

Productive performance and physiological responses of growing rabbits fed diets supplemented with different levels of neem leaves and oil - a review.

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Abstract

Recently, rabbit production in Egypt has proliferated, mainly to accommodate the increasing demand for fresh meat for human consumption and as a source of additional revenue for Egyptian households or small farms. Modern trends in feeding rabbits on herbs because of their great importance in the physiological responses of the body to produce and strengthen production, the neem tree (*Azadirachta indica*) plant is atypical example of herbs, as it has many benefits because all parts of the neem tree- leaves, flowers, seeds, fruits, roots and bark have been used traditionally for the treatment of inflammation, infections, fever, skin diseases and dental disorders. The medicinal utilities have been described especially for neem leaves. Neem leaf and its constituents have been demonstrated to exhibit immunomodulatory, anti-inflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties. This review summarises the wide range of pharmacological activities of neem leaf. Therefore, recent research has proven great important for the application of neem and its products from leaves, flowers, fruits, oil and bark in animal nutrition.

Keywords: Herbs; Production; Neem; Rabbits.

1. Introduction

Recently, rabbit production in Egypt has proliferated, mainly to accommodate the increasing demand for fresh meat for human consumption and as a source of additional revenue for Egyptian households or small farms (Abdel-Wareth *et al.*, 2015). Moreover, that rabbit meat is rich in protein (22g/100g) and low in cholesterol (50g/100g), fat (4g/100g), and energy (124 Kcal/100g) Pla *et al.* (2004). The meat has a complete amino acid profile, a pleasant flavour, and is readily digestible (Cheeke, 1986; Owen, 1981). In rabbit production, feeding is the main cost, so feed intake control could adjust the diet and

nutritional requirements to manage the growth performance (Yakubu *et al.*, 2007).

Accordingly, diet can be enhanced by supplementation of a broad range of elements, including herbal medicines or extracts (Wenk *et al.*, 2000; Grela *et al.*, 2007). Consequently, Research in recent years has been focused on herbal supplementation to animal diets with fewer side effects and is environmentally friendly to manage infectious illnesses. One of these substances is neem which is a widely recognized plant.

Several articles demonstrated the efficiency of using herbal medicines in livestock diets due to its outstanding nutritional and therapeutic values, including antibacterial, anti-viral, anti-inflammatory, and anti-oxidant properties (Klusek *et al.*, 1997; Grela and Semeniuk,


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2006). It has been reported that these substances can stimulate appetite, regulate digestion and metabolism, have anti-diarrheal properties, and stimulate hormonal and immune systems (Grela and Semeniuk, 2006). Herbal extracts are potentially beneficial as growth promoters in diets and play a good role as therapeutic agents to treat certain diseases and disorders. They can replace antibiotics, enhancing immunity, and fight pathogenic bacteria and viral infections (Alimon, 2009). Neem has many incredible medicinal benefits Because it contains many the active componenets like limonoids, azadirachtin, meliantriol, salannin, Nimbin, Nimbidin and deacetylazadirachtinol.

but one of the most important things is that it kills cancerous cells and helps destroy harmful bacteria. Researchers have found that neem products are virtually nontoxic and are compatible with beneficial insects, pollinators and bees. They are environmentally benign, sustainable, renewable and affordable, it also significantly enhances metabolism. Traditionally, neem leaves are used to treat head lice, skin diseases, wounds or skin ulcers. External application of neem also finds merit as a mosquito repellent. Neem is probably the world's oldest skin softener and has been used for this purpose for millennia. Neem leaves can be boiled in water and the water can be strained and stored for use as a skin ointment.

2. Effects of Neem on feed intake and growth performance of rabbits

2.1. Productive performance:

2.1.1. Live body weight and weight gain

Body weight is concerning as the most important item in meat type rabbits production. Almost the aim of each research is to maximize body weight, feed conversion and minimize the production cost per unit. The current study aimed to evaluate adding neem leaves or oil levels on rabbits' productive performance as well as physiological responses.

Oluwafemi and Oluwayinka (2020) reported that, supplementation different levels of neem oil at (0.1 ,0.2 , 0.3 and 0.4%) to rabbits diets significantly ($P<0.05$) increased final body weight compared to control group.

