MEDWIN PUBLISHERS
Committed to create value for Researchers

Open Access Journal of Veterinary Science & Research
ISSN: 2474-9222

Comparative Effects of Honey and Multivitamin on the Immune Response of Cockerel Chicks to Vaccination Against new castle Disease (ND) Using Live Attenuated and "Lasota" Vaccine

Lawal JR[1]*, Bello AM[1], Wakil Y[1], Balami SY[2], Audu Y[1], Lekko YM [1], Mshelia ES [1], Korzerzer RM[1] and Ibrahim UI[1]

[1]Department of Veterinary Medicine, University of Maiduguri, Nigeria

[2]Poultry Unit, Veterinary Teaching Hospital, University of Maiduguri, Nigeria

Research Article

Volume 1 Issue 2

Received Date: July 25, 2016

Published Date: August 31, 2016

***Corresponding author:** Jallailudeen Rabana Lawal, Department of Veterinary Medicine, University of Maiduguri, P.M.B 1069, Maiduguri, Borno State, Nigeria, Tel: +2348032886428; Email: rabanajallailudeen@yahoo.com

Abstract

The comparative effects of honey and commercially available multivitamin (Vitaflash®, Kepro B.V. Holland) on the haemagglutination inhibition (HI) antibody response of cockerel chicks to oral vaccination against Newcastle disease (ND) at 3 weeks of age using live attenuated ND 'LaSota' vaccine was investigated. Sixty (60) day-old cockerel chicks were purchased and reared to 2 weeks of age. The chicks were randomly divided into six groups (A, B, C, D, E_1 and E_2) with 10 chicks per group. Chicks in group A were placed on honey for 7 days prior to vaccination and 7 days following vaccination, while chicks in group B were placed on honey for 7 days following vaccination. Similarly chicks in group C were placed on multivitamin (Vitaflash®, Kepro B.V. Holland) for 7 days prior to vaccination and 7 days following vaccination while chicks in group D were placed on the multivitamin for 7 days following vaccination. Chicks in group E_1 (positive control) were place on plain drinking water ad libitum but were vaccinated at 3 weeks of age, while chicks in group E_2(negative control) received neither treatment nor vaccination but were fed and placed on plain drinking water add libitum until the end of the experiment. Chicks in group A exhibited the highest ND HI antibody titre with a geometric mean titre (GMT) of 181.0, followed by chicks in group C that exhibited a significantly ($P<0.05$) lower ND HI titre with a GMT of 127.9. Chicks in groups E_1 and E_2 did not show any significant antibody titre throughout the period of the experiment. The high antibody titre demonstrated by chicks that were treated with honey before and after vaccination is an indication that honey could be a possible immune enhancer. A more detailed study is therefore required on the quantitative assessment of honey for antibody response in chicks.

Keywords: Honey; Multivitamin; Immune Response; Cockerel Chicks; Newcastle Disease Vaccine

Introduction

The rapidly increasing human population in developing countries has necessitated the need for increase in animal production more especially poultry to meet the increasing animal protein requirements [1,2]. The rapid growth of the human population has also resulted in increasing demand for creation of job opportunities. The demand for animal protein and job opportunities in developing countries like Nigeria were to a large extent met by an increase in poultry production both in the form of large scale units and increased productivity among semi-commercial small holder farmer [3,4]. Poultry are widely accepted by people from a wide variety of cultures and religious backgrounds [5-7]. The types of poultry that are commonly reared in Nigeria are chickens, ducks, guinea fowls, turkeys, pigeons and more recently ostriches. Those that are of commercial or economic importance are chickens, guinea fowls and turkeys, amongst which the chickens predominate [8].

With the global spread of Highly Pathogenic Avian Influenza (HPAI) across several countries since 2003 and especially, the confirmation of the epidemic in Nigeria in February 2006, there is a new attention focused on the importance of poultry in the health and livelihood of households [2]. One of the most important constraints limiting successful poultry production in Nigeria is Newcastle disease (ND) [9].

Newcastle disease (ND) is an acute, infectious and highly pathogenic disease of poultry [10] is reported to be one of the most important viral disease of both commercial and village chickens in most parts of the world including developing countries like Nigeria [9,11,12]. The simplest and logical preventive measure against ND is vaccination [13]. But problem of maintaining minimum viral titre of live attenuated ND vaccines from point of production to the point of administration among others has been reported to play significant role in ND vaccination failures [13-15].While it is true that prevention of ND in endemic areas such as Nigeria is best achieved by vaccination, there is still much work to be done if we are to achieve a significant reduction in poultry production losses, notably by developing new vaccines and medicines [16-18].

