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Research Article

Effect of dietary supplementation of vegetable oil and quercetin on haematological indices and gut attributes in broiler chickens

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Abstract

Quercetin is a flavanol compound having strong antioxidant along with lipid metabolism modulating potential. Present biological experiment was conducted to assess the effect of dietary supplementation of vegetable oil with or without quercetin on haematological indices and gut attributes in broilers. Total 192 number 7 days old Vencobb 400 strain broiler chicks were randomly assigned in to four dietary treatment groups (48 chicks/group) comprise of four replicates in each for 35 days (7th to 42nd day). T1 serves as control, fed basal diet without any supplementation. Basal diet incorporated with quercetin (1 g/kg), vegetable oil (to raise the 10% of ME) and combination of both were supplied to group T2, T3 and T4, respectively. Results shown that the haematological indices were positively influenced ($p<0.05$) by quercetin supplementation compare to control (T1) and inclusion of vegetable oil (T3). Combination of quercetin and vegetable oil (T4) revealed intermediate findings and found comparable with either groups. The length of intestinal segments and caecal pH were not affected ($p>0.05$) with the dietary treatments. Thus, inclusion of vegetable oil (with 10% raised ME level) resulting an alteration in haematological indices. The quercetin supplementation effectively counteracts the altered haematological indices on dietary inclusion of oil in broilers. However, either of supplementation did not have any effect on gut attributes.

Key words: Broilers, Caecal, Haematological, Quercetin, Vegetable Oil

Highlights

- Inclusion on vegetable oil (with 10% raised ME level) adversely alter the haematological indices in broilers.
- Quercetin supplementation (1 g/kg feed) alleviated the alteration in the haematological indices due to supplementation of higher rate of vegetable oil.
- Quercetin alone (1 g/kg feed) supplementation improved the haematological indices.
- Vegetable oil or quercetin or its combination had no effect on gut attributes in broiler chickens.

INTRODUCTION

Fat is often supplemented in the form of animal fat or vegetable oils in the poultry diet, as a concentrated source of metabolizable energy (ME) increasing the energy available to the birds in an attempt to increase utilization, growth performance and productivity (Donaldson *et al.*, 2017). Fat is the natural component of the feed mixture improving the consistency and tastiness of the feed, same time reducing the feed consumption and increasing the feed efficiency in poultry birds (Tufarelli *et al.*, 2015). The vegetable oils like soybean oil, sunflower oil and rice bran oil while animal fat sources like beef tallow, bone and poultry fat are common sources utilized in poultry production (Saraee *et al.*, 2014). Broilers have a greater ability to utilize a considerable amount

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of dietary fat as an energy source with a high capacity for lipid absorption and biosynthesis, though the utilization efficiency largely depends on the fatty acids composition of the source (Saraee *et al.*, 2015). The digestibility of fat is directly influenced by the degree of fatty acid saturation and chain length of added dietary fat (Donaldson *et al.*, 2017). Researchers stated that the saturated fatty acid rich animal fats are not easily digestible in the gut of poultry as compared to either saturated or unsaturated fatty acid dense vegetable oils (Poorghasemi *et al.*, 2013).The type and sources of fat not only have an effect on blood biochemical indices but also on the metabolic process of various organs (Krasnodebska-Deptaand Koncicki, 2000). Recently, hydrogenated vegetable oils are preferred over animal fats used in the poultry diet due to their solid nature at room temperature, better physical properties, shelf life and flavor stability. However, the hydrogenation process resulted in trans-fatty acid formation which may cause an adverse impact on health (Alpha *et al.*, 2011).

