EFFECT OF MEDICINAL PLANTS (NEEM AND MORINGA) ON THE EGG LAYING PERFORMANCE AND MICROBIAL LOADS IN JAPANESE QUAIL

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*This is to certify that the thesis entitled, "**EFFECT OF MEDICINAL PLANTS (NEEM AND MORINGA) ON THE EGG LAYING PERFORMANCE AND MICROBIAL LOADS IN JAPANESE QUAIL**" Submitted to the Department of Poultry Science, Faculty of Animal Science and Veterinary Medicine, Sher-E-Bangla Agricultural University, Dhaka, in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE (MS) in Poultry Science embodies the result of a piece of bona-fide research work carried out by Md. Moniruzzaman Mim, Registration No. 19-10087 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed of during the course of this investigation has been duly acknowledged by him.

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TO

BELOVED PARENTS

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ABBREVIATIONS AND ACRONYMS

MLE	Moringa Leaf Extract
NLE	Neem Leaf Extract
SEM	Standard Error of the Means
NS	Non-significance
BCSRI	Bangladesh Council of Scientific Research Institute
cm	Centimeter
CV %	Percent Coefficient of Variation
DMRT	Duncan's Multiple Range Test
et al.,	And others
e.g.	exempli gratia (L), for example
etc.	Etcetera
g	Gram (s)
i.e.	id est (that is)
Kg	Kilogram (s)
LSD	Least Significant Difference
m^2	Meter squares
ml	MiliLitre
M.S.	Master of Science
No.	Number
SAU	Sher-e-Bangla Agricultural University
var.	Variety
^oC	Degree Celsius
%	Percentage
NaOH	Sodium hydroxide
GM	Geometric mean
mg	Miligram
P	Phosphorus
K	Potassium
Ca	Calcium
L	Litre
Mg	Microgram
Kcal	Kilo calories
ANOVA	Analysis of Variance
CD8	Cluster of Differentiation 8
CFU	Colony Forming Unit
Vit	Vitamin
FCR	Feed Conversion Ratio
PBS	Phosphate Buffer Solution
ISA	Institute of Selection Animals
CNS	Central Nervous System

ABSTRACT

The study was carried out to determine the comparative efficacy of Neem (*Azadirachta indica*) and Moringa (*Moringa oleifera*) leaf powder on the productive performance, health status and microbial population of faeces and egg of commercial layer Japanese quail. A total of 160 Japanese quails of 27 day-old were reared in Sher-e-Bangla Agricultural University Farm, Dhaka, Bangladesh. Quails were divided randomly into 5 experimental groups each contained 4 replications and each replication contained 8 quails. These groups were allotted to five treatment designated as T_1, T_2, T_3, T_4 and T_5 Groups. T_1 was offered basal feed without any supplementation and served as a control. Whereas, group T_2, T_3, T_4 and T_5 were offered basal feed supplemented with Antibiotic, Neem + moringa – 1.2%, Neem + moringa – 1.0% and Neem + moringa – 0.75% respectively. T_1 (control) showed the highest live weight (182.38 ± 5.16g) at 15th week of age. But the results showed that the weekly egg production by number and weight and % HDEP were maximum in T_5 (Neem + moringa – 0.75%) at the end of 15th week of age (6.78 ± 3.01, 11.58 ± 0.07^{a} g/egg and 91.58 ± 2.92, respectively) compared control as well as other treatments. But highest feed consumption was found in T_2 (Antibiotic) which was (241.95 ± 5.92^{a} g/bird) weekly. Again, T_5 (Neem + moringa – 0.75%) showed the best FCR (2.52 ± 0.03^{d} at 15th week of age) whereas T_4 (Neem + moringa – 1.0%) gave highest FCR (3.925^{a}±0.388) at 10th week of age which was not acceptable. Similarly, T_4 (Neem + moringa – 1.0%) showed highest survivability (100%) whereas the least (94.00^{b}±3.50) was from T_2 (Antibiotic). T_5 (Neem + moringa – 0.75%) showed the maximum number of egg (6.78 ± 3.01 per week) along with the maximum egg weight (11.58 ± 0.07^{a} g/egg) at the 15th week of age. So it could be said that neem and moringa leaf extract at 0.75% with water can improve the production performance. T_3 (Neem + moringa – 1.2%) showed the lowest (4.15 ± 0.41^{c}) *Salmonella* sp. population on faecal sample whereas treatment T_5 (0.25% neem + 0.25% moringa– 0.75%) showed the highest (8.22 ± 0.32^{ab}) *Salmonella* sp. population contains. In conclusion, it can be said that Neem + moringa – 0.75% can positively affect the production performance of Japanese laying quail but not significantly affect the microbial population in faeces.

Keyword: *Salmonella* sp, neem, moringa, egg production, antibiotic, gut health.

CHAPTER I

INTRODUCTION

The Japanese quail, previously known as old world quail, is also named as coturnix quail as its' scientific name is *Coturnix japonica*. This bird had been domesticated in Japan in early 11th century. But this species is also commonly found in various part of East Asia including India, Korea and China, and Russia. This species was used as research animal since about 1940s. But after 1957, researchers were being more interested in the Japanese quail to use as research animal. So in this study, the Japanese quail species had been chosen. However, in recent days, farmers have commonly used antibiotics to prevent diseases and to increase meat production and egg yield. There are different types of herbal or medicinal plants around us such as moringa, neem, nyshinda, garlic, broccoli, fennel, thyme, caraway etc. It was found that *Moringa oleifera* leaves have potential prebiotic effects and potentially it has antioxidant phytochemicals named chlorogenic, caffeic acid. *Moringa oleifera* leaf meal which is available in many tropical countries, that is also a good source of antioxidant compounds which are commonly known as ascorbic acid, flavonoids, phenolic and carotenoids (Caicedo-Lopez, *et al*., 2019). The plant is also very well-known as Drumstick tree and Horse-reddish tree. Using of antibiotics continuously in feeds results in drug resistance in poultry, remaining drug in the chicken's body and also causes loss of the natural balance of intestinal microflora (Awad, *et al*, 2009). It was also found that the herbal extracts causes a significant shortage in the intestinal bacterial population of laying Japanese quails. In this case thyme extract has the most decreasing potential on the intestinal bacterial population (Behnamifar, *et al*, 2015). Al-kirshi, *et al*., (2010) reported that better result in the term of egg production will be achieved with up to 10% of mulberry leaf meal in diets of egg laying hens without adversely affecting the performance and egg quality. Many researchers have used seeds of different medicinal plants with dietary feed supplement for increasing production performances for both meat and egg yield. Al-Ashoor *et al.*, (2020) reported a significant increase not only in the egg production but also found better results in the egg quality, fertility and hatchability. Besides Moringa, many others have worked to observe the effect of neem leaf too. It was investigated that there is a great effect of neem and nishyinda leaves together on the weekly live weight gain, feed consumption and feed conversion ratio