Previous studies have shown that oral honey stimulates higher antibody production in chicks against ND and in mice against thymus-dependent and thymus-independent antigens [19-22]. Certain nutrients are capable of modulating the function of the immune system through a variety of mechanisms [23-25]. The immune response of chickens has been shown tobe influenced by number of nutrients which regulate animal immune response [26-28]. The medical properties of some natural products have some consideration such as herbal extracts [29,30], Probiotics [31], Prebiotics [32] and enzymes 2000) [33]. There is an increase demand for using natural biological compounds as feed additives. The present study examined the comparative immune responses of chicks that were orally treated with either honey or multivitamins before and after vaccination, and only after vaccination against ND using live attenuated ND 'laSota'vaccine obtained from the National Veterinary Research Institute (NVRI) Vom, Plateau State.

Therefore, this study aimed to determine the effects of oral honey on the immune responses of chicks to oral vaccination against Newcastle disease (ND) using live attenuated ND laSota vaccine. The results which will provide baseline data that could be used in future research work on the use of honey as a possible immune enhancer in poultry and other livestock species.

Materials and Methods

Experimental birds

Sixty (60) day-old cockerel chicks obtained from the RNT Farms Gujba, Yobe State were used for this experiment. The chickens were fed chick mash from day old to 8 weeks, growers mash from 9 weeks to the end of the experiment.

Source of vaccine

The vaccine used for this study was live attenuated ND "LaSota" vaccine obtained from the National Veterinary Research Institute (NVRI) Vom, Nigeria.

Source of honey

The honey used for this experiment was purchased from the Maiduguri Monday Market. One bottle containing 200mls of honey was used for the experiment.

Experimental Procedure

At two weeks of age, the chicks were divided into 6 groups (A, B, C, D, E_1, and E_2) of 10 chicks each and were housed separately in pens. At three weeks of age, one vial of 200 doses of the vaccine was bought and used in vaccinating the chicks. The vaccine with batch number 18 /2007 was used. The vaccine was reconstituted in 2 litres

of water to which skimmed milk was added. Each chick was given 10ml of the reconstituted vaccine water.

Experimental Design

Group	Treatment
A	Honey 7 days before and 7 days after vaccination
B	Honey 7 days after vaccination.
C	Multivitamin 7 days before and 7 days after vaccination.
D	Multivitamin 7 days after vaccination.
E_1	Vaccinated but no treatment (Positive control)
E_2	Not vaccinated and not treated (Negative control)

- **Groups A**: Was given 5 millilitres of honey in drinking water for 7 days before vaccination and for another 7 days after vaccination. Thereafter, the birds were placed on plain drinking water until the end of the experiment.
- **Group B**: The chicks in this group were placed on plain drinking water before vaccination. On the 8th day 5 millilitres of honey was administered in their drinking water for 7 days. After 7 days of treatment with honey, the birds were then placed on ordinary drinking water.
- **Group C:** Chicks in this group were given multivitamin (Vitaflash®, Kepro B.V. Holland) in drinking water, at 1gm per litre of water daily for 7 days before vaccination. After vaccination on the 8th day, the same treatment with the multivitamin continued for another 7 days. Thereafter the birds were placed on plain drinking water.
- **Group D:** Multivitamin (Vitaflash®, Kepro B.V. Holland) at 1g/litre was administered orally in drinking water for 7 days after vaccination. There after the birds continued taking plain drinking water until the end of the experiment.
- **Group E:** This group was sub-divided into E_1 and E_2, which represented the positive control and negative control groups respectively.

Chicks in the E_1 sub-group were placed on plain drinking water throughout the experiment but were vaccinated at 3 weeks of age.

Chicks in the E_2 sub-group were also placed on plain drinking water throughout the experiment and were neither vaccinated nor treated.

Collection of Blood Sample

Four chicks each were randomly bled from the 6 different groups prior to the commencement of treatment or vaccination. The chicks were again bled every 7 days after vaccination until the 56th day (8 weeks) after vaccination.

Blood samples were collected through the wing vein of the chicks using a 2ml syringe and needle. Two milliliters (2mls) of the blood was collected in a sterile plain sample bottle. The blood sample in each case was centrifuged at 1,500 rpm for 5 minutes to separate the sera. All the samples were individually stored in serum tubes (nunc tubes) at – 20 ^{o}C until tested.