Non-nutritive plant secondary metabolites called phytochemical such as alkaloids, flavonoids, tannins, cyanogenic glycosides and flavanones are synthesized by organisms of the plant kingdom and play a role in the defense mechanisms, microbial control, stressful conditions and physical damage (Kamboh *et al.*, 2018). Quercetin, is a major concern compound that belongs to the polyphenolic flavonoid group having strong antioxidant properties as free radical terminators. It is found in various plant sources such as vegetables, tea, fruits, apples, onions and tomatoes, and is known to exert a positive effect on poultry production and health (Saeed *et al.*, 2017). The previous investigation suggested, quercetin being a powerful free radical scavenger, ameliorates the organ damage, inhibits the effect of oxidative stress (Yi *et al.*, 2011; Sikder *et al.*, 2014), and possess immunomodulatory and anti-inflammatory properties with health improving potential in different species including poultry (Serafini *et al.*, 2012). Thus, with back drop of this, for promoting quercetin as a herbal feed additive the present study aimed to evaluate the effect of dietary supplementation of vegetable oils and quercetin and its combination on hematological indices and gut attributes of broiler chickens.

MATERIALS AND METHODS

Ethical statement: All the trial protocols applied for animal care and use were approved by the Institutional Animal Ethics Committee (IAEC) (IAEC/065-VCN-ANN-2018), College of Veterinary Science and A.H., Navsari Agricultural University, Navsari, Gujarat (India).

Dietary compounds: The dietary supplements, quercetin (a solid high performance liquid chromatography (HPLC) graded powder) was procured from Sigma Aldrich Life Science PVT. Ltd. UK and 100% hydrogenated vegetable oil (Lijjat Vanaspati; Ozone Proconn PVT. Ltd., Gujarat) was purchased from local market.

Birds and experimental diets: One hundred ninety two, Vencob-400 strain broilers (7 day old) chicks were randomly allotted in to four dietary treatment groups comprised of 4 replicates containing with 12 birds per replicate (48 birds/group) for a growth trial of next 35 days of period. The birds were kept under standard managemental conditions with free access to water. A corn-soya based basal diet was formulated for starter and finisher phase (BIS, 2007). The treatments included were the control group (T1) supplied with basal diet without any supplementation, in T2 group along with basal diet quercetin at 1g/kg was supplemented, in T3 group vegetable oil was incorporated in the basal diet to increase the 10% of ME (Kcal/kg) than the recommendation (BIS, 2007) and in T4 group along with basal diet vegetable oil and quercetin in combination at same dose rate were supplemented. The birds were kept under standard managemental condition and supplied with *ad lib* water and weighed amount of feed with ensuring left

Table 1. Layout of experimental designs and treatment regimen

Particulars		Treatment groups			
		T1 (CON)	T2 (Q)	T3 (HFD)	T4 (HFD+Q)
No of Birds		48	48	48	48
Supplement	Diet	Basal diet	Basal diet	Basal diet	Basal diet
Vegetable oil	Starter diet (8-22 days)	-	-	(34 g/kg)**	(34 g/kg)**
	Finisher diet (23-42 days)	-	-	(35 g/kg)**	(35 g/kg)**
Quercetin	Starter diet (8-22 days)	-	1 g/kg	-	1 g/kg
	Finisher diet (23-42 days)	-	1 g/kg	-	1 g/kg

(T: Treatment; CON: Control; Q: Quercetin; HFD: High fat diet)

over. Nutrient requirement of birds at various physiological stage were fulfill as per BIS (2007) feeding standard.The layout of the experimental designs is given in Table 1.

Chemical analysis: Chemical composition of offered feeds was analyzed as per standard techniques of AOAC (2008). Calcium (Ca) in feed samples was analyzed by the method of (Talapatra *et al.*, 1948) and phosphorus (P) was estimated calorimetrically as per (Taussky and Shorr, 1953). The ingredients and nutrient composition of treatment feeds (Starter and Finisher feeds) is being presented in Table 2.

Hematological indices: About 2.5 mL of whole blood was collected from 8 birds per replicate from each treatment groups in K3 ethylene diamine tetra acetic acid (EDTA) coated vial for carrying out hematological assay during the last week of experiment. Hematological indices such as hemoglobin (Hb), total leukocyte count (TLC) and differential leukocyte count (DLC) was estimated by standard methods described by Jain, (1986). The Hematocrit (%), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated by the recommended formula (Jain, 1986).

