meat.

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Therefore, this study was performed to assess the effect of using skipjack industry waste towards egg quality of native chicken.

MATERIALS AND METHODS

Experiment animals and diets: A total of 180 head native hen 5-6 months aged were used in this study. The animal average body weight was 861 g with±9.19 coefficient of variability. At the time before the study the average of HDP was 0%. The animals were placed individually into 180 units of bamboo battery cage sized 50 x 33 x 25. Each cage was a repeat treatment consisted of 4 hens and it was equipped with drinking and feeding tools and hollow egg nest as well.

During three weeks prior to conducting the experiment, the animals experienced a period of flushing. This aims to eliminate the effect of a given feed earlier. The diets given during the flushing based on the need and age of the animals and it was not the treatment diets. Subsequently, it was followed by an adaptation period of 2 weeks to introduce the new diets and thereafter, the experimental diets were applied to the experiment hens, where the aged of hens at the time lasts were range 27-34 weeks old. All experimental diets were isonutrients and isocalorics, based on corn, rice bran, soybean meal, coconut cake, $CaCO_3$, oil, salt and top mix.

There were three types of skipjack waste used as main treatment diets, namely Head and Entrails (HE) (S1), bones (S2) and arachon (S3). The treatment diets have five levels of skipjack waste each, hence the 15 treatment diets combination which consisted of one diet without skipjack waste (control) and the others four containing 5, 10, 15 and 20% skipjack wastes each. The ingredients and fatty acids composition of experimental diets is shown in Table 1 and 2, respectively.

The experiment was conducted for 8 weeks for applying the treatment diets. The experimental hens were maintained with diets and drinking water provided *ad libitum* daily. Feed consumption were measured weekly, whereas weight and egg production recorded daily.

During the entire experiment (8 weeks), two eggs from each experimental unit were collected at weeks 2, 4, 6 and 8 for the analysis of egg quality. At week 8, three eggs were taken from each replicate, then weighed and broke it. The yolks separated from the whites and the yolks were then pooled. The total of egg used consists of 45 eggs obtained from 180 hens fed HE, Bones and Arachon.

Process Skipjack Industry Waste (Heads and entrails (HE), Bones and Arachon): Fresh skipjack washed and separated by size, thereafter the separation between the fish meat and the parts that were not used, involves part of the head and entrails (HE), the rest of filleting (bone) and the company sorting residuals (Arachon). At the time of boiling at the 80°C temperature for 30 minutes three parts HE, Bone, Arachon were separated and it was then drained in the fumigation room at 80-100°C temperature until moisture content down to 10 % HE, Bones, Arachon were then milled made into native hen feed.

Table 1: Average of HDP, egg weight, egg yolk color, of the native hens that giving dietary treatments of HE, Bones and Arachon for 8 weeks

Effect of Types	HDP (%)	Egg weight (g/egg)	Egg yolk color
HE	39.35±0.14	39.96±0.01	11.47±0.02
Bone	40.42±0.13	40.15±0.15	11.11±0.05
Arachon	37.86±0.08	39.95±0.08	11.51±0.03

Description: The same superscript in the same row indicates no significant effect (P>0.05)

Table 2: Effect of Different Level of HE, Bones and Arachon on HDP, egg weight, egg yolk color

Treatments		HDP (%)	EW (g/ekor/hari)	Egg Color
HE	0	34.68±0.01	40.07±0.02	11.50±0.02
	5	36.76±0.06	39.68±0.01	11.34±0.02
	10	44.22±0.12	39.56±0.02	11.38±0.04
	15	38.84±0.12	40.18±0.01	11.46±0.02
	20	42.26±0.19	40.30±0.01	11.67±0.01
Bones	0	40.18±0.04	40.06±0.01	11.42±0.02
	5	35.27±0.06	40.52±0.01	11.25±0.04
	10	40.77±0.16	40.61±0.02	10.88±0.04
	15	47.62±0.12	39.94±0.01	10.88±0.09
	20	38.25±0.02	39.60±0.01	11.13±0.07
Arachon	0	38.10±0.04	40.25±0.00	11.79±0.02
	5	37.08±0.11	39.86±0.00	11.25±0.02
	10	41.97±0.09	39.84±0.02	11.42±0.02
	15	35.86±0.05	39.83±0.00	11.25±0.05
	20	36.31±0.03	39.99±0.01	11.84±0.03

Description: The same superscript in the same row indicates no significant effect (P>0.05). EW: Egg weight

The experiment was carried out based on a completely randomized design arranged by a nested pattern with three factors of treatments. The A factor was the type of skipjack waste treatments, consisted of head and entrails (HE) (S1), Bones (S2), Arachon (S3), while the B factor was the levels under the A factor treatments (S1, S2, S3), namely 0, 5, 10, 15 and 20% each. The levels of B nested in factor A, in which each treatment was repeated three times.