Serology

Newcastle disease antigen was used for this experiment. This was obtained from the National Veterinary Research Institute (NVRI) Vom Nigeria. The positive serum had a titre of 2^5, negative serum titre was 2^3 and the antigen had a titre of 2^7. Serum samples were tested for Newcastle disease virus specific antibodies using a modification of the Hemagglutination Inhibition (HI) test as previously described by Baba et al. [34].

Results

The response of cockerel chicks to oral vaccination against Newcastle disease (ND) using ND "LaSota" vaccine following administration of honey at different times is presented in Table 1. The HA titre of the ND 'laSota' vaccine used for this work was 1:64. At day zero (pre-vaccination and pre-treatment), baseline antibody screening results showed geometric mean titres (GMT) of 11.3, 10.0, 11.3, 7.9, 4.0 and 2.0 for birds in group HN1, HN2, MTVN 1, MTVN 2, positive control and negative control respectively.

The baseline antibody screening results showed that 83.3% of the birds screened were positive for ND HI antibodies at day 7 post vaccination, the highest GMT of 31.9 was recorded in the group of cockerel chicks that were given honey via drinking water 7 days before and 7 days after vaccination. This was followed by 20.1, 15.9. 11.3 and 2.8 for the groups HN1, MTVN 1, HN 2, MTVN 2 and positive control respectively.

At day 14 post vaccination, the highest GMT of 127.9 was obtained in the HN1 group. This was followed by GMTs of 101.5, 63.9, 50.7 and 16.0 in MTVN1, HN2, MTVN2 and positive control groups respectively.

At day 21 post vaccination, the highest GMT of 181.0 was obtained in the HN1 group followed by GMTS of 127.9, 101.5 and 16.0 in MTVN1, HN2, MTVN2 and positive control groups respectively.

At day 28 post vaccination, the highest GMT of 101.5 was obtained in the HN1 group followed by GMTS of 90.5, 50.7 and 11.3 in MTVN1, HN2, MTVN2, and positive control groups respectively.

At day 35 post vaccination, the highest GMT of 80.6 was obtained in the HN1 group followed by GMTS of 50.7, 31.9 and 12.6 in MTVN1, MTVN2, HN2 and positive control groups respectively.

At day 42 days post vaccination, the highest GMT of 31.9 for groups HN1, and MTVN1, followed by 20.1 and 11.3 in MTVN 2 and HN2 groups respectively.

At day 56 days post vaccination, the highest GMT recovered was 20.1 for HN1 group followed by GMTS of 12.6 and 11.3 for groups MTVN1, HN2 and MTVN 2 respectively.

The result of this work has shown that the group of birds which received honey in drinking water before and after vaccination maintained a consistently high GMT values with the highest titre at the third week after vaccination.

The group which did not receive honey nor multivitamin maintained a consistently low titre with the highest titre of 16.0 at third week after vaccination which began to drop by fourth week after vaccination and at the fifth week after vaccination, the birds in this group tested negative for HI.

Days (Pre and Post Vaccination)	HN 1	HN 2	MTVN 1	MTVN 2	Positive control	Negative Control
Pre- vaccination titres	11.3	10	11.3	7.9	4	2
7	31.9	15.9	20.1	11.3	2.8	Negative
14	127.9	63.9	101.5	50.7	16	Negative
21	181	101.5	127.9	101.5	16	Negative
28	101.5	50.7	90.5	50.7	11.3	Negative
35	80.6	12.6	50.7	31.9	Negative	Negative
42	31.9	11.3	31.9	20.1	Negative	Negative
56	20.1	11.3	12.6	11.3	Negative	Negative

TABLE 1: Geometric mean titers (GMT) of antibody response of cockerel chicks to vaccination against Newcastle Disease (ND) using ND
'laSota' vaccine following treatment with honey or multivitamin before and after vaccination or only after vaccination

- **KEY**

HN 1 = Honey administered in drinking water for 7 days before and 7 days after vaccination.
HN2 = Honey administered in drinking water for 7 days after vaccination
MTVN 1 = Multivitamin (Vitaflash®, Kepro B.V., Holland) administered in drinking water for 7 days before and 7 days after vaccination
MTVN 2 = Multivitamin (Vitaflash®, Kepro B.V., Holland) administered in drinking water for 7 days after vaccination.