(FCR) (Haque, 2018). Neem has a bitter taste which can be increased the appetite of birds (Service, n.d.). On the other hand moringa has flavonoids, phenolic acid, vitamin A, vitamin B and vitamin C which play an important role in increasing gut health. Extracts from the leaves are used to treat malnutrition (Gopalakrishnam *et al*., 2016). Both neem and moringa have antimicrobial properties, and moringa has anti-proliferative properties also. Moringa leaves have anthelmintic properties which improve the gut health (Abbas, 2013). That's why this study had been conducted to try to find the effect (if any) of moringa and neem leaf extract, feeding together in the same ratio with water to balance the diet level, on the egg production, feed consumption efficiency and microbial population in the faeces and internal egg of Japanese laying quails.

Objectives:

1. To evaluate the effect of the moringa and neem leaf extract mixture on the egg production performance of Japanese quail.
2. To find out the result of moringa and neem leaf extract on the microbial load in egg and faeces of Japanese quail.

CHAPTER II

REVIEW OF LITERATURE

There are many works with different herbal plants, leaves or extract of them which have been conducted by many researchers all over the world. It was found in different journals like European Journal of Animal Science, Iranian Journal of Veterinary Medicine, Nigerian Journal of Animal Science, American Journal of Veterinary and Animal Sciences, International Journal of Science and Business, Journals from MDPI, Al-Qadisiyah Journal of Veterinary Medicine Sciences and many more. But it was hardly found publications on the effect of Moringa and Neem together on layer quail with a satisfactory result. That's why it was decided to conduct a research work about the effect of moringa and neem leaf extract on Salmonella growth in faeces and egg of layer quail. Here are some background of effects of different herbal treatments on layer quails as well as chickens which had been conducted earlier.

2.1 Review on Japanese quail

2.1.1 The Japanese quail: a review

Vali, (2008) reviewed a study obout Japanese quail which belongs to the order Galiformes, family Phasidae, genus Coturnix and species japonica. He reported several aspects account for the utility of this bird such as, firstly the bird has acquired economic importance by producing meat and eggs which are very delicious and tasty. In Japan & Southeast Asia, egg production is very important, whilw meat is the main product in Europe. Secondly, there is low maintenance cost, small sized body (80-300g) coupled with its short generation interval (3-4 generation yearly), resistance to to diseases & high egg production, rendered it as an excellent laboratory animal. Thirdly, it was reported that this bird is the smallest avian species reared for meat and egg production. Therefore, it has been used as an experimental bird in many sudies. It was also reported that Japanese quail is bred for egg and meat production. (Vali, 2008)

2.1.2 As a laboratory animal model

Huss, *et al.*, (2008) reported that it was 50 years past, the Japanese quail (*Coturnix japonica*) has been a popular animal model in numerous fields of research. He also mentioned that quail has 16-d developmental period and easily accessible embryo which make *Coturnix japonica* a convenient model for studies of developmental biological science. Because its lifespan is relatively short other than other poultry species and its physiology is comparable to that of humans. It was also reported that the adult quail is useful for studies of aging and disease (Huss, *et al.*, (2008). It was reported by Baer, *et al.*, (2015) that *Coturnix japonica* is used as a laboratory animal model for multiple areas of scientific inquiry such as developmental biology, endocrinology, immunology, behaviour studies and a variety of human genetic disorders. The author reported that various significant experimental advantages for the study of amniotes, such as modest size of breeding adults, rapid reproductive maturation, resilience to research manipulations, ease of breeding in laboratory animal facilities, availability of transgenic lines, tools for molecular manipulations and a fully sequenced genome. (Baer, *et al.*, 2015)

2.2 Nutritional and medicinal queries about neem and moringa

2.2.1 Pharmaceutical importance of neem (*Azadirachta indica*)

Neem, rich source of antioxidant, has a valuable role as health promoting action. It can be said that the herb has been used as different types of medicines like Chinese, Ayurvedic, Unani all over the world. Especially this herbal plant as well as its' leaves are being used in the Indian subcontinents in different treatment and prevention of different kind of diseases. This study was evaluated the therapeutic implications of neem and its different ingredients in the health management such as antioxidant activity, anti-cancerous activity, anti-inflammatory activity, wound healing effect, hepato-protective effect, anti-diabetic activity, antimicrobial effect, dentistry role of neem, anti-nephrotoxicity effect, neuroprotective effect etc. Neem and its constituents have also a great role on angiogenesis, apoptosis, oncogene etc. (Alzohairy, 2016)

2.2.2 Nutritional value of MLE and NLE

Moringa leaf and neem leaf contain different kinds of chemical such as protein, lipid, ash, fibre, fat, carbohydrates, magnesium, potassium, calcium, iron, phosphorous etc. The chemical compositions are shown in Table 1. Eid, *et al*., (2017) proved that neem leaf (*Azadirachta indica*) contains a high fibre content.

Table 1 Nutritional value of MLE and NLE (Eid, *et al*., 2017; Amabye, 2016; Obikaonu, 2012)

Elements	**Value of MLE**	**Value of NLE**
Lipid (%)	6 ± 2.5	-
Ash (%)	9 ± 7.45	-
Fibre (g)	19.2	19.34
Fat (%)	2.3	9.43
Protein (%)	24 ± 5.8	-
Carbohydrate (%)	17.6	-
Magnesium (mg)	368	127
Potassium (mg)	1324	254
Iron (mg)	28.2	17.09
Calcium (mg)	2003	510
Phosphorous (mg)	204	80
Energy value (Kcal)	36 ± 9.2	416

N.B. Values are elicited per 100 g leaf powder.

2.2.3 Active ingredients of Neem leaf

Azadirachtin is the most vital active ingredient and the others are nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinate, gedunin, salannin, and quercetin. Alzohairy, (2016) reported that leaves contain ingredients such as nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol and amino acid, 7-desacetyl-7-benzoylazadiradione, 7-desacetyl-7-benzoylgedunin, 17-hydroxyazadiradione, and nimbiol. Quercetin and -sitosterol, polyphenolic flavonoids, were purified from neem fresh leaves and were known to have antibacterial and antifungal properties and seeds hold valuable constituents including gedunin and azadirachtin.

2.2.4 Active ingredients of Moringa leaf

Flavonoids and phenolic acid are polyphenolic compounds majorly present in the dried leaves, roots, flower stem, pods, the seed of Moringa oleifera. Flavonoids are the secondary metabolites and are the most common phyto-constituents present in plants (Jain *et al*., 2020).