Kharde and Soujanya (2014) found that supplementation neem leaves powder on broiler chicks diets at 2g/kg significantly ($P<0.05$) improved the body weight.

Landy *et al.* (2011) revealed that supplementation neem fruits powder on broiler chickens diets at 7 gm and 12 gm /kg diets significantly ($P<0.05$) increased live body weight compared to control group especially by 7gm/kg diets from neem fruits powder.

Kumari *et al.* (2014) reported that supplementation neem leaves to chicken diets at 2.5% significantly ($P<0.05$) increased body weight and weight gain compared to control group.

Odunsi *et al.* (2009) found that, supplementation neem seeds on the cockerel chicken's diets significantly ($P<0.05$) increased final body weight compared to control group.

Supplementation of different level of neem leaf at (5, 10 and 15%) significantly ($P<0.05$) decreased final body weight and weight gain on rabbits compared to control group. (Obgbuewu *et al.*, 2010; Unigwe *et al.*, 2016; Ubua *et al.*, 2019).

Kyere *et al.* (2018) found that, supplementation of neem leaves to pearl guinea fowls diets at (3 ,6 and 9%) significantly ($P<0.05$) decreased final body weight at 3% and 9%. On the other hand, neem leaves at 6% significantly ($P<0.05$) increased final body weight compared to control group.

Khalil (2003) found that, supplementation of neem leaves extract to rabbits significantly ($P<0.05$) decreased body weight compared to control group.

Nnenna and okey (2013) reported that, supplementation of aqueous neem leaf extract to broiler chicks water at 20, 40 and 60 ml per 1 litre of water significantly ($P<0.05$) decreased

final body weight at the level of 20 ml/litre, however aqueous neem leaf extract at 40ml and 60ml /litre significantly ($P<0.05$) increased final body weight compared to control group.

Zuraini *et al.* (2006) found that rats fed treated with Neem leaf extract at doses of 50 and 300 mg/kg/day orally did not show any significant effect.

Ekine and Samuel (2020) reported that supplementation of neem leaves to broiler diets at (1, 2, 3 and 4%) did not show any significant different in body weight gain.

2.1.2. Feed intake and Feed conversion ratio

Many studies indicated that feed intake was decreased in rabbit fed different levels of neem leaves or oil, for example, Ozung *et al.* (2019) who found that feed intake was significantly ($P<0.05$) decreased and improved feed conversion ratio in rabbit fed diet supplemented with 5, 10 and 15% neem leaves compared to control group. Likewise, Unigwe *et al.* (2016) addition of 5, 10 and 15 % neem leaf to rabbit diets resulted in a significantly decreased in a total feed intake and there were inance in feed conversion ratio compared to control group.

Landy *et al.* (2011) found that supplementation of neem fruits powder to broiler chickens diets 7 and 12 gm neem fruits powder /kg diets and revealed that neem fruits powder at 7gm/kg significantly ($P<0.05$) increased fed intake and feed conversion ratio of chickens and there were decreased in feed conversion ratio compared to control group.

Kyere *et al.* (2018) found that supplementation of neem leaves to pearl guinea fowls diets at (3, 6 and 9%) neem leaves significantly ($P<0.05$) decreased total feed intake and feed conversion ratio compared to control group.

Odunsi *et al.* (2009) found that supplementation neem seeds on the cockerel chickens diets at 10% and 20% neem seed cake (CNSC) significantly ($P<0.05$) increased total feed intake compared to control group.

Kumari *et al.* (2014) observed that supplementation of neem leaves to chicken at 2.5% neem leaves significantly ($P<0.05$) improved feed intake and there were no significant differences in feed conversion ratio compared to control group.

Kharde and Soujanya (2014) reported that supplementation of neem leaves powder on broiler chiches diets at 0.5, 1 and 2 gm/kg significantly ($P<0.05$) decreased feed conversion ratio compared to control group.

3. Effects of Neem on Blood biochemestery of rabbits

3.1. Haemoglobin

Ogbuewu *et al.* (2010) found that supplementation of neem leaves to rabbits diets at (5, 10 and 15%) did not show any significant difference on haemoglobin concentration on rabbits blood.

Kyere *et al.* (2018) reported that supplementation of neem leaves to pearl guinea fowls diets at (3, 6 and 9%) significantly ($P<0.05$) decreased haemoglobin concentration of pearl guinea fowls blood compared to control group.

