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Khushboo Yadav
Research Scholar, Department of Livestock Production Management, A.N.D.U.A.T, Kumarganj, Ayodhya, Uttar Pradesh, India

Ajeet Kumar Verma
Assistant Professor Department of LPM, ANDUAT, Kumarganj, Ayodhya, Uttar Pradesh, India

PS Pramanik
Professor and Head, Department of LPM, ANDUAT Kumarganj, Ayodhya, Uttar Pradesh, India

Manoj Kumar Verma
Assistant Professor, Department of AGB, ANDUAT, Kumarganj, Ayodhya, Uttar Pradesh, India

SS Chauhan
Department of LPM, ANDUAT, Kumarganj, Ayodhya, Uttar Pradesh, India

Amit Kumar
Research Scholar, Department of Animal Husbandry Extension Education ANDUAT, Kumarganj, Ayodhya, Uttar Pradesh, India

Corresponding Author:
Khushboo Yadav
Research Scholar, Department of Livestock Production Management, A.N.D.U.A.T, Kumarganj, Ayodhya, Uttar Pradesh, India

Effect of herbal supplementation of neem (*Azadirachta indica*) and moringa (*Moringa oleifera*) leaf meal against coccidiosis in the diet of Kadaknath layers

Khushboo Yadav, Ajeet Kumar Verma, PS Pramanik, Manoj Kumar Verma, SS Chauhan and Amit Kumar

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Abstract
This investigation aimed to assess the effect of incorporating neem leaf meal (NLM), moringa leaf meal (MLM), and their combination (MNLM) into the diet on the number of fecal oocysts in Kadaknath layers. A total of 72 Kadaknath laying hens, aged 42 weeks, were randomly allocated into four groups. Each group consisted of eighteen birds, and three replications were conducted for each treatment. The experimental groups consisted of the control group, referred to as T_C, which was administered a basal diet (BD) without any dietary supplements. The T_M group received a combination of BD and 2% moringa leaf meal (MLM), the T_N group received a combination of BD and 2% neem leaf meal (NLM), and the T_{MN} group received a combination of 1% MLM and 1% NLM (MNLM) supplement. Fecal samples were collected at 15-day intervals over a period of two months and examined for the existence of fecal oocysts. A statistically significant difference was seen between the T_C group and the groups that received herbal supplementation ($p<0.05$). At the conclusion of the experiment, the T_{MN} group, which received a combination of 1% moringa leaf meal and 1% neem leaf meal, exhibited the lowest average Oocyst per gram count of 83.33. This was followed by the T_N group, which had a mean Oocyst per gram count of 100, and the T_M group, which had a mean OPG count of 116.67. The study determined that the combination of 1% moringa and 1% neem leaf meal exhibited a greater antiparasitic effect on the eggs.

Keywords: Moringa leaf meal, neem leaf meal, Kadaknath layers, coccidiosis, Oocyst per gram

Introduction

The Indian poultry industry has various challenges, with coccidiosis emerging as a prominent issue due to its significant impact on bird mortality and morbidity. It plays a significant role in the decrease of meat and egg output. In the prevention and treatment of this condition, the utilization of anticoccidial drugs plays a pivotal role. The potential implications of the emergence of cross and multi-resistances to anticoccidial medications extend to avian populations, human well-being, and ecological systems. Nevertheless, medicinal plants have recently been increasingly popular as a feasible substitute that has demonstrated the ability to decrease the occurrence of diseases and enhance the production of meat and eggs. In recent times, there has been an increase in the interest in utilizing phytogenic feed additives in poultry to improve the production of the birds (Hashemi and Davoodi, 2011; Khan *et al*., 2012) [6, 8]. Neem and Moringa are highly beneficial herbal plants for promoting health.

Neem, scientifically referred to as *Azadirachta indica, A Juss*., belongs to the Meliaceae family. The neem tree, known for its versatility and usefulness in tropical regions, is highly regarded as a 'village dispensary' in India (K.Girish *et al*., 2008) [7]. The significance of the neem tree was acknowledged by the US National Academy of Sciences in 1992, as evidenced by the publication of a paper entitled "Neem- a tree for solving global problems" (Biswas K *et al*., 2008) [3]. According to Mitra *et al*. (2000) [9], the primary bioactive constituents found in neem leaves include nimbin, nimbinene, 6-desacetylnimbiene, nimbandiol, nimbolide, and quercetin. The majority of plant constituents, such as fruit, seed, leaf, bark, root, and others, contain compounds that possess antiseptic, antibiotic, antiviral, antipyretic, anti-inflammatory, anti-ulcer, antifungal, and hypocholesterolemic properties (Onyimonyi *et al*., 2009) [10].

