Research Article

Omega-3 as a Dietary Supplement in Rabbits: Effect on the Growth Rate, Blood Parameters and Lipid Profiles

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Abstract | This study aimed to investigate the influence of Omega-3 on the growth rates, blood parameters and lipid profiles in New Zealand (albino) rabbits. Twelve male rabbits aged 6-7 months with an average initial weight of 1308.00±39.97 g were used in this study. The rabbits were divided into three groups; the control group was treated orally with distilled water, and the second and third groups were treated orally with 150 or 300 µl Omega-3, respectively. The rabbits' body weight significantly increased in both Omega-3 treated groups, as well as red blood cells, haemoglobin, packed cell volume, lymphocytes and monocytes, after 60 days, relative to the control group. On the other hand, total white blood cells, including serum cholesterol, triglycerides, low-density lipoproteins and the aspartate aminotransferase (AST) and alanine transaminase (ALT), were significantly decreased in both Omega-3 treated groups compared to the control. In conclusion, the supplement with Omega-3 (150 and 300 µl) induced the growth rate, and liver enzymes, and reduced their lipid profiles, suggesting it would be a beneficial dietary supplement for rabbits.

Keywords | Omega-3, Polyunsaturated fatty acids, Dietary supplement, Albino rabbits, Animal feeding.

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INTRODUCTION

Omega-3 polyunsaturated fatty acids (PUFA) are gaining interest as dietary supplements for animal feeding because of their several roles and benefits in health promotion and disease lessening (DiNicolantonio and O'Keefe, 2018; Alagawany et al., 2019). They have been proved to be efficient in the treatment of rheumatoid arthritis (Lee et al., 2019), reduction of platelets and endothelial cells, and are useful for their anti-thrombotic and anti-inflammatory effects (Kanakri et al., 2017; Kanakri et al., 2018). Salman (2017) showed that omega-3 has an important role in increasing the total amount of white blood cells, and the lipid profile. Recent research revealed that omega-3 could decrease the activity of the nuclear factor, which is important for controlling gene expression during inflammatory responses and has been linked to the pathogenesis of the cardiovascular disease (De Winther et al., 2005; Adkins and Kelly, 2010; Schmid-Lausigk and Aurich, 2014).

Oils containing these fatty acids (FAs) originate primarily from plant sources or are improved in plants, with nearly 80% of them being of plant origin, whereas some of them are also obtained from marine, algal, and single-cell sources (Shahidi and Ambigaipalan, 2018). The predominant FAs present in plant oils are saturated and unsaturated

compounds with straight aliphatic chains of carbon atoms (Abdel-Khalek et al., 2019).

Mammals have a limited ability to synthesize Omega-3 FAs, which are necessary to provide health benefits (Ravindran et al., 2016). Particularly, rabbits can't synthesize Omega-3, and therefore these kinds of fatty acids have to be provided in their diet. Despite it is well known the positive effects of Omega-3 in humans and many other species of animals, in rabbits little information is available. Thus, this study aimed to investigate the effect of Omega-3 as a dietary supplement on the growth rate, blood parameters, and lipid profile of male New Zealand (albino) rabbits.

MATERIALS AND METHODS

Animals and experimental design

This study was performed at the animal housing within the departments of animal production at college of Agriculture, Tikrit University, following guidelines for the use of animals that were approved by the Animal-Ethic Committee (No.AS-3085P). Twelve New Zealand (albino) male rabbits, aged 6-7 months, with an average weight of 1308.00 ± 39.97g were used in the study. All of the animals were fed *ad libitum* with a balanced food based on barley meal 30%, yellow corn 30%, soybeans 25%, wheat bran 12.3%, vitamins 0.2%, limestone 2%, sodium chloride 0.5%, protein content therein 18.78%, with a total of 2514.4 calories/kg. They were randomly divided into 3 groups (n = 4 in each group). The control received distilled water by gavage needle; whereas the experimental groups were administered with Omega-3 (Scitron Nutrition, India) also by gavage needle, with one group receiving 150 µl and the other 300 µl. The commercial available Omega-3 fatty acids used in this study consist of natural fish oil dissolved in liquid form, containing (180 mg) of EPA (eicosapentaenoic acid) and (120 mg) of DHA (docosahexaenoic acid), gelatin, and glycerin.