Discussion

Most of the commonly used ND vaccines in Nigeria are live attenuated among which the ND vaccines, lentogenic 'laSota' strain is described as the best in terms of high

spreading potential and capability of production protecting antibody titres by all routes of administration [35].Honey has been reported to enhance the immune response of chicks vaccinated against Newcastle disease (ND) [7,36,37].

The present study conducted to investigate the effects of honey on the immune response of cockerels chicks from 7-42 days of age that were vaccinated against ND, using ND "laSota" vaccine obtained from the National Veterinary Research Institute (NVRI) Vom, Nigeria. However, the effect of supplement of honey in drinking water on the immune response of cockerel chicks was evaluated. The results from this present study revealed that young cockerels placed on honey before and after vaccination appeared to develop a better haemagglutination inhibition (HI) antibody response as compared to those placed on only multivitamin or the control group that were placed on ordinary drinking water. At three weeks post vaccination, the highest GMT of 181.0 was recorded in chickens that were given honey via drinking water before and after vaccination whereas GMT of 16.0 was recorded in chickens that were given ordinary drinking water. The multivitamin group also maintained a reasonably high antibody titre with the GMT of 101.5 which indicated that the multivitamin used in this study had some level of immune enhancing properties. Also, from the results, it was observed that the group of birds placed on either honey or multivitamin 7 days before and 7 days after vaccination maintained a higher titre than those placed on either of these only 7 days after vaccination. This could suggest that honey and multivitamin act slowly on the immune system and therefore a considerable length of time should be maintained in order to achieve the best results. Since honey has been reported to have antimicrobial property, the high immune responses demonstrated by chicks that were placed on honey in this experiment is an indication that there could be some degree of contamination of the reconstituted vaccine.

Propolis has been reported as a natural resinous mixture produced by honey bees from substances collected from many plants sources [38]. Due to the many biological activities of propolis, such as antimicrobial, anti-inflammatory, antioxidant and immunostimulatory, which are attributed to its chemical composition, including flavonoids, aromatic acids, diterpene acids and phenolic compounds [39,40]. According to Hegazi et al. [36] and Fan *et al.* [41] propolis is able to enhance lymphocyte proliferation, and this can reflect in the lymphoid organs weight, impacting on immune function and disease resistance ability. However, previous studies have shown that flavonoids have an immunossupressor effect on the lymphoproliferative [42,43]. The findings of this study is in accordance with previous report of Hegazi et al. [36,37] who in a similar study demonstrated total antibody response of the 10% honey additives as supplement to broiler chicks vaccinated with (NDV vaccine. Broiler chicks fed with honey had significantly higher antibody response as compared with the control group. The similarity in these researches may be due the chemical composition of honey which includes huge mixture of flavonoids and phenolic acids which act as antimicrobial agent [37,44].It is clear that the similarity in performance that is associated with an immune response is due to the diversion of nutrients away from growth or egg production to be used by the immune system. Improvements in immunity or functions that support immunity are associated with Zn, Mn, Cu and Se [44,45].Honey in drinking water may be considered as a new natural additive due to their components of micronutrients which enhanced and developed the immune system. The use of honey as a cockerel immune-stimulant for poultry farming purposes needs validation with further studies, using different levels and other poultry species as previous suggested by Hegazi et al. [37]. Serum antibody titer is the indicator of humoral immunity. Results in this study have showed that the antibody titers in most of treatment groups at each time point were higher than that from control group, suggesting that they could promote humoral immunity.

Conclusion

From the findings from this study, it could be concluded that the honey supplementation increases antibodies response of exotic breed cockerel chicks when administered before and after ND vaccination. This may be due to the immunostimulatory activity of natural honey. It is there assumed that this group of poultry could be better protected against ND if given honey orally via drinking water before and after vaccination.

Recommendation

Advanced similar researches should be extended using natural honey as supplement in drinking water pre and post ND vaccinations in other exotic poultry breeds and also evaluate the quantity of honey to be used in order to achieve an excellent immune stimulation to vaccination.

References

1. Abdelqader A, Wollny C B, Gauly M (2007) Characterization of local chicken production systems and their potential under different levels of management practice in Jordan. Trop Anim Health Prod 39(3): 155-164.

2. Ayele G, Asare-Marfo D, Birol E, Roy D (2009) Investigating the Role of Poultry in Livelihoods and the Impact of HPAI in Ethiopia. Controlling Avian Flu and Protecting People's Livelihoods in Africa and Indonesia. International Food Policy Research Institute (IFPRI) with the International Livestock Research Institute (ILRI) and Royal Veterinary College (RVC) Pp. 12.