Oyagbemi *et al*. (2012) [11] reported that neem has been shown to effectively combat internal parasites and coccidiosis. The study also indicated that neem can boost immunity against both clinical and subclinical infections.

Moringa oleifera, a rapidly growing tree belonging to the Moringaceae family, is indigenous to the Indian subcontinent and exhibits resilience in arid environments. Moringa is commonly known by various different names, including horseradish, drumstick, ben oil, and benzoil tree. The antibacterial properties of Moringa are attributed to the presence of several phenolic compounds, such as flavonoids, saponins, tannins, and other compounds (Bukar *et al*., 2010) [4]. Prior studies have provided evidence that various parts of the plant, including its leaves, seeds, roots, barks, and flowers, possess distinct bioactive chemicals that possess both nutritional and therapeutic properties (Fahey *et al*., 2005) [5]. According to Fahey (2005) [5] and Wang (2016) [18], the Moringa plant has been employed in the treatment of several parasitic ailments such as malaria, leishmaniasis, trypanosomiasis, schistosomiasis, dracunculiasis, and filariasis. These findings suggest that the plant exhibits inherent antiparasitic properties. Extensive research has demonstrated its utility as poultry feed (Mahfuz and Piao 2019) [14]. Moringa leaves have the potential to be utilized in veterinary medicine for the treatment of avian coccidiosis.

The present investigation aimed to assess the potential efficacy of including moringa and neem leaf meal, either individually or in combination, into the dietary regimen of Kadaknath layers in mitigating the occurrence of coccidiosis.

Materials and Methods

Experimental design and diets

The experiment was carried out at Livestock Farm Complex, C.V.Sc & A.H., A.N.D.U.AT., Kumarganj, Ayodhya. The study was conducted on a sample of 72 Kadaknath laying hens, aged 42 weeks. The hens were randomly assigned to four groups, with each group consisting of 18 layers. Each treatment group was replicated three times. The experiment spanned a period of 60 days, commencing on December 15, 2021, and concluding on February 14, 2022. The study had four treatment groups, wherein all Kadaknath birds in each group were raised under identical environmental and managemental conditions. The birds were housed in a deep litter system and managed in accordance with regular procedures and a measured quantity of feed was given on daily basis.

T_C = basal diet (diet without supplementation).

T_M = basal diet + 2% moringa leaf meal (MLM)

T_N = basal diet + 2% neem leaf meal (NLM)

T_{MN} = basal diet + 1% moringa leaf meal + 1% neem leaf meal (MNLM)

Preparation of moringa and neem leaf meal involved laying out the leaves on a sanitized floor and allowing them to dry in a well-ventilated room for a period of 4-5 days. Subsequently, they were placed in a hot air oven and heated for 1 hour at a temperature of 37°C. The desiccated foliage was pulverized using an electric grinder and subsequently stored in hermetically sealed polythene bags individually. These bags were subsequently employed as an additive in layers feed.

Table 1: Composition of experimental diets (g/100 g) for laying birds

Ingredients Diets				
	T_C	T_M	T_N	T_{MN}
	Basal diet	Moringa	Neem	Moringa+ neem
Maize	64.10	64.10	64.10	64.10
Soyabean meal (44%)	25.57	25.57	25.57	25.57
Salt	0.30	0.30	0.30	0.30
Vegetable oil	1.15	1.15	1.15	1.15
Diacalcium phosphate	1.08	1.08	1.08	1.08
Layer premix*	0.25	0.25	0.25	0.25
Methionine	0.30	0.30	0.30	0.30
Lysine	0.20	0.20	0.20	0.20
Limestone	7.05	7.05	7.05	7.05
Moringa	-	2.00		
Neem	-		2.00	
Moringa+neem	-			2.00
Total Calculated composition	100.00	100.00	100.00	100.00
Crude protein (%)	16.05	16.05	16.05	16.05
Crude fiber (%)	3.90	3.90	3.90	3.90
Metabolisable energy	2619	2619	2619	2619
Lysine	1.02	1.02	1.02	1.02
Methionine	0.56	0.56	0.56	0.56
Calcium	3.72	3.72	3.72	3.72
Phosphorus	0.82	0.82	0.82	0.82

Vitamins 12,000,000, vit. E 30,000 mg, vit. K 2,500 mg, folic acid 1,000 mg, niacin 40,000, folic acid 1,000 mg, vit B 5000 mg, vit B 12 20 mg, vit B6 3,500 mg, biotin 80mg and antioxidant 125,000 mg, cobalt 250 mg, Selenium 250 mg, iodine 1,200 mg, iron 40,000 mg, manganese 70,000 mg, copper 8,000 mg, zinc 60,000 mg, chloride 200,000 mg

Fecal sample collection and Examination

Fecal parasite counts were determined at the commencement of the experiment and subsequently at 15-day intervals. In each replication, a total of two grams of excrement were collected from three birds that were randomly selected. The fecal examination was conducted in the following manner:

Fecal oocyst count (FOC)

To ensure the preservation of the feces samples, they were subjected to a cooling process prior to examination. The FOC was determined using the modified Mac Master Technique. Initially, a quantity of two grams of feces was gathered and combined with 30 ml of a salt solution that was fully saturated. Upon completion of the mixing process, the solution was carefully transferred into one half of a Mac Master slide using a pipette. For an approximation of the total number of eggs in the sample, we multiplied the sum of the counts from each side of the chamber by 50. The data was presented in terms of oocyst per gram (OPG).