Jain *et al*., (2020) reported that *Moringa oleifera* leaf extract contains vitamin**s** such as vitamin A, vitamin B, and vitamin C at the level of 80 μg, 2.324 mg and 8.6 mg, respectively.

Jain *et al*., (2020) also mensioned that *Moringa oleifera* contains two potent glucosinolates namely α-4 rhamno-pyranosyloxy-benzyl glucosinolate and acetyl-α-4-rhamno-pyranosyloxy-benzyl glucosinolate which also possess potent bioactivity against cancerous cells. An uncommon glucosinolate named glucomoringin is also possessed in *Moringa oleifera* leaf extract which is a potent anti-proliferative and antimicrobial agent.

2.2.5 Pharmaceutical importance of Moringa

Moringa oleifera is one of the most valuable and important plant which is broadly ploughed in India. It is extensively used all over the world as nutritional herb. The plant also hold pearl pharmacological operation such as antioxidant, anti-ulcer, anti-asthmatic, hepatoprotective, anti-diabetic, anti-cancer, anti-fertility, CNS activity, anti-allergic effect, anti-inflammatory, analgesic, wound healing, cardiovascular activity and anti-pyretic activity. It was reported Paikra, *et al*., (2017) that every part of the plant contains a valuable medicinal function. It was also reported that the plant holds rich source of various vitamins like vitamin A, vitamin C and milk protein. It was also found that many different active phyto-constituents are present in Moringa plant like protein, saponins, tannin, steroids, alkaloids, oils, fat etc. (Paikra, *et al*., 2017)

2.3 Herbal effect of Quail production

2.3.1 Effect of thyme, garlic and caraway

This study were conducted to find a congenial and harmless feed additive to increase the quality and quantity of poultry egg production. The study was acted by Behnamifar,*et al.*, (2015) in the poultry farming unit of the Department of Poultry Science, Faculty of Agriculture, Tarbiat Modares University. They used a total of sixtyfour (64), 10-week old laying Japanese quails to conduct this study (male to female ratio of 1:3). Herbal substances were added to drinking water treatments in the ratio like 1:1000 and used a control group containing no additives in their water. They measured number and weight of produced eggs daily, feed consumption on weekly basis and the egg quality, yolk cholesterol, hatchability and intestinal bacterial population at the end of experiment after 8 weeks. Finally, there was no overall effect of herbal extract on quality and quantity of egg production in comparison with control; however, the thyme and garlic extracts abated the cholesterol of serum and yolk comparative to the control. The hatchability of fertile eggs was not affected significantly by the herbal treatment. The intestinal bacterial population of laying quails was decreased significantly by the herbal extracts used in this study. (Behnamifar,*et al.*, 2015)

Table 2 Effects of herbal extracts on the performance of Japanese quail including feed conversion and egg weight (g). (Behnamifar,*et al.*, 2015)

Treatment	**FCR (feed intake/kg mass)**	**Hen day egg production (%)**
Control	4.31	68.81
Thyme	4.83	67.51
Caraway	4.00	66.10
Garlic	4.53	69.25
P value	NS	NS
SEM	0.143	1.061

NS=Non-significance

2.3.2 Effect of Adding Broccoli Leaves *(Brassica oleracea)*

This experimental study was carried out to determine the effects of using broccoli leaves extract to drinking water on egg production and intestinal microorganism (total bacteria, lactobacilli and *Escherichia coli*) of breeder Japanese quails. A total 132 birds were used to conduct this experiment. To determine the effect of broccoli leaf, the broccoli leaves extract as 100, 200 and 300 mg. l-.1 were added to bird's drinking water of the second, third and fourth groups respectively. Fresh caecal and duodenum samples from euthanized (three females of treatment) were diluted 10-fold by weight in buffered peptone water after 45 days from study begins. The results showed a momentous advancement in eggs production (HD %), accumulative eggs per 30 days and eggs mass in the third and fourth groups compared to the other groups. Caecal and duodenum *E. coli* exhibited a great decrease in third and fourth groups compared to the others. On the basis of the presented results can be terminated that alcoholic extract of broccoli leaves at levels 200 and 300 mg. l-1 could enhance productive characteristics (eggs production HD %, accumulative eggs per 30 days and eggs mass). On the contrary, duodenum lactobacilli was increased and caecal and duodenal *E. coli* population was decreased of breeder Japanese quails. (Al-Ashoor *et al*., 2020)

2.3.3 Quails Response to Aqueous Extract of Bush Marigold Leaf

The outcomes of insertion of aqueous extract of Bush marigold (*Aspilia africana*) leaf in quail diet were investigated. 150 Japanese quail chicks were used in the experimental study. The birds were assigned to five treatments of; 0, 2.5, 5.0, 7.5 and 10% insertion of aqueous extract of Bush marigold leaf in this 14-weeks feeding experiment. It was indicated in the final result that feeding Aqueous extract of Bush Marigold Leaf (AeBML) did not affect daily weight gain, FCR and carcass yield. Mortality rate and percentage cracked eggs were decreased. Dietary supplementations with AeBML affected on the feed consumption and dressed weight of growing quails momentously. Egg number and hen day production developed as level of AeBML raised, though egg weight reduced. Feeding AeBML developed shell thickness, yolk colour and albumen weight when compared to the control group. (Agiang, 2011)

2.3.4 Effects of natural ingredients contained in the plant material

This study aimed to examine of antimicrobial features for aqueous extracts of cinnamon (C), oregano (O), ginger (GR) and garlic (GC) as safe and alternative resources for traditional disinfectant of hatching eggs The materials consisted of 2400 hatching eggs (10.5 g) gained from Japanese quails layers at 14 weeks of age. 5% aqueous extracts of GR, GC, O, and C were used for disinfection in treatment groups according to the treatment group. They collected egg samples for microbial screening on 14th day of experiment. They placed 12 eggs per each group in sterile boxes containing 50 ml of phosphate-buffered saline (PBS) with 3 drops of TWEEN 80. They diluted the sample serially in PBS solution and plated on sterile medium apropos to obtain the total number of yeast, bacteria and fungi. They counted colonies and presented as CFU/1ml of liquid from the egg after incubation. They performed a microscopic examination as well as Gram’s staining method and API biochemical tests to identify the bacterial colonies. All disinfectant groups and NC did not momentously differ in eggshell conductance constant (*K*). Disinfectant solution of C led to reduction of weight loss of fertile eggs (%) in weight loss of fertile eggs. (Al-Shammari, 2017)

Table 3 Microbial counts with aqueous solutions of plant extracts (Al-Shammari, 2017)