Nnenna and okey (2013) found that supplementation of aqueous neem leaf extract to broiler chicks water at 20, 40, 60 ml per 1 litre of water significantly ($P<0.05$) increased haemoglobin concentration of broiler chicks blood compared to control group.

Odunsi *et al.* (2009) found that supplementation of neem seeds on the cockerel chickens diets significantly ($P<0.05$) increased haemoglobin concentration on cockerel chickens blood compared to control group.

Oluwafemi and Oluwayinka (2020) reported that supplementation of different levels of neem oil at (0.1, 0.2, 0.3 and 0.4%) to rabbits diets significantly ($P<0.05$) increased haemoglobin concentration on rabbits blood compared to control group.

Ozung *et al.* (2019) found that supplementation of neem leaves at (5, 10 and 15%) to rabbits diets significantly ($P<0.05$) decreased haemoglobin concentration on rabbits blood, however neem leaves at 10% did not show any significant different in haemoglobin concentration on rabbits blood compared to control group.

Unigwe *et al.* (2016) reported that supplementation of different levels of neem leaf (5, 10 and 15%) significantly ($P<0.05$) increased haemoglobin concentration on rabbits blood compared to control group.

Khalil (2003) found that supplementation of neem leaves extract to rabbits significantly ($P<0.05$) decreased haemoglobin concentration on rabbits blood as compared to control group.

Kumari *et al.* (2014) found that supplementation of neem leaves to chicken diets 2.5% significantly ($P<0.05$) decreased haemoglobin concentration, however in other addition groups significantly ($P<0.05$) increased haemoglobin concentration on chicken blood compared to control group.

3.2. Total protein concentration, Albumin and Globulin

Ogbuewu *et al.* (2010) found that supplementation of neem leaves NLM at (5, 10 and 15%) to rabbits diets significantly ($P<0.05$) decreased total protein and albumin concentration, However at 10% and 15% NLM significantly increased albumin concentration compared to control group.

Ogbuewu *et al.* (2015) reported that supplementation neem leaves at (5, 10 and 15%) to rabbits diets significantly ($P<0.05$) decreased total protein concentration and globulin, however albumin levels increased on rabbits blood compared to control group.,

Bratte *et al.* (2015) reported that supplementation neem leaves at (2.5, 5, 7.5 and 10%) to chicken diets significantly ($P<0.05$) increased total protein concentration on chicken blood compared to control group, except at 2.5

and 7.5% total protein concentration decreased significantly ($P<0.05$) on chicken blood compared to control group.

Oluwafemi and Oluwayinka (2020) reported that supplementation of different levels of neem oil (0.1, 0.2, 0.3 and 0.4%) to rabbits diets did not show any significant changes on total protein but significantly ($P<0.05$) increased globulin concentration on rabbits blood compared to control group.

Ogbuewu *et al.* (2010) found that supplementation of neem leaves to rabbits diets at (5, 10 and 15%) did not show any significant changes on total protein concentration but increased albumin. However, globulin levels decreased on rabbits blood compared to control group.

Kyere *et al.* (2018) reported that supplementation of neem leaves to pearl guinea fowls diets at (3, 6 and 9%) significantly ($P<0.05$) increased total protein, albumin and globulin concentration on blood as compared by control group.

Nnenna and okey (2013) reported that supplementation aqueous neem leaf extract to broiler chicks water at 20, 40 and 60 ml per 1 litre of water significantly ($P<0.05$) increased total protein, albumin and globulin concentration on broiler chicks blood compared to control group.

Kumari *et al.* (2014) found that supplementation neem leaves to chicken at 2.5% significantly ($P<0.05$) increased total protein concentration on chicken blood, however other neem treatments did not show any significant in total protein concentration compared to control group.

Bratte *et al.* (2015) supplemented neem leaves at (2.5, 5, 7.5 and 10%) to chicken diets and revealed that neem leaves at 2.5% significantly ($P<0.05$) increased albumin concentration on chicken blood, however at 7.5% and 10% neem leaves significantly ($P<0.05$) decreased albumin concentration on chicken blood and there was no significant difference at 5% neem leaves compared with control group.

El-Zaiat *et al.* (2021) supplemented neem seeds oil on sheep diets at 20 and 40 ml/ sheep per day and revealed that neem seeds oil significantly ($P<0.05$) increased globulin concentration on sheep blood compared to control group.