Variables: The variables studied were the physical characteristics of eggs, namely: egg weight (Keshavarz and Austic, 2004), Hen Day Production (HDP) (Dadang, 2006), yolk weight and yolk color (Stadelman and Cotteriil, 1994; Parmar *et al.*, 2006; Monira *et al.*, 2003), the content of n-3 fatty acids (Folch *et al.*, 1957).

Sampling was performed in three weeks after the hens were treated, a number of eggs were collected each week on a specific day (Tuesday) for 8 weeks consecutively, in the next morning the collected eggs were then individually weighed and broken out. The internal quality of egg observed were: weighing the eggs, its external condition was recorded (wholeness, cleanliness and egg shape). Yolk color was determined by matching with one of the matching bands score of the yolk colour fan (Hunton, 1987; Parmar *et al.*, 2006; Monira *et al.*, 2003). Having separated the whites of the eggs and egg yolks and both were then weighed to determine the weight each. The eggs yolk part were analyzed to determine the fatty acids by direct methylation method according to Folch (1957). Methylation of fatty acids (IUPAC, 1987 and AOCS Official Method Ce 1b-8, 1992).

Statistical analyses: Data were analyzed using Nested ANOVA Random Complete design (CRD) If the there are any significant different between treatments, it followed Duncan's Mean range Test (DMRT).

RESULTS AND DISCUSSION

Effect of HE, bones, arachon on HDP and eggs characteristics: The dietary treatments of HE, bone and Arachon with several administration levels turned out to give a positive effect on the production of native chicken. The analysis of variance showed there were no significant effects (p>0.05), due to the dietary skipjack wastes (HE, bone and arachon) of HDP. National Research Council (1994) suggested that the level of provision of fish meal were range 10-15% in diets. Egg production varies depending on many factors, such as strain hens, feed consumption and environmental temperature (North and Bell, 1990).

The use of HE, Bone and Arachon in native chicken diet had no significant effect (P>0.05) on egg weight. The previous researchers Baucells *et al.* (2000) reported that administration of 4% fat sources such as fish oil, sunflower oil, animal fat and other fat sources in the feed of laying hens did not affect weight eggs. The use of HE, bone and Arachon in the diets trial had no significant effect (P>0.05) on egg yolk color. The higher the value of the egg yolk, the yellow color of the egg yolk even close to red and vice versa. The main factors that affect the color of egg yolk was the pigment contained in the feed material constituent. Some feed ingredients that have been known to contain pigments that can affect the color of egg yolk was yellow corn. Yellow corn contains quite high of pro-vitamin A (carotene). Grobas *et al.* (2001) stated that the addition of oil or fat in the diet may improve egg yolk color index, this maybe caused by oxycarotinoid contained in the feed in the form of fat soluble, that is lard, soybean oil, flaxseed oil and olive oil.

Effect of the usage level of HE, bone, arachon in dietary treatments on HDP, egg weight, egg yolk color: The results of the average level of HDP, Egg Weight, Yolk Weight, Yolk Color, are shown in Table 2.

The Hen Day Production (HDP) was not significantly affected (P>0.05) by any level of the HE, Bones, Arachon in the diets. NRC (1994) suggested that the level of provision of fish meal were ranged of 10-15%. Egg production varies depending on many factors, such as hens strain, feed consumption and environmental temperature (North and Bell, 1990).

Table 3: Mean fatty acid content of Egg Yolk: SAFA, MUFA, Σn-3, Σn-6 and Ratio n-3: n-6 (mg/g)

	Types		
Variables	HE	Bones	Arachon
SAFA (mg/g)	23.24±0.55	40.91±0.30	45.03±0.30
MUFA (mg/g)	70.72±0.18	63.11±0.27	67.26±0.24
Σn-3 (mg/g)	8.75±0.48	6.47±0.40	8.35±0.54
Σn-6 (mg/g)	25.18±0.17	22.97±0.32	23.17±0.22
Ratio n-3 : n-6	1 :5.95±1.2	1 :4.30±0.58	1 :6.38±1.3

Description: -SAFA: Saturated Fatty Acid; MUFA: Mono Unsaturated Fatty Acid; Σn-3: total n-3 fatty acids; Σn-6: total n-6 fatty acids (amount of linoleic, EPA and DHA).