Bacterial spp	NC	PC	GR	GC	O	C	SEM
Salmonella ***spp.***	7.60	8.30	23.5	0.00	0.00	0.00	0.000

NC – negative control, PC – positive control, GR – ginger, GC – garlic, O – oregano, C – cinnamon

2.3.5 Efficacy of *Moringa oleifera* (Lam) Leaf meal on Egg production

In a study, 90 12-week old Japanese quail hens were involved in a completely design (CRD) to 3 treatments with 3 replications consisted of 10 birds each which were fed ad libitum for 42 days. In the diets, the researchers used a ration formulation which contained 19.8% crude protein (CP) and 2856.2 Kcal/kg metabolic energy (ME) and (diet-1) T_1, (diet-2) T_2 and (diet-3) T_3 contained 0%, 1% and 2% *Moringa oleifera* leaf meal (MOLM), respectively. They used a register to note daily feed consumption and egg production. After investigating the weekly feed consumption, it was revealed that the hens fed diet-2 under T_2 (1% leaf meal) (23.75g/day) significantly less compared with those of birds fed diet-1 under T_1 (control, without leaf meal) ($p<0.05$) (25.37g/day). And it was similar ($p < 0.05$) to the feed consumption of birds fed diet-3 under T_3 (2% leaf meal) (22.78g/day) in compared with T_1. The hen-day production did not significantly vary ($p<0.05$) among the treatments. The feed conversion ratio (FCR) followed similar propensity as the feed consumption and hen-day production. (Abu & Akangbe, 2017)

Table 4 Performance characteristics of layer quails fed graded levels of moringa leaf (Abu & Akangbe, 2017)

Parameters	**T_1**	**T_2**	**T_3**	**SEM**
Feed Intake/bird/week (g)	25.37	23.75	22.78	0.44
Egg Weight/bird/day (g)	9.67	9.79	9.38	0.10
No of eggs/bird/week	5.02	4.96	5.07	0.17
Hen day production (%)	23.88	23.77	24.26	0.79
Feed Conversion Ratio (g)	3.67	3.45	3.37	0.09

2.3.6 Robusta Lampung coffee extract feeding evaluation

Now-a-days many treatments consider herbs which are apprehended to reduce the negative effects of antibiotics and are environmentally amicable, one of which is coffee. Green coffee is apprehended to have many active components such as tannin, alkaloids, chlorogenic acid (CGA), polysaccharides and polyphenols which are useful as anti-inflammatory, antimicrobial and ameliorates immune system of the body. This study was involved 60 heads ISA (Institute of Selection Animals) Brown day-old chicks (layer strain) chickens manually inoculated with *Salmonella enteritidis* bacteria with concentration of 10^8 CFU/ml. The researchers supplied with coffee extracts to the chickens on the 3rd Day to 15th Day of experiment, then the chickens were infected with 0.50 ml bacteria on the 16th Day and they necropsied the 60 chickens at the 18th Day. They calculated the data on relative levels of CD8 T cells using the flow cytometer test and analysed quantitatively using the One Way ANOVA (Analysis Of Variance) with a confidence level of 95% while they observed jejunum histopathology using a microscopy and analysed explicatively. From the results, it showed that Robusta coffee extract from Lampung can act as an antibacterial by enhancing CD8 + T cells and repairing jejunum histopathology. (Qosimah, 2019)

2.3.7 Results of adding Marjoram plant (*Origanum vulgarae*) on feed

This experiment was conducted in the field quail to study the effect of different levels of dietary Marjoram (*Origanum vulgarae*) and (*Rosmarinus officinalisL*) on some productive Japanese quail. In this study, the researchers used 450 chicks to conduct the entire experiment. They used 10 treatments (T_1 to T_{10}) in this study and designed each treatment with 45 birds. They found a significant ($p \leq 0.05$) gain in the final body weight and significant increase of cumulative weight also. They also found a momentous ($p \leq 0.05$) improvement in the cumulative feed conversion ratio compared with control treatment, while no significant ($p \leq 0.05$) differences were found in the amount of feed consumption. (Mossa *et al*., 2019)

2.3.8 Neem and Nishyinda Leaves

The aim of this work was to investigate the effects of Nishyinda (*Vitex nigundo*) and Neem (*Azadirachta indica*) leaves on growth performance and hematologic parameter in quail. A total of 40 14th day old Japanese quail, randomly assigned into 4 groups, were used to conduct this study. They designed the groups like group T_0 as control (normal feed), group T_1 (2% Neem with diet), T_2 (2% Nishyinda with diet) and T_3 (2%

mixture of 1%Neem and 1% Nishyinda with diet). At the end of the study, they found a significant increase in the body weight in the treatment group compared to the initial body weight. They obtained highest body weight in T_3 followed by T_0 which differ significantly ($P<0.01$) from each other. During 2 to 6 weeks of age, they found a momentous differ in the FCR for the T_3 and T_1 from the control group which improved significantly which were 4.17±0.09 and 4.38±0.05 respectively. They also concluded that there were great enhance of live body weight with 2% of combined (1%) Neem and (1%) Nishyinda leaves powder supplementation with the diet in treatment groups and also weekly weight gain, feed consumption and FCR were increased as compared to that of control group of quail. (Haque, 2018)

2.3.9 Moringa leaf extract effect in different parameter

The study presented by the researchers of Poultry Department of Zagazig University, Egypt, aimed to investigate the dietary effect of *Moringa oleifera (M. oleifera)* seed and leaves powder on laying Japanese quails in different parameters such as egg production, egg quality and reproduction. They used a total 168 Japanese quails (male: female= 2:5) of eight weeks of age which were in laying period. Birds were distributed to four treatment groups, with six replication in each group. In each replication, they distributed seven birds (male: female= 2:5). They maintained the first group as control group and served feed with *M. oleifera* leaves meal (MLM) (1 g/kg MLM) in the second group, feed with *M. oleifera* seed meal (MSM) (1 g/kg MSM) in the third group and the forth group was served the combination of MLM and MSM (1 MLM g/kg + 1 MS g/kg (MSL). Lastly, they did not find any change affected by the dietary treatments in relation with feed consumption, feed conversion ratio. However, egg production was greatly enhanced. They also found in all treatments with ML, MS, and MSL that the triglycerides and the total cholesterol were decreased ($p < 0.05$), with no significant differences in alanine aminotransferase (ALT), albumin, total protein, globulin, and A/G ratio among dietary treatment. Their results clearly indicated that the inclusion of *Moringa oleifera* seeds in Japanese quail diet momentously enhanced egg production, along with some egg quality parameters. (Ashour, *et al*., 2020)