Gut attributes: The 32 birds (8 birds/treatment) were euthanized at 42nd days of age to study the different aspects of gastrointestinal attributes (morphometry and caecal pH). The obtained segments were squeezed and washed in normal saline. The morphometry, *viz.*, length and mass of these segments were assessed by using measuring tape and electron balance, respectively. The caecum of respective birds was collected immediately after evisceration of euthanized birds and their contents were drained out through caecal canal in a small beaker (25 mL). The pH of collected content was measured with the help of digital battery operated portable water proof pH meter (pH Tester 30, Eutech Instruments Oakton, Thermo Fisher scientific, USA).

Statistical analysis: The generated data were analyzed using SPSS, version 20.0 following one way ANOVA. The means were compared using Duncan's multiple range test (Duncan, 1955).

RESULTS

Hematological indices: The influence of dietary supplementation of vegetable oil and quercetin on different hematological indices of broilers is presented in Table 3. Higher ($p<0.05$) hemoglobin concentration, and hematocrit (%) ($p>0.05$) were recorded in birds fed T3 diet.The Hb, MCV, MCH, MCHC and HCT values were

Table 2. Ingredients (g/kg) and nutrient composition (%) of treatment feeds (starter and finisher feeds)

Ingredients (g)	Starter (T1 and T2) (8-22 days)	Finisher (T1 and T2) (23-42 days)	Starter (T3 and T4) (8-22 days)	Finisher (T3 and T4) (23-42 days)
Maize	500	500	500	500
Maize gluten	75	52	75	52
Deoiled rice bran	90	100	90	100
Rice polish	50	95	50	95
De-oiled soya cake	87	50	87	50
Ground nut cake	90	75	90	75
Vegetable oil	-	-	34	35
Protolive	79	99	79	99
Mineral mixture[1]	1	1	1	1
DCP	25	25	25	25
Salt	1.5	1.5	1.5	1.5
Biometh	0.3	0.3	0.3	0.3
Lysine	0.3	0.3	0.3	0.3
Toxin binder	0.5	0.5	0.5	0.5
Choline	0.3	0.3	0.3	0.3
Meriplex	0.1	0.1	0.1	0.1
Nutrient composition%**				
Dry matter	91.21	91.79	90.42	91.14
Crude protein	23.16	20.25	22.98	20.65
Ether extract	4.49	4.43	5.95	5.88
Crude fibre	5.02	5.11	5.16	5.22
NFE	60.78	63.32	59.23	61.46
Total ash	6.55	6.89	6.68	6.79
Calcium	1.23	1.20	1.30	1.26
Phosphorus	0.58	0.62	0.58	0.62
ME*, Kcal/kg	3080.56	3185.25	3385.45	3490.31
L- lysine**	1.22	1.31	1.45	1.36
DL-methionine**	0.55	0.49	0.51	0.57

[1]Tracemin CB: Each 1 kg contain–Manganese - 90 g, Zinc - 80 g, Iron - 90 g, Copper -15 g, Iodine - 2 g and Selenium - 300 mg; DCP- Dicalcium phosphate; **calculated values

improvised ($p<0.05$) with quercetin in T4 group of birds. The mean values for TLCs were greater ($p<0.05$) in T3 (42.62±2.22) and T4 (42.37±1.54) groups over the T1 (35.00±1.19) and T2 (34.87±2.09) groups. The mean concentration of heterophils (%) was 23.12±0.71, 21.75±0.61, 24.25±0.81 and 22.87±0.63 in T1 to T4 groups, respectively. The mean heterophils percentage was higher in T3 group followed by T4, T1 and low in T2 group. The lymphocytes, monocytes, eosinophils and basophils (%) were not affected with dietary treatments. Although, the ratio of heterophils to lymphocytes (H : L) was greater ($p<0.05$) in T3 group followed by T1, T4 and lowest in T2 group.