3.3. Urea and creatinine concentration

Ogbuewu *et al.* (2015) found that supplementation neem leaves to rabbits diets at (5, 10 and 15%) significantly ($P<0.05$) decreased urea concentration on rabbits blood, however no significant differences on creatinine concentration were found.

Ozung *et al.* (2019) found that supplementation of neem leaves at (5, 10 and 15%) to rabbits diets did not show any significant difference in urea and creatinine concentration among groups. Nnenna and okey (2013) reported that supplementation aqueous neem leaf extract to broiler chicks water at 20, 40 and 60 ml per 1 litre of water significantly ($P<0.05$) increased urea concentration, although there were significantly ($P<0.05$) decreased on the levels of creatinine concentration compared to control group.

3.4. cholesterol concentration

supplementation of neem leaves at (5, 10 and 15%) to rabbits diets significantly decreased cholesterol concentration on rabbits compared to control group (Ogbuewu *et al.*, (2010) and Ogbuewu *et al.*, (2015))

Bratte *et al.* (2015) supplemented neem leaves at (2.5, 5, 7.5 and 10%) to chicken diets and revealed that neem leaves at 5% ,7.5% and 10% significantly ($P<0.05$) increased cholesterol concentration, however at 2.5% cholesterol concentration significantly decreased on chicken blood compared to control group.

Oluwafemi and Oluwayinka (2020) indicated that supplementation of different levels of neem oil at (0.1 , 0.2, 0.3 and 0.4%) to rabbits diets significantly($P<0.05$) decreased cholesterol concentration compared to control group.

El-Zaiat *et al.* (2021) found that supplementation neem seeds oil on sheep diets at 20 and 40 ml/ sheep per day significantly ($P<0.05$) decreased cholesterol concentration compared to control group.

Ogbuewu *et al.* (2015) found that supplementation neem leaves to rabbits diets at (5, 10 and 15%) significantly($P<0.05$) decreased cholesterol concentration compared to control group.

Nnenna and okey (2013) reported that supplementation aqueous neem leaf extract to broiler chicks water at 20, 40 and 60 ml per 1 litre significantly($P<0.05$) decreased cholesterol concentration compared to control group.

Ekine and Samuel (2020) found that supplementation neem leaves on broiler diets at (1, 2, 3 and 4%) significantly ($P<0.05$) increased total cholesterol concentration compared to control group.

Khalil (2003) found that supplementation neem leaves extract to rabbits significantly ($P<0.05$) decreased total cholesterol concentration compared to control group.

Kumari *et al.* (2014) found that supplementation neem leaves to chicken diets at 2.5% significantly ($P<0.05$) decreased cholesterol concentration compared to control group.

3.5. glucose concentration

Ogbuewu *et al.* (2010) supplemented neem leaves at (5, 10, 15%) in rabbits diets and noted that neem leaves at 10 and 15% decreased significantly ($P<0.05$) glucose concentration, however at 5% (NLM) increased significantly ($P<0.05$) glucose concentration on rabbits compared with control group.

Ogbuewu *et al.* (2010) reported that supplementation of neem leaves at (5 ,10 and 15%) to rabbits diets and observed that neem leaves at 5% and 10% there were no significant differences on glucose concentration on rabbits blood ,however there is significantly ($P<0.05$) decreased glucose concentration on rabbits blood

at 15% neem leaves compared with control group.

Oluwafemi and Oluwayinka (2020) reported that supplementation of different levels of neem oil at (0.1 , 0.2, 0.3 and 0.4%) to rabbits diets significantly ($P < 0.05$) decreased glucose concentration on rabbits blood compared to control group.

Ogbuewu *et al.* (2015) found that supplementation of neem leaves to rabbits diets at (5, 10 and 15%) neem leaves and observed that neem leaves at 15% significantly ($P < 0.05$) decreased glucose concentration, however neem leaves at 5% and 10% there were no significant differences on glucose concentration compared to control group.

Nnenna and okey (2013) reported that supplementation aqueous neem leaf extract to broiler chicks water at (20, 40, 60) ml aqueous neem leaf extract per 1 litre significantly ($P < 0.05$) decreased glucose concentration on broiler chicks blood compared to control group.