2.3.10 Potentiality of medicinal plants and algae

This study was conducted with 675 Japanese quails to investigate the effect of *Rhus coriaria*, *Gracilaria pulvinata*, *Punica granatum* and *Sargassam cristafolium* dried powder with dietary feed on their some egg quality characteristics and productive performance. The birds used in this study were 7 weeks of age. The researchers randomly distributed the birds into 9 groups contained 3 replicates of 25 birds in each. The found that the treatments did not affect egg weight, albumen weight, shell weight and shell thickness. They also found that the effects effect of medicinal plants on weekly egg production vary depending on the dietary medicinal plant type and dosage. The researchers found that the birds feeding diet with supplemented *Sargassum cristaefolium* had more weekly egg production comparison to others. It was investigated that the diet supplemented with *S. cristaefolium* increased yolk weight. In addition, greater albumen protein and TBA (Thiobarbituric acid) component of the tenth day's eggs were discerned in birds fed diet supplemented with *G. pulvinata*. In this study, it was found that the cholesterol was the lowest in level in the egg yolk of *R. coriaria* and *G. pulvinata* groups and in the control group, cholesterol level was in the most amounts. Based on the observed data, mixture of 0.5% and 2% of *R. coriaria*, *S. cristafolium*, *P. granatum* and *G. pulvinata* peel to the diet directed to be increased in egg production rate and enhanced the egg quality in Japanese quail. (Habibi *et al,* 2019)

2.3.11 Efficacy of *Mentha spicata* on diet

In this experiment, three hundreds 7-day old Japanese quail chicks were used to investigate the effect of dietary *Mentha spicata* on growth performance, carcass attributes, meat quality and antimicrobial antivity. Here, *M. spicata* was used at the rate of 0%, 1%, 2%, 3% and 4% of diet. While considering the experiment, the FCR was not significantly attacked by the experimental treatment. Any momentous effect on growth performance and carcass attributes was not found during experiment. In statistical analysis, FCR was not also affected by the treatment. But beginning of the experiment, due to high dose of the treatment, loss of body weight and less feed consumption were observed. *M. spicata* reduced *E. coli* in the gut of quail in which the treatment was 3% of diet, while the Lactobacilli population had been risen. Finally, it was found that, the lower level of *M. spicata* in diet could be improved the meat quality and higher level of *M. spicata* could be increased the microbial ecosystem of small intestine of quail. (Ghazaghi, *et al*, 2014)

2.3.12 Morical extract effect on Japanese quail

To conduct this study, the researchers used Morical with dietary feed at the level of 2%, 4% and 8% of the diet. They maintained deep litter system and standard ration with adlib water. Birds fed with 4% Morical with dietary feed showed highest egg production compare to that of other treatment. The FCR was also showed highest result compared to that of other group including control group. But best egg weight and best total yolk weight was found in the group C which contained 8% Morical powder. The researchers reached in a conclusion that Morical powder increased the rate of egg production and quality of egg in Japanese quail. (Sunder, *et al.*, 2013)

2.3.13 Assessment of antimicrobial effect of Moringa

A group of researchers of an African institute investigated the assessment of antimicrobial effect of Moringa. They used disc diffusion method to investigate the study. They used ethanolic extract of leaves, seeds and roots of Moringa peregrine. They tested against three bacterial species such as *Escherichia coli (E. coli), Klebsiella pneumoniae* and *Staphylococcus aureus*. They found that the Moringa extracts showed significant antimicrobial effect. It was noticed that the inhibition areas were 14 to 30, 8 to 19 and 9 to 22 mm in diameter against *E. coli, S. aureus* and *K. pneumonia* respectively. At the end of the study they found that the best potential result showed against microbial components was for the roots of Moringa. (El-Moez, *et al*., 2014)

2.4 Effect of essential oil on Quail production

2.4.1 Results of using Essential Oils and *Salmonella* Infection analysis

The experimental study was allocated challenging Lohmann White laying hens with *Salmonella enteritidis* (SE). In this study, the researchers fed the birds a diet supplemented with an essential oils-based phytobiotic named Intebio®. At 1st and 7th day post-inoculation, they examined the effects of eight genes related to immunity, transport of nutrients in the intestine, and metabolism. It was also revealed about the caecal microbiome composition and blood biochemical/immunological indices and egg production traits were recorded as data. As a result, *Salmonella enteritidis* (SE) challenge of laying hens and Intebio® application had either a concealing or activating effect on the manifestation level of the investigated genes (e.g., *IL6* and *BPIFB3*), the current resounding mammalian tissue-specific manifestation. They also found momentous differences between control and experimental subgroups in egg production traits (i.e., egg weight/number/mass). The phytobiotic application which was used in this experimental study, suggested a bona fide effect on the welfare and productivity of poultry. (Laptev, *et al*., 2021)

2.4.2 Blending essential oil components on Salmonella growth

Researchers from a university investigated that the outcome of a specific blend of essential oil (EO) and this blended EO mixed with sodium-butyrate on growth performance and *Salmonella* colonization in chickens. They used 480 one-day old male chicks and distributed in 5 treatments (8 pens per treatment, 12 birds per pen) and brought up. They used dietary treatments with different doses of EO (0 mg/kg; 50 mg/kg and 100 mg/kg) and a mixture of EO with 1 g/kg of sodium-butyrate. They inoculated orally with 10^8 CFU of *Salmonella enteritidis* on 7th day. They measured individual body weight and feed intake per pen at arrival and on a weekly basis. They apprehended *Salmonella* prevalence and enumeration in faeces per treatment at 72 hours post-infection and on 23rd and 37th day. They cultured caecal content and liver samples from 16 birds per treatment for *Salmonella*. They did not notice any difference on growth performance among treatments. They observed that all faecal samples were positive for *Salmonella*. At slaughter, they found lower *Salmonella* contamination (positive samples) in cecum in birds fed blended essential oil with sodium-butyrate compared with the other treatments ($P < 0.05$). But they noticed higher colonization rates on the bird's caecal sample from control unit. Thus, finally they came in a conclusion that essential oil or its combination with sodium-butyrate did not affect growth performance. (Cerisuelo, *et al.*, 2014)

Table 5 Salmonella prevalence (% positive faecal cultures) and Salmonella counts (log10 CFU/g of faeces) in samples of faeces. (Cerisuelo, *et al.*, 2014)

Parameters	Dietary treatment				
	Control	EO50	EO100	EOB50	EOB100
Prevalence (% positive)					
10th Day (72 h post-infection	100	100	100	100	100
23th Day	100	100	100	100	100
37th Day	100	100	100	100	100