Body weight measurements

Rabbitts' body weight (g) was calculated weekly for 60 days, after cutting the feed for 12 hours, using an electronic scale (ACS-A9, DahongYing, China).

Blood sample collection

The blood samples were collected from 6 of the bare vein in the ear directly with a 10 ml wine syringe and divided into two parts. The first vial (1 ml) contained heparin to help determine haematological parameters and separate the serum. The second vial (9 ml) did not contain an anticoagulant to obtain serum. The samples were centrifuged at 3000 rpm for 12 minutes to separate the blood serum from the remaining components, and then separated and stored at (–20 °C) until analysis.

Haematological Parameters determination

Red blood cell (RBC) and white blood cell (WBC) counts were measured according to Natt and Herrick (1952); the latter, was calculated using a hemocytometer and followed Hean's (1995) method, and packed cell volume (PCV) was determined according to Dacie and Lewis (1975). The differential counting of white blood cells (lymphocytes and mononuclear cells) by light microscopy using an oil lens (X 100) (Sood, 1985). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and the mean corpuscular haemoglobin concentration (MCHC) were calculated according to Coles (1980).

Biochemical and Antioxidants parameters determination

Serum lipids profiles, including cholesterol, triglycerides, and high-density lipoproteins (HDL) were determined by the enzymatic method using the kit REF 11505 (Biomeriux, Biolabo, France). The low-density lipoproteins (LDL) were estimated according to Friedewald et al. (1972), whereas the concentration of the very-low-density lipoproteins (VLDL) was calculated by dividing the score for triglycerides by 5. Albumin concentration was measured according to Doumas et al. (1971); globulin concentration by the separation of albumin from total protein, and glucose present in the serum using the enzymatic-based kit (Biosystems Kit, No. REF11533, Spain). The concentration of the liver enzymes aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine transaminase (ALT) was calculated calorimetrically according to Reitman (1957). The concentration of urea and creatinine was determined using a spectrophotometer (type pd 303, Apel, Indian) and a ready-to-use kit (BioSystems, Linear Chemicals, Spain) following Young's (1997) instructions.

Statistical analyses

The assays were performed using a complete randomized design (CRD) in one way. Data coming from the assays were analysed using the software Statistical Analysis System (SAS; VERSION 9, USA; SAS, 2004). ANOVA analyses were performed to detect significant variances and were followed by Duncan's multiple range test (Duncan, 1955) at a 95% or 99% confidence level.

RESULTS

Body weight

The results of the effect of feeding with Omega-3 on the final body weight of male New Zealand (albino) rabbits are shown in Figure 1. Rabbits that were fed with 300 µl of Omega-3 (third group) significantly increase their body weight (1578.67 g) compared to the control group fed only with distilled water (1415.33 g). Whereas no significant difference was detected between the final body weight of

Table 1: The feeding with Omega-3 affected most of the blood parameters of the rabbits

Item	Omega-3			Significant
	Control	150 µl	300 µl	
RBC (×10⁶/µL)	10.94±0.25 b	12.08±0.24 a	12.15±0.28 a	**
PCV (%)	39.77±0.62 b	47.38±0.60 a	47.41±0.49 a	**
Hb (g/100mL)	13.16±0.20 c	13.89±0.25 b	14.96±0.21 a	**
MCV (femtoliter)	36.41±0.88	39.26±0.94	39.10±1.13	ns
MCH (pictograms/ cell)	12.05±0.26	11.52±0.44	12.33±0.25	ns
MCHC (%)	33.14±0.98 a	29.32±0.56 b	31.59±0.77 ab	*
WBC (×10³/µL)	9.74±0.60 a	7.90±0.34 b	7.76±0.36 b	*
Lymphocyte (%)	39.09±3.43 b	49.13±0.26 a	53.70±1.82 a	**
Monocyte (%)	6.11±0.36 b	6.34±0.78 b	8.64±0.46 a	*

Mean values ± standard deviation are represented. ANOVA analyses were performed to detect significant variances among all the interactions, followed by the Duncan test at 95% confidence (*) or 99% confidence (**) levels. Different letters denote statistical differences among treatments. References: RBC (red blood cell); PCV (packed cell volume); Hb (haemoglobin); MCV (mean corpuscular volume); MCH (mean corpuscular haemoglobin); MCHC (mean corpuscular haemoglobin concentration); WBC (white blood cell); ns (no statistically significant differences).