3. Seeberg D S (2002) Money in My Hand: An Anthropological Analysis of the Empowerment of Women through Participation in Participatory Livestock Development Project in Northwest Bangladesh. Field Report Based on Ethnographic Fieldwork in Bangladesh. Aarhus University Denmark, Pp. 62.

4. Riise J C, Permin A, Kryger K N (2005) Strategies for developing family poultry production at village level - experiences from West Africa and Asia. World Poult Sci J 61: 15-22.

5. Kim S F, David S, Erdogan B Z (2004) The Influence of religion on attitudes towards the advertising of controversial products. Eur J Marketing 38(5/6): 537-555.

6. Nam K C, Jo C, Lee M (2010) Meat products and consumption culture in the East. Meat Sci 86(1): 95-102.

7. Seleshe S, Jo C, Lee M (2014) Meat Consumption Culture in Ethiopia. Korean J Food Sci Anim Resour 34(1): 7-13.

8. Adene D F, Oguntade A E (2006) Nigeria's Poultry Population 2003. In: The structure and importance of the commercial and village based poultry industry in Nigeria. FAO (Rome) study, October 2006 Pp 29.

9. Ibrahim U I, Lawal J R, El-Yuguda A D (2016) Level of Newcastle disease vaccination awareness and its effects on village chicken production in Gombe State, Nigeria. Direct Research Journal of Agriculture and Food Science 4(3): 48-54.

10. Alexander D J, Bell J G, Alders R G (2004) Technology review: Newcastle disease with special emphasis on its effect on village chickens. FOA Animal production and Health Paper No.161: Pp. 63.

11. Zeleke A, Sori T, Gelaye E, Ayelet G (2005) Newcastle disease in village chickens in Southern and Rift valley Districts in Ethiopia. Inter J Poult Sci 4(7): 507-510.

12. Aziz A G T, Ahmed T A (2010) Serological survey of Newcastle disease in domestic chickens in Sulaimani Province. J ZankaySulaimani 13(1): 31-38.

13. Marangon S, Busani L (2006) The use of vaccination in poultry production. Revue Science Technology Officiale Des Epizooties 26(1): 265-274.

14. Ibrahim U I, EL-Yuguda A D, Tambari P S (2000) Trials of Feed base Vaccine Vehicles. Nig J Expt Appl Biol 1: 83-86.

15. Ibu O J, Ogunsola D, Aba–Adulugba E P, Echeonwu G O N (2002) Quality control assessment of two lentogenic Newcastle Disease Vaccines imported into Nigeria and the locally produced "laSota". Trop Vet 20: 8-10.

16. Humphrey B D, Huang N, Klasing K C (2002) Rice expressing lactoferrin and lysozyme has antibiotic-like properties when fed to chicks. J Nutr 132(6): 1214-1218.

17. Nair M K M, Vasudevan P, Venkitanarayanan K (2005) Antibacterial effect of black seed on Listeria monocytogenes. Food Control 16: 395-398.

18. Echeonwu B C, Ngele M B, Echeonwu G O N, Joannis T M, Onovoh E M, et al. (2008) Response of chickens to oral vaccination with Newcastle disease virus vaccine strain I_2 coated on maize offal. African Journal of Biotechnology 7(10): 1594-1599.

19. Ambali A G, El-Yuguda A D, Atiku S A (1995) Effects of Ascorbic acid and honey on the immune response of domestic chickens to vaccination with NDV – V4 vaccine.

20. Al-Waili N S, Haq A (2004) Effects of honey on antibody production against thymus dependent and thymus independent antigens in primary and

secondary immune responses. Journal of medicinal food 7(4): 491-494.

21. Monsuru O A, Kabir B O, Titilayo E A, Kayode E O, Oluwatosin O A (2012) Growth Mineral Deposition and Physiological Responses of Broiler Chickens Offered Honey in Drinking Water during Hot-Dry Season. International Journal of Zoology Article ID 4035026 pages.

22. Ahmed H, Amr M A, Fyrouz A A (2013) Influence of Honey on Immune Response Against Newcastle Disease Vaccine. International Journal of Basic and Applied Virology 2(1): 1-5.

23. Korver D, Klasing K (2001) Influence of nutrition on immune status of the bird. Proceedings of the 24th Technical Turkey Conference Pp: 43.

24. Abdel-Mohsein H S, Mahmoud M A M, Mahmoud U T (2014) Influence of propolis on intestinal microflora of Ross broilers exposed to hot environment. Advances in Animal and Veterinary Sciences 2(4): 204-211.