Table 3. Influence of dietary vegetable oil and quercetin supplementation on hematological indices of broilers

Parameters	Treatment groups			
	T1	T2	T3	T4
Hb (g/dl)	10.31±0.23^{c}	11.93±0.22^{b}	14.62±0.32^{a}	12.18±0.28^{b}
RBCs (*10^{6}/µl)	1.89±0.04	1.93±0.17	2.10±0.04	2.00±0.04
MCV(fl)	157.99±5.95^{b}	182.64±15.17^{ab}	208.27±4.71^{a}	184.07±5.69^{ab}
MCH (pg)	54.81±2.18^{b}	64.97±5.51^{a}	69.58±1.54^{a}	61.18±2.26^{ab}
MCHC (g/l)	34.74±0.87^{ab}	35.72±1.20^{a}	33.41±0.17^{ab}	33.20±0.33^{b}
HCT (%)	29.78±0.87^{d}	33.56±0.76^{c}	43.75±0.82^{a}	36.68±0.63^{b}
TLCs (*10^{3}/µl)	35.00±1.19^{b}	34.87±2.09^{b}	42.62±2.22^{a}	42.37±1.54^{a}
Heterophils (%)	23.12±0.71^{ab}	21.75±0.61^{b}	24.25±0.81^{a}	22.87±0.63^{ab}
Lymphocytes (%)	66.75±1.06	68.12±0.76	65.62±0.82	67.37±0.70
Monocytes (%)	7.00±0.53	7.25±0.41	6.62±0.37	6.75±0.31
Eosinophils (%)	0.25±0.16	0.25±0.16	0.37±0.18	0.12±0.12
Basophils (%)	2.87±0.29	2.62±0.26	3.12±0.39	2.87±0.29
H: L	0.34±0.01^{ab}	0.32±0.01^{b}	0.37±0.01^{a}	0.33±0.01^{ab}

Data represents as Mean ± SE (n=8 birds/treatment); Means with different superscripts in a row differ significantly (p<0.05); Hb- Hemoglobin, RBCs- Red blood corpuscles, MCV- Mean corpuscular volume, MCH- Mean corpuscular hemoglobin, MCHC- Mean corpuscular hemoglobin concentration, TLCs- Total leukocyte counts, H- Heterophil, L- Lymphocyte

Gut attributes: The influence of dietary supplementation of vegetable oil and quercetin on gut attributes, *viz.*, length of intestinal segments and caecal pH of broilers is shown in Table 3. Either vegetable oil or quercetin or both in combination had no significant effect on length of intestinal segments or caecal pH. The mean values (p>0.05) for length of duodenum, jeunum, ceacum and colon were 31.25±2.28, 30.62±0.82, 30.62±0.65 and 29.75±1.20; 128.25±5.89, 127.50±5.81, 125.50±5.85 and 122.87±6.44; 16.00±0.59, 17.25±0.45, 15.75±0.77 and 16.62±0.56; 8.12±0.78, 8.00±0.59, 7.87±0.81 and 7.37±0.37, respectively for T1, T2, T3 and T4 groups. The caecal pH values were greater numerically in T1 (7.29±0.12) group followed by T2 (7.22±0.12), T4 (7.09±0.16) and T3 (7.01±0.15) groups.

DISCUSSION

Hematological indices: Hematological indices (Hb, MCV, HCH, MCHC, HCT, TLCs, H, H : L ration) were significantly influenced by the dietary treatments. Highest (p<0.01) hemoglobin and hematocrit (%) level was observed in the T3 group. Further, among differential leucocytes count the heterophils (%) was also high (p>0.05) in T3 which resulted in a higher H : L ratio in T3 group.

However, the RBCs, monocytes, eosinophils and basophils were found comparable (p>0.05) and not affected by dietary supplementation. Elevated Hb concentration in T3 group might be due to increasing oxygen requirement associated with high metabolic activity for utilization of high dietary fat (Ani *et al.*, 2015). Elevated oxygen requirement is compensated by raised Hb concentration of the blood, resulting from increased oxygen carrying capacity which is also linked with increased uptake of iron by persuading the mucosal block theory and thereby enhancing the bioavailability of iron in the intestinal mucosa (Ani *et al.*, 2015).

Values for RBCs (*$10^6/\mu L$) were found comparable and in the standard normal range from 1.5 to 2.5 (Al-Nedawi, 2018), however, numerically higher values for RBCs (*$10^6/\mu L$) were observed in the group (T3) fed energy dense diet.