Table 2: The feeding with Omega-3 affected most of the lipid profiles of the rabbits

Item	Omega-3			Significant
	Control	150 µl	300 µl	
Cholesterol (mg/dL)	180. 40±4.91 a	152.35±5.31 b	143.37±2.08 b	**
Triglyceride (mg/dL)	127.30±1.21 a	109.40±3.78 b	108.85±3.08 b	**
HDL(mg/dL)	15.98±0.39	17.05±0.93	17.68±0.45	ns
LDL (mg/dL)	69.08±5.78 a	60.00±0.74 b	52.20±4.87 b	*
VLDL (mg/dL)	25.46±0.24 a	21.88±0.76 b	21.77±0.61 b	**

Mean values ± standard deviation are represented. ANOVA analyses were performed to detect significant variances among all the interactions, followed by the Duncan test at 95% confidence (*) or 99% confidence (**) levels. Different letters denote statistical differences among treatments. References: HDL (high-density lipoproteins); LDL (low-density lipoproteins); VLDL (very low-density lipoproteins); ns (no statistically significant differences).

Table 3: The feeding with Omega-3 affected most of the kidney and liver function indicators

Item	Omega-3			Significant
	Control	150 µl	300 µl	
Urea (g/dL)	66.33±2.52 a	64.57±0.25 a	56.99±2.83 b	*
Creatinine (µ mol\L)	1.85±0.28	1.36±0.25	1.35±0.25	ns
AST (U/L)	29.08±0.76 a	27.75±1.85 a	18.75±0.66 b	**
ALT (U/L)	61.00±0.80 a	59.00±0.83 a	51.65±0.78 b	**
ALP (King /100 ml)	71.93±3.53	72.68±0.92	71.38±0.48	ns

Mean values ± standard deviation are represented. ANOVA analyses were performed to detect significant variances among all the interactions, followed by the Duncan test at 95% confidence (*) or 99% confidence (**) levels. Different letters denote statistical differences among treatments. References: AST (aspartate aminotransferase), ALP (alkaline phosphatase), and ALT (alanine transaminase); ns (no statistically significant differences).

Table 4: The feeding with Omega-3 affected all of the biochemical serum parameters of the rabbits

Item	Omega-3			Significant
	Control	150 µl	300 µl	
Glucose (mg/dl)	190.85±0.41 a	184.35±2.91 a	164.20±10.03 b	*
Total protein (g/dl)	7.21±0.20 b	7.83±0.09 a	8.35±0.22 a	**
Albumen (g/dl)	3.75±0.13 b	4.15±0.14 ab	4.23±0.11 a	*

Globulin (g/dl)	3.46±0.12	b	3.68±0.20	ab	4.13±0.19	a	*

Mean values ± standard deviation are represented. ANOVA analyses were performed to detect significant variances among all the interactions, followed by the Duncan test at 95% confidence (*) or 99% confidence (**) levels. Different letters denote statistical differences among treatments.

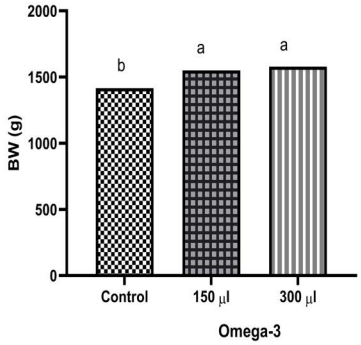

Figure 1: Effect of the feeding with Omega-3 on the final body weight of male New Zealand (albino) rabbits

rabbits fed with 150 µl and 300 µl Omega-3 (second and third groups).