25. Mahmoud M A M, Abdel-Mohsein H S, Mahmoud U T (2014) Effect of Chinese propolis supplementation on Ross broiler chicks: Microbial population in fecal matter and litter. Journal of Advanced Veterinary Research 4: 77-84.

26. Wallace F A, Miles E A, Calder P C (2000) Activation state alters the effect of dietary fatty acid on pro-inflammatory mediator production by murine macrophages. Cytokine 12(9): 1374-1379.

27. Cunningham-Rundles S (2001) Nutrition and mucosal immune system. Current Opinion in Gastroenterology 17(2): 171-176.

28. Emadi M, Jahanshiri F, Kaveh K, Hair-Bejo M, Ideris A, et al. (2011) Nutrition and immunity: the effects of the combination of arginine and tryptophan on growth performance, serum parameters and immune response in broiler chickens challenged with infectious bursal disease vaccine. Avian Pathol 40(1): 63-72.

29. Hegazi A G, Ragaa H M, Amal M E (2006) Antimicrobial Activity of Some Medicinal Plants Egypt. J Vet Sci 40: 41-48.

30. Chang H F, Lin Y H, Chu C C, Wu S J, Tsai Y H (2007) Protective Effects of Ginkgo biloba, Panax ginseng and Schizandra chinensis Extract on Liver Injury in Rats. Am J Chin Med, 35(6): 995-1009.

31. Nichols, Andrew W, (2007) Probiotics and athletic performance: A systematic review. Current Sports Medicine Reports 6(4): 269-273.

32. Gibson G R, Roberfroid M B (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 125(6): 1401-1412.

33. Eisenthal R, Danson M J (2002) Enzyme Assays: A Practical Approach. Oxford University Press.

34. Baba S S, El Yuguda A D, Baba M M (1998) Serological evidence of mixed infection with Newcastle disease and Egg drop syndrome 1976 viruses in Village chicken in Borno state, Nigeria. Tropical Veterinarian 16: 137-141.

35. Nawathe D R (1988) Problems of Newcastle Disease Control. In: Viral disease of Animals in Africa. OAU/STRC Scientific Publication, Lagos, Nigeria. Pp. 301-311.

36. Hegazi A, Abdou A M, Allah F A (2012) Egyptian propolis 9- Its effect on chicken productivity and immune response against Newcastle disease vaccine. Br Poult Sci. 1(3): 25-30.

37. Hegazi A, Abdou A M, Allah F A (2013) Influence of Honey on Immune Response Against Newcastle Disease Vaccine. International Journal of Basic and Applied Virology 2(1): 1-5.

38. Salatino A, Fernandes-Silva C C, Righi A A, Salatino M L F (2011) Propolis research and the chemistry of plant products. Nat Prod Rep 28: 925-936.

39. Lofty M (2006) Biological activity of bee propolis in health and disease. Asian Pac J Cancer Prev 7(1): 22-31.

40. Trusheva B, Popova M, Bankova V, Simova S, Marcucci M C, et al. (2006) Bioactive constituents of Brazilian red propolis. Evid Based Complement Alternat Med 3(2): 249-254.

41. Fan Y, Yu L, Deyun W, Liu J, Song X, et al. (2013) Effect of epimedium polysaccharide-propolis flavone

immunopotentiator on immunosuppression induced by cyclophosphamide in chickens. Cell Immunol 281(1): 37-43.

42. Namgoong S Y, Son K H, Chang H W, Kang S S, Kim H P (1994) Effects of naturally occurring flavonoids on mitogen-induced lymphocyte proliferation and mixed lymphocyte culture. Life Sciences 54(5): 313-320.

43. You K M, Son K H, Chang H W, Kang S S, Kim H P (1998) Vitexicarpin, a flavonoid from the fruits of Vitexrotundifolia, inhibits mouse lymphocyte proliferation and growth of cell lines in vitro. PlantaMedica 64(6): 546-550.

44. Hegazi A G, Faten K A (2009) Influence of Honey on the Suppression of Human Low Density Lipoprotein (LDL) Peroxidation (In-vitro). Journal of Evidence Based Complementary and Alternative Medicine 6(1): 113-121.

45. Eyng C, Murakami A E, Santos T C, Silveira T G V, Pedroso R B, et al. (2015) Immune Responses in Broiler Chicks Fed Propolis Extraction Residue-supplemented Diets. Asian Australas J Anim Sci 28(1): 135-142.