The level of usage of HE, bone and Arachon in the diet did not significantly affect (P>0.05) on egg weight. Wahju (1988) suggested that egg weight is influenced by genetics, stage of maturity, age, medications and nutrients in the diet, especially the amino acid methionine and linoleic fatty acids. Feed that contains sufficient amino acids and fatty acids derived from HE, bones and Arachon will produce the same weight of eggs.

The use of any level of HE, bone and Arachon in the diets had no significant effect (P>0.05) on egg yolk color. Leeson and Summers (2001) stated fish oil is a good source of vitamin A with a content of 750 IU/g of lemuru fish oil. Carotene as the precursor of vitamin A may affect yolk color index.

Effect of HE, bones, arachon on egg yolk fatty acids: The results of average of SAFA, MUFA, Σn-3, Σn-6 and ratio n-3: n-6 of the eggs, due to the treatments of HE, bone and arachon in the hen diets are shown in Table 3.

The use of HE, Bone and Arachon in native chicken diet had no significant effect (P>0.05) on SAFA of the eggs. Rusmana (2010) showed that administration of palm oil which is rich in saturated fatty acids and mono-unsaturated fatty acids in laying hens diets led the high content of saturated fatty acids and mono-unsaturated fatty acids in egg yolks.

The MUFA of the eggs was not significantly affected (P>0.05) by the treatment diets. Baucells *et al.* (2000) suggested that the administration of 4% animal fats rich in saturated fatty acids and 4% oil-rich rapeseed rich in mono-unsaturated fatty acids in laying hens diets causes the egg yolks contain a lot of saturated fatty acids and mono-fatty acid unsaturated fatty acids.

The use of HE, Bone and Arachon in the hens diets had no significant effect (P>0.05) on Σn-3 FA of the eggs. Rusmana (2010) reported that laying hens which fed diets containing n-3 can increase the content of n-3 in the eggs.

The Σn-6 of the eggs was not significantly affected (P>0.05) by the treatment diets.

Table 4: Mean fatty acid content of Egg Yolk: SAFA, MUFA, Σn-3, Σn-6 and Ratio n-3: n-6 (mg/g)

Treatments		Variables				
		SAFA (mg/g)	MUFA (mg/g)	Σn-3 (mg/g)	Σn-6 (mg/g)	Ratio n-3: n-6
HE	0	46.86±0.01	67.73±0.13	1.54±0.02	30.89±0.01	20.14±0.02
	5	20.19±0.41	49.10±0.01	11.68±0.03	17.98±0.01	1.54±0.02
	10	14.96±0.01	71.25±0.02	7.37±0.00	26.80±0.01	3.64±0.01
	15	16.92±0.01	87.36±0.01	10.34±0.06	26.01±0.01	2.52±0.05
	20	17.27±0.08	78.19±0.00	12.83±0.03	24.26±0.01	1.90±0.03
Bones	0	44.67±0.01	72.60±0.01	3.59±0.12	30.35±0.01	8.53±0.13
	5	53.74±0.01	77.29±0.12	7.14±0.00	25.78±0.01	3.61±0.01
	10	34.04±0.78	51.69±0.78	4.26±0.78	20.40±0.01	4.80±0.00
	15	41.32±0.00	61.40±0.00	7.78±0.10	22.81±0.78	2.94±0.01
	20	30.79±0.01	52.57±0.01	9.62±0.00	15.49±0.00	1.61±0.01
Arachon	0	44.32±0.01	72.62±0.00	1.37±0.01	31.57±0.01	23.06±0.00
	5	40.31±0.12	49.06±0.31	5.54±0.00	20.23±0.00	2.90±0.00
	10	26.30±0.78	49.10±0.01	11.68±0.03	17.97±0.30	1.54±0.02
	15	63.38±0.00	87.36±0.00	10.34±0.06	26.01±0.02	2.52±0.05
	20	50.85±0.01	78.19±0.00	12.83±0.03	24.26±0.00	1.90±0.03

Description: -SAFA: Saturated Fatty Acid; MUFA: Mono Unsaturated Fatty Acid; Σn-3: total n-3 fatty acids; Σn-6: total n-6 fatty acids (amount of linoleic, EPA and DHA). The same superscript in the same row indicates no significant effect (P> 0.05).