Haematological Parameters

The administration of both doses of Omega-3 (150 and 300 µl) led to a significant increase in red blood cell (RBC), packed cell volume (PCV), haemoglobin (Hb), white blood cell (WBC) count, lymphocytes, compared to the control group of rabbits (Table 1). For all these haematological parameters no significant differences were detected between rabbits treated with both doses of Omega-3, except for the Hb, in which the group that received 300 µl presented a higher value than the one that received the lower dose. For example, the administration of 150 µl or 300 µl of Omega-3 led to increases ranging around 10% for RBC, 19% for PCV, and between 5.5 % - 13.7% for Hb, 18.9% - 20.3% for WBC, and 25.7% and 37.4% for the lymphocyte count. Regarding the mean corpuscular haemoglobin concentration (MCHC), only those rabbits that received 150 µl of Omega-3 presented a significantly lower value compared to the control group (11.5%), whereas those rabbits that received 300 µl of Omega-3 presented a significantly higher count of monocytes (41.4%). No significant differences were observed in mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) values among the three groups.

Lipids profiles

The lipids profile was significantly affected in those rabbits that received both doses of Omega-3 throughout the study period (60 days), and a decrease in the values was observed for all the parameters evaluated compared to the control group, except for the HDL, which registered no significant differences (Table 2). For example, the administration of 150 µl or 300 µl of Omega-3 led to a decrease between 15.5% and 20.5% of cholesterol, 14% of triglycerides, 13.4 and 24% for LDL, and around 14% for VLDL, depending on the dose administered.

Kidney and liver function

The effect of the administration of Omega-3 on rabbits' kidney and liver function was studied by measuring some key indicators (Table 3). The highest dose of Omega-3 (300 µl) was the only one that showed an effect on rabbits' kidney and liver function. For example, it led to the lowest level of urea, aspartate aminotransferase (AST) and alanine transaminase (ALT) enzymatic activities, compared to the control group. No significant difference was detected in creatinine level and alkaline phosphatase (ALP) activity among the groups.

Biochemical parameters

The administration of 300 µl Omega-3 significantly affected the levels of serum glucose, albumin, and globulin compared to the control group (Table 4); it reduced by 14% the levels of glucose and increased by 11.3% and 19.4% the levels of albumin and globulin, respectively. Only the total proteins were significantly augmented by both doses of Omega-3 (8.6% and 15.8% increases with 150 µl and 300 µl of Omega-3, respectively).

DISCUSSION

In the present study, we demonstrated that when male New Zealand (Albino) rabbits were treated orally with 150 or 300 µl of Omega-3, their body weight improved. Similar to what we observed, Rebollar et al. (2014), Kowalska (2015), and Salman (2017) also reported that the use of fish oils as a dietary supplement of Omega-3 had a positive effect on the final body weight of rabbits. Some possible explanations for this result are that Omega-3 may positively affect the appetite of rabbits, resulting in an increased feed intake and improved intestinal absorption, as stated by Okeke et al. (2011); more efficient utilization of nutrients from food and further conversion into muscle protein is also possible (Risso et al., 2016; Rodrigues et al., 2017). Another valid explanation is that rabbits fed with Omega-3 gained body weight because the increased TSH levels stimulated thyroid hormones and increased rabbits' metabolism, as reported in a previous study (Habeeb et al., 2021).

It is well known that Omega-3 can stimulate anti-inflammatory cells and reduce physiological stress (Deuel et al.,

2012). Thus, the significant effect Omega-3 had on red blood cell (RBC), haemoglobin (Hb), packed cell volume (PCV), and white blood cell (WBC) lymphocytes and mononuclear cells, may be explained due to its capacity to increase or decrease these kinds of cells when functioning as an antioxidant or an anti-inflammatory supply, and due to its role in increasing lymphocytes and reducing neutrophils by stimulating the immune system (Saiyed et al., 2015; Ravindran et al., 2016). These results agree with those reported by Abbas (2013) and Salman (2017).