Red blood cell indices (MCV, MCH and MCHC) were influenced ($p<0.05$) in association with energy dense diet or quercetin supplementation. The observed values were higher than the control group suggesting that high triglycerol or fatty acid levels increase the concentration of erythrocytes in the circulatory system (Souza *et al.*, 2014). However, in contrast no alterations were observed for RBCs, Hb, MCH and MCHC on high fat diet and alcohol supplementation in the rat model (Chandra *et al.*, 2018). Higher ($p<0.01$) level of HCT% in T3 may reflect increasing oxidative stress due to the dietary burden of higher oil supplementation, which was ameliorated by quercetin in group T4. Similar comparable findings for HCT% in FLHS induced birds on high fat diet and hormone supplementation were observed by Shini, (2014), who suggested that birds usually have a good tolerance for loss, up to 10% of circulating blood volume.

White blood cells are directly correlated with the immune system and play a crucial role in body defense mechanism. The significant increase in the values of TLCs (*$10^3/\mu L$) on oil supplementation (T3 and T4) was an indication of inflammation, infection and stress to major vital organs. Similarly, an increase in TLC concentration on supplementation of palm and groundnut oil incorporated diet in rat was observed by Ani *et al.* (2015). In the present study, elevated levels ($p<0.05$) of heterophils (%) in T3 followed by T1 and T4, were in response to NAFLD. To accomplish immune response, heterophils use complicated defense mechanisms towards antigenic reactions. A similar pattern was also observed for the H : L ratio indicating the birds in the group T3 fed energy dense diet were under metabolic stress. Alike to present findings, Kumar *et al.* (2014) also suggested that increased heterophils (%) and H : L ratio have been owed to be a stress condition.

Basophil concentration was comparable among treatment groups however the trend showed that the value was increasing in T3, indicated that the birds in the group had an inclination towards hyperlipidemia (El-bialy *et al.*, 2015). Overall findings of hematological parameters suggested that the quercetin was an attempt to relieve the metabolic stress generated by energy dense diet.

Gut attributes: The influence of dietary supplementation of vegetable oil and quercetin on gut attributes,*viz.*, length of intestinal segments and caecal pH of broilers is shown in Table 4. Either vegetable oil or quercetin or both in combination did not influence ($p>0.05$) on the length of intestinal segments or caecal pH. A trend for the apparent reduction in caecal pH in T3, is pointed out that increase in the energy density of ration may manipulate the caecal pH towards acidity. Similar findings for gut parameters and caecal pH were observed on 6% and 12% level of extruded linseed supplementation in broilers (Gheorghe *et al.*, 2020). These findings are partially in agreement with those reported by Marzoni *et al.* (2014) who reported that the addition of a mixture of natural antioxidants did not exert any impact on the majority of broiler carcass traits. Ashour *et al.*, (2021) also reported that supplementation of pyocyanin powder (a secondary blue redox-active metabolite), is one of these natural antioxidants increased the intestinal length in broiler birds. The supplementation used in the present experiment either vegetable oil or quercetin might not favor the fatty acid metabolism in a way to produce required saturated fatty acids and signals for gut epithelial cell proliferation. Thus, the gut attributes were not influenced with dietary treatments.

On the basis of findings, it was concluded that feeding of high energy dense diet might lead to an increase in metabolic activity and oxidative stress, consequently the adverse

Table 4. Influence of dietary vegetable oil and quercetin supplementation on gut attributes [Length (cms) of intestinal segments and caecal pH] of broilers

Parameters	Treatment Groups			
	T1	T2	T3	T4
Duodenum	31.25±2.28	30.62±0.82	30.62±0.65	29.75±1.20
Jejunum	128.25±5.89	127.50±5.81	125.50±5.85	122.87±6.44
Ceacum	16.00±0.59	17.25±0.45	15.75±0.77	16.62±0.56
Colon	8.12±0.78	8.00±0.59	7.87±0.81	7.37±0.37
Caecal pH	7.29±0.12	7.22±0.12	7.01±0.15	7.09±0.16

Data represents as Mean ± SE (n=8 birds/treatment)

changes in hematological indices. Quercetin supplementation revealed better hematological indices along with the potential to cover oxidative stress generated by high energy diet.The gut attributes were remained unaltered with either quercetin (1 g/kg diet) or vegetable oil (>10% ME than the BIS recommendation) supplementation at these levels.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: ABP, VRP: Involved in investigation, data generation, preparing original draft; ABP, VRP, YDP: Engaged in conceptualization, data curation, supervision and final editing; ABP, VRP, APR: Involved in statistical analysis and methodology; ABP, VRP, SDR, SSP: Data collection.