Omega-6 consists of arachidonic (C20:4) and linoleic (C18:3). Linoleic acid is one of the essential fatty acids that is absolutely necessary in the body. In contrast to the opinion, Haumann (1997) states that the linoleic acid that has a combination of 9-cis and 11-trans called Conyugated Linoleic Acid (CLA) may be role as anticarcinogenic and can reduce the risk of atherosclerosis. The high linoleic acid content in the eggs tend to increase the production and quality of eggs. The ratio of n-3: n-6 of the eggs were not significantly affected (P>0.05) by the treatment diets (P>0.05). It can be explained that, to date into consideration the experts usually is the ratio between omega-3 fatty acids to omega-6 fatty acids. In this study the ratio of n-3: n-6 were 1:5.9 of the HE treatment and 1:4.43 of the bone treatment. It was approaching to the ratio that recommended by World Health Organization (WHO) and Food Agriculture Organization (FAO), that is 1:5 (Newton, 1996).

Effect of the usage level of HE, bone, arachon in dietary treatments on SAFA, MUFA, Σn-3, Σn-6 and ratio n-3: n-6 (mg/g): The analysis of variance showed that there were no significant effects (p>0.05) against SAFA, MUFA, Σn-3, Σn-6 and ratio n-3: n-6 of the eggs, due to the usage level of HE, bone and arachon in the hen diets (Table 4).

The level of usage of HE, bone and Arachon in the diet did not significantly affect (P>0.05) on the eggs SAFA. Montesqrit (2008) in the research of using lemuru fish oil 0.5-4% with saturated fatty acid content of 24.86-28.11% in the hen diets, found that the content of Saturated Fatty Acids (SAFA) of egg yolks were increased when food containing these fatty acids given in the feed of laying hens.

Influence the level of HE, Bones and Arachon not significantly affect on the eggs MUFA (P>0.05). Montesqrit (2008) in the fish oil study lemuru 0.5-4% unsaturated fatty acid content of 32.10-33.28. Content of unsaturated fatty acids (MUFA) and increases in feed ingredient egg yolk when food containing these fatty acids given in the feed of laying hens. he results showed that the content of Mono-Unsaturated Fatty Acids (MUFA) as the effect of the level of using HE, Bones and Arachon in the diets were range 57.34-78.71 mg/g. Unsaturated fatty acids (MUFA) are very beneficial as a source of essential fatty acids in humans. This value is very influential in controlling blood cholesterol levels, thus tended to avoid from the atherosclerosis.

The Σn-3 of the eggs was not significantly affected (P>0.05) by the level of the treatments in the diets (P>0.05). Comparing to the study of Farrell (1993) with the Σn-3 contained in the eggs of 0.014 g/egg yolk or by calculation of±0.84 mg/g yolk. Provision of 8% flaxseed in diet cause the content of n-3 fatty acids, especially linoleic acid with increased five times Cherian and Sim, 1992). In this study, the increasing of the usage level of skipjack waste by 0-20% in the diets, the total n-3 of fatty acids of eggs content were increased.

The effect of any level of HE, Bones and Arachon on Σn-6 were not significant (P>0.05). Comparing to the study of Farrell (1993), the omega-6 contained in eggs was 0.701 g/egg yolk or by calculation of 41.97 mg/g egg yolks, while the results of this study found content of omega-6 were ranged of 19.93-30.93 mg/g yolk, it was lower than those study.

The ratio of n-3: n-6 was not significantly affected (P>0.05) by the any level of the treatments in the diets. The results in this study showed that the ratio of n-3: n-6 less than 1:5 at usage levels of skipjack wastes of 5, 10, 15 and 20% in the diets. While at usage level of 0% of skipjack waste (control), the ratio of n-3: n-6 was higher than 1:5. According to Newton (1996) the recommendations of the WHO (World Health

Organization) or FAO (Food Agriculture Organization) that the ratio of n-3: n-6 is 1:5.

Conclusion: It is concluded that the use of skipjack industrial waste of HE, Bones and Arachon can produce optimal productivity of native hen. The level usage of HE, Bones and Arachon in the diets that suggested to increase the productivity of native hen was at 15% level.

REFERENCES

Baucells, M.D., N. Crespo, S.C. Barroeta, S. Lopez-Ferrer and M.A. Grashorn, 2000. Incorporation of different polyunsaturated fatty acids into eggs. Poult. Sci., 79: 51-59. http://ps.fass.org/content/79/1/s1.full.pdf. Tanggal akses 8- Sepetember-2012.

Cherian, G. and J.S. Sim, 1991. Preferential Accumulation of n-3 fatty acids in the brain of chicks from eggs enriched with n-3 fatty acids. Poult. Sci., 71: 1658-1688.