Parameters	Control	EO50	EO100	EOB50	EOB100
Bacterial count (log_{10} CFU/g of faeces)					
10th Day (72 h post-infection	3.66	4.12	4.47	4.71	4.33
23th Day	4.11	3.82	3.69	4.22	4.10
37th Day	5.77	6.86	5.00	4.59	6.03

Birds were supplied a feed supplemented with or without essential oil [EO; 0 mg/kg (control), 50 mg/kg (EO50), and 100 mg/kg (EO100)] or a combination of EO with 1 g/kg of sodium-butyrate (B; EO50 + B, EOB50 and EO100 + B, EOB100)

2.4.3 Herbal essential oil mixture as a dietary supplement

To conduct the study Çabuk, *et al*., (2014) used 180 7-week-old laying quail. Birds were fed various diets more than 12-week period. The diet, without essential oil mixture (EOM) or antibiotics (ANTs), used as control; diet including EOM (24 mg/kg feed), and a basal diet including an ANT (Avilamycin, 10 mg/kg feed) were supplied. Metin (2014) provided diets (in mash form) and water for consumption. The essential oil mixture was made with 6 different essential oils derived from the following herbs: myrtle leaf (*Myrtus communis*), sage leaf (*Salvia triloba* L.), laurel leaf (*Laurus nobilis* L.), oregano (*Origanum* sp.), fennel seeds (*Foeniculum vulgare*), and citrus peel (*Citrus* sp.). In the final result the researchers observed that the egg production was increased in the EOM and ANTs diet supplied group in comparison with the control diet. However, they also found that in the EOM and ANT groups, egg production was similar. Again, they also noticed no differences between the treatment groups with regard to egg weight. FCR was significantly improved by EOM and ANT supplementation. Thus, Çabuk, *et al*., (2014) concluded that essential oil mixture has beneficial effects as a dietary supplement on egg production and FCR.

Table 6 Effect of the dietary inclusion of essential oil mixture or an antibiotic on egg production (Çabuk, *et al*., 2014)

Parameter	**Control**	**EOM**	**Antibiotic**
Egg production, %	86.52	89.44	88.34
Egg weight, g	11.80	11.69	11.77
Feed intake, g/quail	29.24	29.14	29.13
FCR	2.88	2.81	2.81

2.5 Effect of organic acid on quail production

2.5.1 Organic Acids on the Performance of Japanese Quails

In this study Khan, *et al*., (2016) were reared 300 day-old quail. They offered organic acid to Japanese quails at the level of 0.5, 1, 1.5 and 2ml per litre of drinking water. They used Aciflex® as organic acid. Significantly it was found that low ($P<0.05$) feed and water intake in 2ml group compared to the control group, but highest weight gain, best FCR, dressing percentage, liver weight and lowest mortality were noticed in 2ml compared to the control. Significantly in the control group, higher feed cost was obtained, while it was the same in all other groups. The researchers observed that the gross return was not affected by organic acids supplementation. It was also noticed that total return was higher in 1.5 and 2ml groups significantly but the least found in the control group. Finally at the end of the study the researchers (Khan, *et al*., (2016) recommended to use organic acid with water supplementation because organic acid supplementation in drinking water at the rate of 2ml/L of drinking water enhanced performance and economic return in Japanese quails.

2.5.2 Effect of combination of probiotic-prebiotic, organic acid & antibiotic

This study, conducted by Çakir, *et al*., (2008), aimed was to compare the effect of different combination of probiotic-prebiotic, organic acid and antibiotics. The prepared a combination of probiotic, prebiotic, phytogenic substances and cell wall fragments (Biomin® IMBO), combination of formic and propionic acid based on an inorganic phyllo-silicate carrier (Biotronic) and a combination of the Biomin® IMBO and Biotronic as feed additives and compared among them with an antibiotic (Avilamycin) and control diet on the growth performance, several serum parameters and digestive villus height of quails. The researchers used a total of 300 1-day-old Japanese quail chicks. The experimental design consisted of 5 dietary treatments such as;

1) Control diet without supplementation
2) Diet with Biomin®IMBO at a level of 0.1 kg/100 kg feed;
3) Diet with Biotronic at a level of 0.4 kg/100 kg feed;
4) Diet with a Biomin®IMBO and Biotronic combination at levels given above;
5) Diet with avilamycin at a level of 10 mg/kg feed.

Birds consuming diet supplemented with dietary treatment 2, 3 & 4 had a significantly ($P<0.05$) higher duodenal villus height. It was observed that the single use of

Biomin®IMBO and Biotronic resulted significantly higher jejunum (P<0.05) villus height too. There was no significant differences in serum parameters and no beneficial effect of dietary supplements on growth performance, feed intake, feed efficiency and absolute and proportional organ weights could be observed among groups according to dietary supplements. Çakir, *et al.*, (2008) recorded that the beneficial effects of such supplements could be more evident in other circumstances, like stress.

2.5.3 Effect of combination of organic acid and essential oil

Matty & Hassan, (2020) conducted the current study to investigate the effects of dietary supplements of essential oil (Gallant$^{+®}$**)** & organic acid on the growth hormone, glutathione, growth performance, some biochemical parameters and intestinal histomorphology in Japanese quails. They used 120 day-old Japanese quails which were distributed randomly into four groups. They marked G1 as control group that was fed on a basal diet and they supplied feed to quails of G2, G3, and G4 with a supplemented diet Gallant$^{+®}$ 300, 600 and 900g/ton, respectively. The researchers observed that supplementation with the combination of Gallant$^{+®}$ 600 g/ton and 900g/ton to quail led to significant decrease in triglyceride while supplementation with 600 g/ton caused decrease in cholesterol. On the other hand, it was noticed that an increase in final body weight and total weight through the duration of the experiment by feeding 300 g/ton, as well as a decrease in total feed consumption. They also observed that the best FCR in all supplementation with 300 g/ton caused a significant growth hormone elevation. The researchers reported that all feed additives didn't affect the level of glutathione. Interestingly, it was reported by Matty & Hassan, (2020) that the addition of different doses of Gallant$^{+®}$ to the diet enhanced villus length and width, crypt depth, villus / crypt ratio, percentage of goblet cell, apparent surface area, and intestinal epithelium thickness compared to the control group. It was concluded by Matty & Hassan, (2020) that growth hormone, growth performance and intestinal histo-morphology in Japanese quails could be improved by dietary supplementation with different doses of Gallant$^{+®}$, and might use as dietary supplementation with organic acid and essential oil as alternatives to the growth promoter of antibiotics.