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REFERENCES

Al-Nedawi AM, 2018. Reference haematology for commercial Ross 308 broilers. Online J Vet Res, 22(7): 566-570, doi: 10.13140/RG.2.2.34589.97768

Alpha RM, Reddy GA, Reddy RA and Adilaxmamma K, 2011. Effect of dietary vanaspati alone and in combination with stressors on sero-biochemical profile and immunity in white leghorn layers. Toxicol Int, 18(1): 31-34, doi: 10.4103/0971-6580.75850

Ani EJ, Nna VU, Obi CE and Udobong NJ, 2015. Comparative effects of thermoxidized palm oil and groundnut oil diets on some hematological parameters in albino wistar rats. Australian J Basic Appl Sci, 9(5): 181-184. Available at: jbasweb.com/old/ajbas/2015/March%202015/181-184.pdf

AOAC, 2008. Association of Official Analytical Chemists. Official methods of analysis 18th edition. Washington D. C. U.S.A. Available at: https://www.aoac.org/official-methods-of-analysis-18th-edition-2008/

Ashour EA, Farsi RM, Alaidaroos BA, Abdel-Moneim AE, El-Saadony MT *et al.*, 2021. Impacts of dietary supplementation of pyocyanin powder on growth performance, carcass traits, blood chemistry, meat quality and gut microbial activity of broilers. Italian J Anim Sci, 20(1): 1357-1372, doi: 10.1080/1828051X.2021.1924087

BIS, 2007. Indian Standard: Poultry feed specifications, 5th revision, Bureau of Indian Standards, New Delhi. Available at: https://law.resource.org/pub/in/bis/S06/is.1374.2007.pdf.

Chandra HK, Mishra G, Sahu N, Nirala SK and Bhadauria M, 2018. Effect of rutin against high-fat diet and alcohol-induced alterations in hematological variables of rats. Asian J Pharm Clin Res, 11(11): 186-189, doi:10.22159/ajpcr.2018.v11i11.27588

Donaldson J, Madziva MT and Erlwanger K, 2017. Metabolic health of guinea fowl and muscovy ducks fed a high-fat diet. Asian J Anim Vet Adv, 12: 274-283, doi: 10.3923/ajava.2017.274.283

Duncan DB, 1955. Multiple ranges and multiple F' test. Biometrics, 11(1): 1-42, doi: 10.2307/3001478

El-bialy BE, Saleh NT and Abou-Elkhair RM, 2015. Potential hazards of feeding albino rats on diet containing repeatedly boiled cooking oil: clinicopathological and toxicological studies. Int J Adv Res, 3(3): 134-147. Available at: https://www.journalijar.com/uploads/729_IJAR-5094.pdf

Gheorghe A, Lefter NA, Idriceanu L, Ropotã M and Hãbeanu M, 2020. Effects of dietary extruded linseed and Lactobacillus acidophilus on growth performance, carcass traits, plasma lipoprotein response, and caecal bacterial populations in broiler chicks. Italian J Anim Sci, 19(1): 822-832, doi: 10.1080/1828051X.2020.1801359

Jain NC, 1986. Scanning electron micrograph of blood cells. In: Schalm's Veterinary Haematology. 4th ed. P. Lea & Febiger. Philadelphia, 463-470. Available at:https://www.scirp.org/%28S%28351jmbntvnsjt1aadkposzje%29%29/reference/referencespapers. aspx?referenceid= 2645457