Dadang, R.A., 2006. Effect of Rice Bran and Phytase Supplementation on Egg Laying Performance and egg quality of Laying Hens. Thesis. Univercity Putra Malaysia. Unpublished.

Farrell, D.J., 1993. Manipulating the composition of the egg to improve human health, RPAN Seminar a new concept in Poultry Feed Technology. Jakarta. 16. Sepetember.

Folch, J.M. Lees and G.H. Sloane Stanely, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226: 497-507. www.cyberlipid.org/exract/extr0005.htm.

Grobas, S., J. Mendes, R. Lazaro, C. De Blas and G.G. Mateos, 2001. Influence of source and percentage of fat added to diet on performance and fatty acid composition of egg yolks of two strains of laying hends. Poult. Sci., 80: 1171-1179. www.poultryscience. org/ps /paperpdfs/01/ps011171.pdf Tanggal akses 29 September 2012 .

Haumann, B.F., 1997. Nutrional Aspects of n-3 fatty acids. inform. 8: 428.

Hunton, 1987. Laboratory evaluations of egg quality.Pages 87-102.in:egg quality current problems and recent advances. Wells, R.G. and C.G. Belyavin (Eds.), Butterwoths, London, UK.

Keshavarz, K. and R.E. Austic, 2004. The Use of Low-Protein, Low-Phosphorous, Amino Acid- and Phytase-Supplemented Diets on Laying Hen Performance and Nitrogen and Phosphorus Excretion. Poult. Sci., 83: 75-83.www.poultryscience.org/ps/paperpdfs/04/p0410075.pdf. Tanggal akses 10-Juni-2011.

Leeson, S. dan J.D. Summer, 2001. Nutrition of the Chicken. Ed ke-4. Guelph, Ontario. University Books.

Leeson, S. and J.D. Summer, 1991. Comercial Poultry Nutritional University Book. Guelph. Ontario. Canada.

Lewis, N.M., S. Seburg and N.L. Flagen, 2000. Enriched eggs a source of N-3 polyunsaturated fatty acids for humans. Poult. Sci., 79: 971. www.ncbi.nlm.nih.gov/pubmed/10901195.

Montesqrit, 2008. Penggunaan Mikrokapsul Minyak Ikan Dalam Ransum Ayam Petelur Terhadap Performan Produksi dan Kualitas Telur. Seminar Nasional Sains dan Teknologi II. Universitas Lampung. http: // www. Scribd.com /ifadri/documents.

Monira, K.N., M. Salahuddin and G. Miah, 2003. Effect of bread and holding period on egg quality diarateristics of chicken. Int. J. Poult. Sci., 2.4: 261-263. www.pjbs.org/ijps/fn 1336.pdf. Tanggal akses 21 april 2013.

National Research Council, 1994. Nutrient Requirements of Poultry. Ed ke-9. Washington DC. National Academy Pr Baucells MD, Crespo N. Barroeta SC, Lopez-Ferrer S, Grashorn MA. 2000. Incorporation of different polyunsaturated fatty acids into eggs. Poult. Sci., 79: 51-59.

Newton, 1996. Long Chain Fatty Acids in Health and Nutrional. J. Food Lipids. Onlinelibrary.Wiley.com/doi/10.1111/j.1745-4522.1996.tb00071.x/abstract.

North, M.O. and D.D. Bell, 1990. Commercial Chicken Production Manual. 4th Edn., An Avi Book Publised by Van Nostrand Reinhold. New York: Chapman and Hall.

Parmar, S.N.S., M.S. Thakur, S.S. Tomar and P.V.A. Pillai 2006. Evalution of egg quality traits in indegeneous kadaknath bread of poultry.Livestock Res. Rural Dev., 18. http://www.irrd.org18/9/pam 18132.htm. Tanggal akses 21 april 2013.

Rusmana, D., 2010. Minyak Ikan Lemuru Tingkatkan kekebalan Ayam. Disertasi . Ilmu Ternak. Institut Pertanian Bogor. http:/cvanekonutrisi.blogspot.Com/2010/09/minyak ikan lemuru tingkatkan kekebalan html.Download 1 januari 2011.

Stadelman and Cotterill, 1994. Egg Science and Technology. Fourth Edition.Food Products Press. United State America.

Wahju, J., 1988. Ilmu Nutrisi Unggas. Gadjah Mada University Press. Yogyakarta. 45-47; 78-110, 287-305.