The serum lipid profile level was lower in the rabbits that received either dose of Omega-3 compared to the control rabbits who only received distilled water. This result may be explained due to the capacity of Omega-3 to inhibit lipogenesis (Sampath and Ntambi, 2005). Moreover, the increased expression of the enzyme lipoprotein lipase in Omega-3-treated rabbits is attributed to its ability to reduce the production of very-low-density lipoproteins, which are responsible for the carriage of triglyceride, phospholipids, and cholesterol (Harris et al., 1990; Abdel-Khalek et al.,, 2019). Our results are in agreement with Abbas (2013), and Salman (2017), who found that Omega-3 reduced lipid profile by impeding the biosynthesis of triglycerides and very low density lipoprotein in the liver.
In the present experiment, the liver enzyme values were lowest in the rabbits that received orally Omega-3 compared to the control. According to Stillwell and Wassall (2003), the essential fatty acids are important in the cell envelopes and, in combination with phospholipids and effective in envelope-binding enzymes. Some results agree with Asaad and Aziz, (2012), and Salman (2017). Moreover, Mohamed et al., (2012) and Salman (2017) found that Omega-3 can affect liver enzyme activity.

Whereas the significant increase in the blood protein levels in Omega-3-treated rabbits may be due to the biological effect it has on increasing feed consumption and inducing a better intestinal absorption (Okeke et al., 2011). Similarly, El-Moghazy et al. (2014) showed that feeding adult New Zealand rabbits with fish oil-enriched Omega-3 polyunsaturated fatty acids significantly increased protein and blood albumin levels.

CONCLUSION

The oral administration of Omega-3 (at both doses of 150 and 300 µl) improved male New Zealand (albino) rabbits' final body weight and the haematological parameters, positively affected liver enzymes, and reduced lipid profiles. Therefore, Omega-3 is a promising dietary supplement with various benefits for rabbits.

NOVELTY STATEMENT

Omega-3 has many benefits for human and animal health, but little information about its effect on rabbits is available. Moreover, rabbits cannot synthesize Omega-3 and need to be provided in their diet. Current findings demonstrated that Omega-3 is a promising dietary supplement with various benefits for rabbits, including the gain of body weight, the improvement of haematological parameters, and lipids profile.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHOR'S CONTRIBUTION

A. Al-Janabi was coordinator of the research and interpreted the data. A. Al-Janabi, M. Alsalami and A. Mohammed in the study were supervisor of data collection and wrote draft manuscripts, A. Al-Dour was assistants of the collection of data.

REFERENCES

Abbas M (2013). Physiological Effects of Omega3 Unsaturated Fatty Acids in Healthy Subjects. Parameters. 160: 171.

Abdel-Khalek A.E., Khalil W.A., El-Sayed R.E. (2019). Effect of Different Dietary Sources of Oils on Growth Performance and Profile of Lipid, Testosterone and Fatty Acids in Rabbit Bucks. J. Anim. Poult. Prod. 10, 297–304. https://doi.org/10.21608/jappmu.2019.54806

Adkins Y., Kelley D.S. (2010). Mechanisms underlying the cardioprotective effects of omega-3 polyunsaturated fatty acids. J. Nutr. Biochem. 21: 781–792. https://doi.org/10.1016/j.jnutbio.2009.12.004

Alagawany M., Elnesr S.S., Farag M.R., Abd El-Hack M.E., Khafaga A.F., Taha A.E., Tiwari R., Yatoo M.I., Bhatt P., Khurana S.K. (2019). Omega-3 and omega-6 fatty acids in poultry nutrition: effect on production performance and health. Animals. 9: 573. https://doi.org/10.3390/ani9080573

Asaad H.R., Aziz F.M. (2012). Protective role of omega-3 fish oil against the toxicity of ifosfamide in male rats. Jordan J. Biol. Sci. 5: 37–346.

Coles E.H. (1980). Veterinary clinical pathology. WB Saunders.

Dacie J.V., Lewis S.M. (1975). Practical Haematology. Churchill Livingstone, London.