Zuraini *et al.* (2006) found that supplementation of neem leaves ethanolic extract on rats did not show any significant changes in glucose concentration while at diabetic group addition of streptozotocin significantly ($P < 0.05$) increased glucose concentration on rats, however at diabetic added neem leaves extract group significantly ($P < 0.05$) decreased glucose concentration on rats compared to control group. Khalil (2003) found that supplementation of neem leaves extract to rabbits significantly ($P < 0.05$) decreased glucose concentration compared to control group.

Kumari *et al.* (2014) found that supplementation of neem leaves to chicken at 2.5% neem leaves significantly ($P < 0.05$) decreased glucose concentration compared to control group.

3.6. Serum aspartate amino transferase (AST) and alanine amino transferase (ALT)

Ogbuewu *et al.* (2010) reported that supplementation neem leaves at (5, 10 and 15%) to rabbits significantly ($P < 0.05$) decreased serum

aspartate amino transferase and alanine amino transferase, however at 5% (NLM) significantly ($P < 0.05$) increased serum aspartate amino transferase and alanine amino transferase compared to control group.

Ogbuewu *et al.* (2015) found that supplementation of neem leaves at (5, 10 and 15%) to rabbits diets the result did not show any significant changes on serum aspartate amino transferase and alanine amino transferase concentration compared to control group.

Seriana *et al.* (2021) revealed that supplementation of neem leaf ethanolic extracts at (100, 200 and 300) ml/kg from rat body weight and indicated that neem leaf ethanolic extracts at 100 ml/kg body weight significantly ($P < 0.05$) decreased serum aspartate amino transferase and increased alanine amino transferase concentration, however neem leaf ethanolic extracts at 200 and 300 ml/kg body weight significantly ($P < 0.05$) increased serum aspartate amino transferase concentration compared to control group.

Ekine and Samuel (2020) found that supplementation of neem leaves on broiler diets at (1, 2, 3 and 4%) and revealed that neem leaves significantly ($P < 0.05$) decreased serum aspartate amino transferase concentration, however levels of alanine amino transferase concentration did not show any significant changes compared to control group.

3.7. High-density lipoprotein (HDL) and low-density lipoprotein (LDL)

Zuraini *et al.* (2006) found that subplementation of neem leaves ethanolic extract on rats significantly ($P < 0.05$) decreased high-density lipoprotein and the low-density lipoprotein levels increased compared to control group.

Ekine and Samuel (2020) found that supplementation of neem leaves on broiler diets at (1, 2, 3 and 4%) significantly ($P < 0.05$) decreased high-density lipoprotein and the low-density lipoprotein levels increased compared to control group.

Khalil (2003) reported that supplementation of neem leaves extract to rabbits significantly ($P < 0.05$) decreased high-density lipoprotein and the low-density lipoprotein levels increased compared to control group.

Kumari *et al.* (2014) found that supplementation neem leaves to chicken diets at 2.5% neem leaves significantly ($P < 0.05$) decreased high-density lipoprotein concentration, however other treatments significantly ($P < 0.05$) increased high-density lipoprotein, although there were improved in low-density lipoprotein levels compared to control group.

3.8. Red Blood Cells and White Blood Cells

Oluwafemi and Oluwayinka (2020) indicated that supplementation of different levels of neem oil at (0.1, 0.2, 0.3 and 0.4%) to rabbits diets there were no significant different on red blood cells and increased white blood cells compared to control group.

Ogbuewu *et al.* (2015) found that supplementation of neem leaves to rabbits diets at (5, 10 and 15%) neem the results show no significant different on red blood cells and white blood cells concentration compared to control group.

Kyere *et al.* (2018) found that supplementation of neem leaves to pearl guinea fowls diets at (3, 6 and 9%) neem leaves significantly ($P < 0.05$) increased red blood cells and white blood cells concentration compared to control group.

3.9. Mean cell volume, Mean cell hemoglobin, Mean cell hemoglobin concentration and packed cell volume

Ogbuewu *et al.* (2010) found that supplementation neem leaves to rabbits diets at (5, 10 and 15%) neem leaves and observed that neem leaves at 5% and 15% significantly ($P < 0.05$) decreased mean cell volume, however neem leaves at 10% there were no significant different and levels of Mean cell hemoglobin

Nnenna and okey (2013) reported that supplementation of aqueous neem leaf extract to broiler chicks water at (20, 40, 60) ml aqueous neem leaf extract per 1 litre significantly ($P < 0.05$) increased red blood cells and decreased white blood cells concentration blood compared to control group.