2.6 Effect of seed meal on quail production

2.6.1 Feedback of using fennel seed supplement in diet

For conducting this experiment 16 weeks aged 96 Japanese quails were used by the researcher to investigate the performance of the dietary supplement, egg quality. The scientific name of the used fennel seed is *Foeniculum vulgare*. The researcher used the treatment group with the supplement at the level of 0.3%, 0.6% and 0.9% of diet. They did not find any great effect of fennel seed on growth performance, egg production, body weight, feed intake and egg weight. But they found that 0.6% of the fennel seed supplement with the diet deteriorated the FCR. They also did not find any great effect of fennel seed on egg quality, yolk index, shell quality, Hough unit. However, it can be said that egg quality may not be affected by the dietary fennel supplement on laying quail. There is no any harmful effect of dietary fennel supplement on egg quality and egg production performance of laying quail. (Buğdayc, *et al*., 2018)

2.7 Oriental herbal medicine feed additive (OHMFA) effect on chickens

In this experimental study, the researchers investigated that the supplemented feed (with 1% OHMFA) effect on the prevention of *Salmonella* spp. colonization and the death occurrence in broiler chickens. They found that *Salmonella*'s frequency in faeces samples experimented with OHMFA (25/239; 10.5%) was momentously decreased ($p<0.05$) than found in control group (83/347; 23.9%). They isolated 108 *Salmonella* spp. belonging to 4 different serotypes from 3 broiler farms. Among the serotypes isolated, it was noticed that *Salmonella typhimurium* was the most prevalent (60.2%) among others like *Salmonella enteritidis* (20.4%), *Salmonella pullorum* (6.4%) and *Salmonella gallinarum* (13.0%). During conducting the experiment, the incidence of chickens death, it was found that the mortality rate of OHMFA group (3.9%) was momentously fewer ($p<0.01$) than the control group (5.7%). From these result, the researchers finally reached in an opinion that the Oriental Herbal Medicine Feed Additives (OHMFA) application might impede Salmonella colonization in the chickens. (Kang, *et al*., 2003)

2.8 Benefits of herbal supplementation

Feed additives contains nutrient and non-nutrient components that assists in developing the feed utilization proficiency and thus decreasing the higher feed cost. Now a days, in poultry production, feed producers are using herbal feed additives other than antibiotics because of banning on the use of certain antibiotics due to their harmful residual effects. Herbs are referred to as a flowering plant in which their stem remains above ground and that's why they does not become woody and referred to as a plant when valued for its medical properties, scent, flavour etc. (Vinus, *et al.*, 2018)

2.9 Research gap

There are many work conducted by the researchers all over the world on different kinds of medicinal plants such as moringa, neem, oleivera, nishinda, thyme, garlic, caraway, broccoli as well as different herbal oils, organic acid and so on. There are lack of research in Bangladesh of the effects of medicinal plants (moringa and neem) on layer Japanese quail in the contrast of the production performance as well as microbial effects on their gut health. Also there are lake of empirical evidence and consensus of this type of research work to recommend such a treatment that could help our farmers to produce antibiotic free meat and egg which is very healthful for human being. So this study was performed to try to find out that kinds of quiescent resources.

CHAPTER III

MATERIALS AND METHODS

3.1 Statement of the experiment

The study was conducted to evaluate the effect herbal treatment on the egg production and microbial growth on faeces and internal-egg. A total of 27 day-old aged 160 commercial layer Japanese quails were used for this study in cage rearing system under cold-dry environment for the period of 10 weeks to assess the effect of herbal extract on the growth performance and egg quality of layer quail using herbal (0.25% neem + 0.25% moringa) extract with water and also the herbal effect on Salmonella of faeces and egg.

3.2 Preparation of the experimental house

The house had been cleaned, washed and disinfected 7 days before bringing the quails. Ceiling, walls and floor were thoroughly cleaned and disinfected. The experimental house was fully covered with curtains due to cold weather. The cage of quail was also disinfected at the same time. PPM solution had been used as disinfectant.

Figure 1 Disinfecting quail cage

3.3 Collection of experimental quails

A total of 160 commercial laying quail were collected from renowned quail farm which is located at Mouchak, Kaliyakoir, Gazipur. After a total 3.5 hours journey, the quails were brought at our Poultry Science research farm of Sher-e-Bangla Agricultural University, Dhaka.

Figure 2 Quail farm

Figure 3 Collected quails had been loaded on pick-up

3.4 Experimental materials

The collected laying Japanese quails were carried to the university poultry farm in the afternoon. After giving 15-20 minutes rest, the birds were distributed randomly in five (5) dietary treatment groups having 32 laying quail in each group and 4 replications of each treatment group having 8 quails in each replication.

Figure 4 Distributed into the cage according to treatment and replication

3.4.1 Experimental treatments

Five treatments including control as follows:

1. T_1 = Control (Normal diet and normal drinking water)
2. T_2 = Antibiotic (Ciproflox) with water
3. T_3 = 0.25% neem + 0.25% moringa – 1.2 % extract
4. T_4 = 0.25% neem + 0.25% moringa – 1.0 % extract
5. T_5 = 0.25% neem + 0.25% moringa – 0.75 % extract

3.4.2 Layout of the experiment

Treatments with Replication (8 birds/replication)				**No. of birds**
T_1R_1 (n=8)	T_1R_2 (n=8)	T_1R_3 (n=8)	T_1R_4 (n=8)	32
T_2R_1 (n=8)	T_2R_2 (n=8)	T_2R_3 (n=8)	T_2R_4 (n=8)	32
T_3R_1 (n=8)	T_3R_2 (n=8)	T_3R_3 (n=8)	T_3R_4 (n=8)	32
T_4R_1 (n=8)	T_4R_2 (n=8)	T_4R_3 (n=8)	T_4R_4 (n=8)	32
T_5R_1 (n=8)	T_5R_2 (n=8)	T_5R_3 (n=8)	T_5R_4 (n=8)	32
Total				**160**

Figure 5 Distributed according to the replication

3.5 Experimental diets

3.5.1 Collection of neem + moringa leaf

Neem leaf (*Azadirachta indica*) and Moringa leaf (*Moringa oleifera*) were collected from trees planted in the university's agriculture garden.

3.5.2 Preparation of Neem Leaf Powder and Moringa Leaf Powder

First of all sun-dry of the leaves was done. Then oven-dry was carried out at 80^{o} C at over-night. Then the dried leaves were grinded with the blending machine. Again oven-dry was done to remove moisture. And finally the grinded powder of neem leaf and moringa leaf was stored in two different air-tight container for further use.