Statistical Analysis

An Analysis of Variance was conducted on all of the experimental data using Snedecor and Cochran (2004). The researchers utilized Duncan's New Multiple Range Test to assess whether there was a statistically significant variation in means.

Results and Discussion

The financial impact of this parasite disease on the global chicken industry is significant, mostly due to production losses and the expenses incurred for treatment and prevention (Allen and Fetterer, 2002; Shirley *et al*., 2005) [1, 15]. The expenses are incurred due to the need for medication for the hens, as well as losses resulting from mortality, sickness, and poor growth in the survivors. Regrettably, this phenomenon has resulted in an increase in the cost of chicken and other poultry commodities. Additionally, the customer may find the presence of medication or antibiotic residue in the poultry product to be unfavorable. The plants Moringa and neem exhibit anti-parasitic properties. Table No.2 displays the effects of MLM, NLM, and MNLM on internal parasites (OPG) in Kadaknath layers at 15-day intervals. On the first day, there was no significant difference observed between any of the groups and OPG values in T_C, T_N, T_{MN} is 516.67 whereas T_M has 483.33. On the 15th day, the average OPG values in T_M (466.67), T_N (433.33), and T_{MN} (400) exhibit a statistically significant difference compared to T_C (533.33). However, these values also demonstrate similarities among themselves. Notably, T_M exhibits a considerable resemblance with T_C, T_N, and T_{MN}. However, after a period of 30-60 days, all the groups that received herbal supplements showed a significant difference (p<0.05) from the control group. However, there was no significant difference observed among them. The findings presented in the table indicate a significant positive impact of dietary supplementation with MLM, NLM, and MNLM on the diets of the T_M, T_N, and T_{MN} groups during the 60-day experimental period. Notably, all the herbal supplemented groups exhibited significant differences compared to the control group (T_C), with the T_{MN} group demonstrating the lowest value of OPG (83.33).

Table 2: OPG count of the fecal sample of Kadaknath layers fed diet supplemented with MLM, NLM and MNLM.

DAYS	T_C group	T_M group	T_N group	T_{MN} group	P- value
Pre treatment	516.67±16.67	483.33±16.67	516.67±16.67	516.67±16.67	0.441
15th day	533.33±16.67^b	466.67±16.67^{ab}	433.33±33.33^a	400±28.87^a	0.028
30th day	500±28.87^b	233.33±16.67^a	183.33±16.67^a	183.33±16.67^a	<0.001
45th day	483.33±16.67^b	167.67±44.09^a	133.33±33.33^a	133.33±33.33^a	<0.001
60th day	500±28.87^b	116.67±16.67^a	100±0.00^a	83.33±16.67^a	<0.001

Means with same superscripts do not differ significantly

In a study conducted by Shola David Ola *et al*. (2013) [16], it was shown that the acetone leaf extract of Moringa exhibited noteworthy anticoccidial properties in broiler chickens. Similarly, Salles *et al*. (2014) [13] demonstrated that the crude aqueous extract of moringa seeds effectively suppressed the hatching of eggs by over 90% when tested against H. contortus. The findings of our study support the results reported by Panday *et al*. (2018) [12], who employed a 2% neem leaf meal to combat coccodia in commercial broiler hens. Their study revealed a significant decrease in OPG levels. In their study, Amin *et al*. (2010) [2] demonstrated that a 10% water extract of neem exhibited a moderate level of effectiveness against gastrointestinal nematodes in sheep. Conversely, betel leaf, devil's tree, jute, and turmeric were found to be comparatively less effective. Similarly, Sujon *et al*. (2008) [17] observed a statistically significant (p<0.05) effect of an ethanol extract of neem on goats, when compared to other plant extracts such as labanga, karolla, and pineapple, when administered at a concentration of 50 mg/ml.

Conclusion

The findings of this study indicate that the inclusion of MLM, NLM, and MNLM in the diet of Kadaknath layers resulted in a considerable reduction in OPG values. Notably, the combination of 1% moringa leaf meal and 1% neem leaf meal (MNLM) yielded the lowest OPG value.

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