Odunsi *et al.* (2009) found that unprocessed neem seed cake significantly ($P < 0.05$) increased red blood cells and white blood cells concentration on cockerel chickens blood.

Ozung *et al.* (2019) found that supplementation of neem leaves at (5, 10 and 15%) to rabbits diets and revealed that neem leaves at 5% and 10% significantly ($P < 0.05$) increased red blood cells concentration, however neem leaves at 15% significantly ($P < 0.05$) decreased red blood cells, while neem leaves at 10% and 15% significantly ($P < 0.05$) increased white blood cells concentration, however neem leaves at 5% significantly ($P < 0.05$) decreased white blood cells concentration compared to control group.

Unigwe *et al.* (2016) reported that supplementation of different level of neem leaf (5, 10 and 15%) and observed that no significant different on red blood cells concentration, while at 10% didn't show any significant different in white blood cells concentration, however neem leaves at 5% and 15% significantly ($P < 0.05$) decreased white blood cells concentration compared to control group.

did not show any significant changes compared to control group.

Unigwe *et al.* (2016) found that supplementation of neem leaves at (5, 10 and 15%) on rabbits diets and revealed that there were any significant different of mean cell volume, Mean cell hemoglobin and Mean cell hemoglobin concentration, however there were increased packed cell volume of rabbits compared to control group.

Ozung *et al.* (2019) found that supplementation of neem leaves at (5, 10 and 15%) to rabbits diets and revealed that neem leaves at 5% and 10%

significantly ($P < 0.05$) decreased Mean cell hemoglobin and increased Mean cell hemoglobin concentration, however neem leaves at 15% there were no significant differences Mean cell hemoglobin and Mean cell hemoglobin concentration, while there were decreased packed cell volume compared to control group.

Ogbuewu *et al.* (2015) founded that supplementation of neem leaves to rabbits diets at (5, 10 and 15%) neem leaves and indicated that there were no significant changes on Mean cell hemoglobin concentration and packed cell volume compared to control group.

Kyere *et al.* (2018) found that supplementation of neem leaves to pearl guinea fowls diets at (3, 6 and 9%) neem leaves significantly ($P < 0.05$) increased packed cell volume percentage concentration on pearl guinea fowls blood compared to control group.

Nnenna and okey (2013) reported that supplementation aqueous neem leaf extract to broiler chicks water at (20, 40, 60) ml per 1 litre significantly ($P < 0.05$) increased packed cell volume compared to control group.

Odunsi *et al.* (2009) found that supplementation of neem seeds on the cockerel chickens diets at 10% and 20% did not show any significant difference compared to control group.

Oluwafemi and Oluwayinka (2020) found supplementation of different levels of neem oil at (0.1, 0.2, 0.3 and 0.4%) to rabbits diets significantly ($P < 0.05$) increased packed cell volume concentration on rabbits blood compared to control group.

3.10. *Lymphocytes and Neutrophil*

Ogbuewu *et al.* (2010) reported that supplemented neem leaves to rabbits diets at (5, 10 and 15%) neem leaves and observed that neem leaves at 5% there were no significant changes, however at 10% neem leaves significantly ($P < 0.05$) increased Lymphocytes, while neem leaves at 15% significantly ($P < 0.05$) decreased on Lymphocytes, although

levels of Neutrophils significantly ($P < 0.05$) decreased compared to control group.

4. Conclusions

This review summarises the wide range of pharmacological activities of neem leaf.

Therefore, recent research has proven great importance for the application of neem and its products from leaves, flowers, fruits, oil and bark in animal nutrition.

Authors' Contributions

All authors are contributed in this research.

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There is no fund in this research.

Institutional Review Board Statement

All Institutional Review Board Statement are confirmed and approved.

Data Availability Statement

Data presented in this study are available on fair request from the respective author.

Ethics Approval and Consent to Participate

This work carried out at Department of Animal and Poultry Production and followed all the department instructions.

Consent for Publication

Not applicable.

Conflicts of Interest

The authors declare no conflict of interest.

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