Kamboh AA, Khan MA, Kaka U, Awad EA, Memon AM *et al*., 2018. Effect of dietary supplementation of phytochemicals on immunity and haematology of growing broiler chickens. Italian J Anim Sci, 17(4): 1038-1043, doi:10.1080/1828051X.2018.1438854

Krasnodebska-Depta A and Koncicki A, 2000. Physiological values of selected serum biochemical indices in broiler chickens. Med Wet, 56(7): 456-460

Kumar M, Sidhu VK, Roy D, Kushwaha R and Vaiswani S, 2014. Application of herbal feed additives in animal nutrition - A review. Int J Livest Res, 4: 1-8, doi: 10.5455/ijlr.20141205105218

Marzoni M, Chiarini R, Castillo A, Romboli I, De Marco *et al*., 2014. Effects of dietary natural antioxidant supplementation on broiler chicken and Muscovy duck meat quality. Anim Sci Papers Reports, 32(4): 359-368

Poorghasemi M, Seidavi A, Qotbi AAA, Laudadio V and Tufarelli V, 2013. Influence of dietary fat source on growth performance responses and carcass traits of broiler chicks. Asian Australas J Anim Sci, 26(5): 705-710, doi: 10.5713/ajas.2012.12633.

Saeed M, Naveed M, Arain MA, Arif M, Abd El-Hack ME *et al*., 2017. Quercetin: Nutritional and beneficial effects in poultry. World's Poult Sci J, 73(2): 355-364, doi: 10.1017/S004393391700023X

Saraee MHA, Seidavi A, Dadashbeiki M, Laudadio V and Tufarelli V, 2014. Effect of dietary supplementation with different levels of green tea powder and fish oil or their combination on carcass characteristics in broiler chickens. Pakistan J Zool, 46(6): 1767-1773

Saraee MHA, Seidavi A, Dadashbeiki M, Laudadio V and Tufarelli V, 2015. Supplementing fish oil and green tea (*Camellia sinensis*) powder in broiler diet: effects on productive performance. Res Opin Anim Vet Sci, 5(2): 99-104

Serafini M, Peluso I and Raguzzini A, 2012. Session 1: antioxidants and the immune system. Flavonoids as anti-inflammatory agents. Proceed Nutr Soc, 69(3): 273-278, doi:10.1017/S002966511000162X

Shini A, 2014. A thesis on "Fatty Liver Hemorrhagic Syndrome in Laying Hens: Field and Experimental Investigations". A thesis submitted for the degree of Doctor of Philosophy at the University of Queensland, School of Agriculture and Food Sciences. Available at: https://espace.library.uq.edu.au/data/UQ_346438/s4185012_phd_submission.pdf/

Sikder K, Das N, Kesh BS and Dey S, 2014. Quercetin and β-sitosterol prevent high fat diet induced dyslipidemia and hepatotoxicity in swine albino mice. Indian J Exp Biol, 52(1): 60-66. Available at: https://pubmed.ncbi.nlm.nih.gov/24617016/

Souza FDCD, Duncan WP and Carvalho RP, 2014. Haematology and plasma biochemistry in rats fed with diets enriched with fatty fishes from Amazon region. Rev Nutr, 27(5): 547-555, doi: 10.1590/1415-52732014000500004

Talapatra SK, Ray SC and Sen KC, 1948. Calcium assimilation in ruminants on oxalate-rich diet. J AgricSci, 38(2): 163-173, doi: 10.1017/S0021859600005426

Taussky HH and Shorr E, 1953. A micro-colorimetric method for the determination of inorganic phosphorus. J Biol Chem, 202(2): 675-685

Tufarelli V, Bozzob G, Perillob A and Laudadio V, 2015. Effects of feeding different lipid sources on hepatic histopathology features and growth traits of broiler chickens. Acta Histochem,117(8): 780-783, doi:10.1016/j.acthis.2015.08.001

Yi L, Jin X, Chen CY, Fu YJ, Zhang T *et al*., 2011. Chemical structures of 4-oxoflavonoids in relation to inhibition of oxidized low-density lipoprotein (LDL) - induced vascular endothelial dysfunction. Int J Mole Sci, 12(9): 5471, doi: 10.3390/ijms12095471

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