De Winther M.P.J., Kanters E., Kraal G., Hofker M.H. (2005). Nuclear factor κB signaling in atherogenesis. Arterioscler. Thromb. Vasc. Biol. 25: 904–914. https://doi.org/10.1161/01.ATV.0000160340.72641.87

Deuel J.W., Lutz H.U., Misselwitz B., Goede J.S. (2012). Asymptomatic elevation of the hyperchromic red blood cell subpopulation is associated with decreased red cell deformability. Ann. Hematol. 91: 1427–1434. https://doi.org/10.1007/s00277-012-1467-5

DiNicolantonio J.J., O'Keefe J.H. (2018). Importance of maintaining a low omega–6/omega–3 ratio for reducing inflammation. Open Hear. 5, e000946. https://doi.org/10.1136/openhrt-2018-000946

Doumas B.T., Ard Watson W., Biggs H.G. (1971). Albumin standards and the measurement of serum albumin with bromcresol green. Clin. Chim. Acta. 31: 87–96. https://doi.org/10.1016/0009-8981(71)90365-2

Duncan D.B. (1955). Multiple range and multiple F tests. Biometrics. 11: 1–42. https://doi.org/10.2307/3001478

El-Moghazy M., Zedan N.S., El-Atrsh A.M., El-Gogary M., Tousson E. (2014). The possible effect of diets containing fish oil (Omega-3) on hematological, biochemical and histopathogical alterations of rabbit liver and kidney. Biomed. Prev. Nutr. 4: 371–377. https://doi.org/10.1016/j.bionut.2014.03.005

Friedewald W.T., Levy R.I., Fredrickson D.S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem., 18(6): 499-502. https://doi.org/10.1093/clinchem/18.6.499

Habeeb A.A.M., Basuony H.A., Michael M.I., Gad A.E. (2021). Role of Omega-3 in the improvement of productive and reproductive performance of New Zealand White female rabbits. Biol. Rhythm Res. 52: 206–217. https://doi.org/10.1080/09291016.2019.1586100

Harris W.S., Connor W.E., Illingworth D.R., Rothrock D.W., Foster D.M. (1990). Effects of fish oil on VLDL triglyceride kinetics in humans. J. Lipid Res. 31: 1549–1558. https://doi.org/10.1016/S0022-2275(20)42339-9

Hean P.J. (1995). Principle of Hematology. Edited by: LH Yong.

Kanakri K., Carragher J., Hughes R., Muhlhausler B., Gibson R. (2018). The effect of different dietary fats on the fatty acid composition of several tissues in broiler chickens. Eur. J. Lipid. Sci. Technol. 120: 1700237. https://doi.org/10.1002/ejlt.201700237

Kanakri K., Carragher J., Muhlhausler B., Hughes R., Gibson R. (2017). In ovo exposure to omega-3 fatty acids does not enhance omega-3 long-chain polyunsaturated fatty acid metabolism in broiler chickens. J. Dev. Orig. Health Dis. 8: 520–528. https://doi.org/10.1017/S2040174417000216

Kowalska D. (2015). Effect of adding rapeseed and fish oils to the diet of rabbits on the fatty acid composition of saddle fat and the degree of carcass fatness. Sci. Ann. Polish Soc. Anim. Prod. 11: 69–78.

Lee S.A., Whenham N., Bedford M.R. (2019). Review on docosahexaenoic acid in poultry and swine nutrition: Consequence of enriched animal products on performance and health characteristics. Anim. Nutr. 5: 11–21. https://doi.org/10.1016/j.aninu.2018.09.001

Mohamed S.S., Mohamed S.R., El-Sedeek L.E., Khalil A.F., Deabes M.M. (2012). The omega-3 ecosa and decosa polyunsaturated long chain fatty acids have a potent effect to protect liver and kindey toxicity in rats. Int. J. Acad. Res. 4.

Natt M.P., Herrick C.A. (1952). A New Blood Diluent for Counting the Erythrocytes and Leucocytes of the Chicken. Poult. Sci. 31: 735–738. https://doi.org/10.3382/ps.0310735

Okeke I.N., Peeling R.W., Goossens H., Auckenthaler R., Olmsted S.S., de Lavison J.-F., Zimmer B.L., Perkins M.D., Nordqvist K. (2011). Diagnostics as essential tools for containing antibacterial resistance. Drug Resist. Updat. 14: 95–106. https://doi.org/10.1016/j.drup.2011.02.002

Ravindran V., Tancharoenrat P., Zaefarian F., Ravindran G. (2016). Fats in poultry nutrition: Digestive physiology and factors influencing their utilisation. Anim. Feed Sci. Technol. 213: 1–21. https://doi.org/10.1016/j.anifeedsci.2016.01.012

Rebollar P.G., García-García R.M., Arias-Álvarez M., Millán P., Rey A.I., Rodríguez M., Formoso-Rafferty N., De la Riva S., Masdeu M., Lorenzo P.L. (2014). Reproductive long-term effects, endocrine response and fatty acid profile of rabbit does fed diets supplemented with n-3 fatty acids. Anim. Reprod. Sci. 146: 202–209. https://doi.org/10.1016/j.anireprosci.2014.02.021

Reitman S, S.. F. (1957). Colorimetric Method for the Determination of serum glutamine Oxaloacetate and Pyruvic Transaminase. Amer. J. Clin. Path. 28: 56–63. https://doi.org/10.1093/ajcp/28.1.56

Risso A., Pellegrino F.J., Relling A.E., Corrada Y. (2016). Effect of long-term fish oil supplementation on semen quality and serum testosterone concentrations in male dogs. Int. J. Fertil. Steril. 10: 223.

Rodrigues A.C., Ruiz C.M., De Nardo C.D.D., Mothé G.B., Rossi F.M., De Sousa D.B., Netto H.A., De Souza F.F. (2017). Effect of dietary supplementation with omega-3 and–6 on fresh and frozen/thawed sperm quality of dogs. Semin. Ciências Agrárias 38: 3069–3076. https://doi.org/10.5433/1679-0359.2017v38n5p3069

Saiyed M.A., Joshi R.S., Savaliya F.P., Patel A.B., Mishra R.K., Bhagora N.J. (2015). Study on inclusion of probiotic, prebiotic and its combination in broiler diet and their effect on carcass characteristics and economics of commercial broilers. Vet. World 8: 225. https://doi.org/10.14202/vetworld.2015.225-231

Salman I.S. (2017). The effect of fish oil and omega-3 fatty acid on some physiological and Biochemical Criteria in Male Rabbits. Al-Nahrain J. Sci. 20: 108–113. https://doi.org/10.22401/JNUS.20.1.15

Sampath H., Ntambi J.M. (2005). Polyunsaturated fatty acid regulation of genes of lipid metabolism. Annu. Rev. Nutr. 25: 317–340. https://doi.org/10.1146/annurev.nutr.25.051804.101917

SAS, S.A.S. (2004). STAT Users Guide for Personal Computers. Release7. 0. SAS Inst. Inc., Cary, NC., USA.

Schmid-Lausigk Y., Aurich C. (2014). Influences of a diet supplemented with linseed oil and antioxidants on quality of equine semen after cooling and cryopreservation during winter. Theriogenology. 81: 966–973. https://doi.org/10.1016/j.theriogenology.2014.01.021

Shahidi F., Ambigaipalan P. (2018). Omega-3 polyunsaturated fatty acids and their health benefits. Annu. Rev. Food Sci. Technol. 9: 345–381. https://doi.org/10.1146/annurev-food-111317-095850

Sood R. (1985). Hematology for students and practitioners. Jaypee Brothers, India. Pp: 243-320.

Stillwell W., Wassall S.R. (2003). Docosahexaenoic acid: membrane properties of a unique fatty acid. Chem. Phys. Lipids. 126: 1–27. https://doi.org/10.1016/S0009-3084(03)00101-4

Young D.S. (1997). Effects of drugs on clinical laboratory tests. Ann. Clin. Biochem. 34: 579–581. https://doi.org/10.1177/000456329703400601