Figure 6 Blending of dried leaves

Figure 7 Moringa leaf powder

Figure 8 Neem leaf powder

3.5.3 Preparation of extract (NLE + MLE)

5g powder (0.25% neem + 0.25% moringa) was added in 1L distilled water and mixed properly with the help of a mixture spoon. Then the solution was heated in oven at 80°C for 2-2.5 hours. A golden-yellow coloured solution was obtained after removing from the oven after 2 hours. At last the solution was filtered and stored in a bottle.

Figure 9 Measuring Neem leaf powder

Figure 10 Measuring Moringa leaf powder

Figure 11 Mixing with water

Figure 12 Putting into oven

Figure 13 After removing from oven

Figure 14 Pouring into flask

Figure 15 Filtered the extract

Figure 16 Stored for further use

3.6 Management procedures

3.6.1 Room temperature and relative humidity

Daily room temperature and humidity was recorded every day with a digital thermo-hygrometer. Average room temperature was 20-24 ^{0}C and relative humidity was 55-60% during the experimental period.

Figure 17 Thermo-hygrometer

3.6.2 Feeding management

Everyday 25g feed per bird was supplied up to 6th week (2nd week of rearing) of age. Then 30g per bird was supplied up to 11th week (7th week of rearing) of age and 10 litter water was supplied to the quals. Average feed consumption was recorded regularly. Broiler grower and layer-layer feed was supplied at the ratio of 2:1. 30% protein concentrate (Protam) was also supplied. All ingredients were mixed properly.

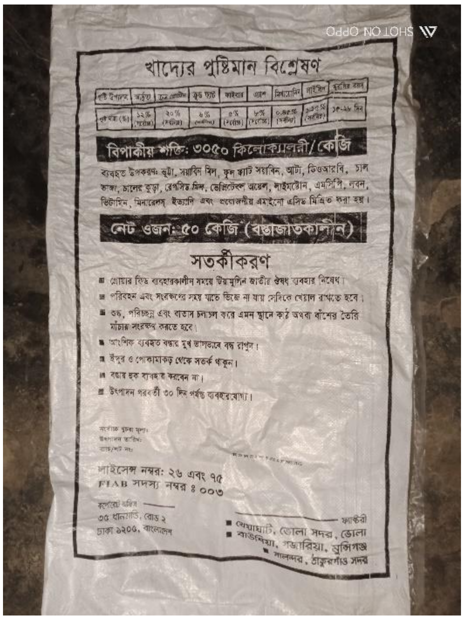

Figure 18 Broiler grower feed

Figure 19 Protein concentrate

Figure 20 Mixing the feed ingredients

Table 7 Ration for quail

Items	**Starter (0-6th week age)**	**Layer (>6th week age)**
Protein (%)	24.0	18.0
Calcium (%)	0.85	2.75
Phosphorus (%)	0.6	0.65
Methionine (%)	0.5	0.45
Vitamin premix (%)	0.25	0.25
Mineral premix (%)	0.10	0.10
Energy (Kcal/kg)	2750	2600

3.6.3 Watering management

Everyday 15 litres water was supplied to the birds. Each treatment was supplied with 3 litres water. Antibiotic and supplementary treatment was supplied with water at a particular dose.

Figure 21 Water supply

Figure 22 Antibiotic supply

Figure 23 Treatment extract measurement

3.6.4 Lighting

14-16 hours lighting per day was maintained. It is a vital part for raising layer quails.

3.6.5 Bio-security measures

Bio-security components was properly maintained during the experimental period. Entry of wild birds and animals were prohibited. A footbath was set just after the door of the farm to avoid the risk of pathogen transmission. PPM solution was used in footbath. Proper hygienic and sanitation program was undertaken in the farm and its premises. Strict sanitary measures were taken during the experimental period. PPM solution was used to disinfect the cage of the bird and the house also.

3.6.6 Hygiene

Proper hygienic management was maintained in the entire experiment. Litters were changed two times in a week. Waterers were cleaned in everyday basis before suppling water.

3.6.7 Vaccination

To prevent diseases among the birds, chicks were vaccinated as per standard vaccination schedule (Table 8).

Table 8 Vaccination schedule

Vaccine	**Age of bird (weeks)**	**Route of administration**
Newcastle (ND)	5-8	Through eye drop
Infectious Bronchitis (IB)	5-8	Through drinking water
Newcastle (ND)	10-12	Through eye drop
Newcastle (ND)	14-16	Through drinking water or Intramuscular
Infectious Bronchitis (IB)	14-16	Through drinking water or Intramuscular
Avian Encephalomyelitis	6-15	Through drinking water or Wing web

Figure 24 ND vaccine

Figure 25 Vaccination

3.6.8 Medication

Mineral supplement was added with water. Calplex® at the rate of 2.5ml per litre of water was used in this case to prevent calcium and vitamin D deficiency. Coxicure, at the dose of 2g per litre water, was used to prevent coccidiosis among the birds. The name of the antibiotic which was used during the experiment was Ciproflox (100ml). The dose of the antibiotic was 1ml in 1L drinking water.

Figure 26 Anti-coccidial drug

Figure 27 Calcium

Figure 28 Antibiotic

3.6.9 Ventilation

The experimental shed was south facing and open-sided. Due to wire net cross ventilation, it was easy to remove polluted gases from the farm.

3.7 Study parameters

3.7.1 Recorded parameters

Data was recorded on weekly live weight, weekly feed consumption, daily feed consumption and death of quail chicks to calculate mortality percentage. FCR was calculated from total egg production and total feed consumption per bird in each replication. Egg production and total egg weight were recorded daily.

3.8 Data collection

3.8.1 Live weight

The initial body weight and weekly body weight of each replication was kept to get final body weight gain per quail.

Figure 29 Live Weight measurement

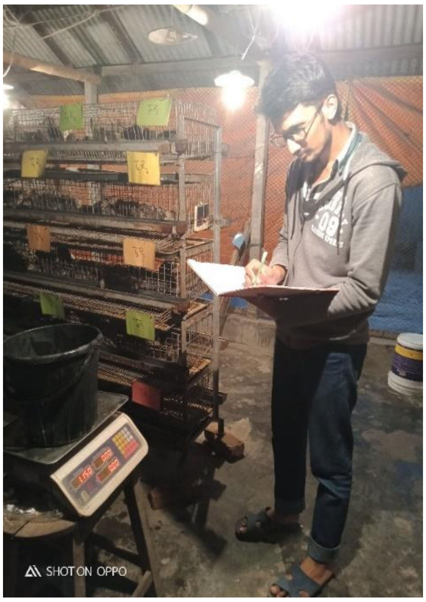

Figure 30 Data collection

3.8.2 Feed consumption

Daily feed consumption of each replication was kept to get weekly FCR and total feed consumption per bird.

3.8.3 Mortality of quail

Death record for each replication was counted to calculate the survivability and mortality.

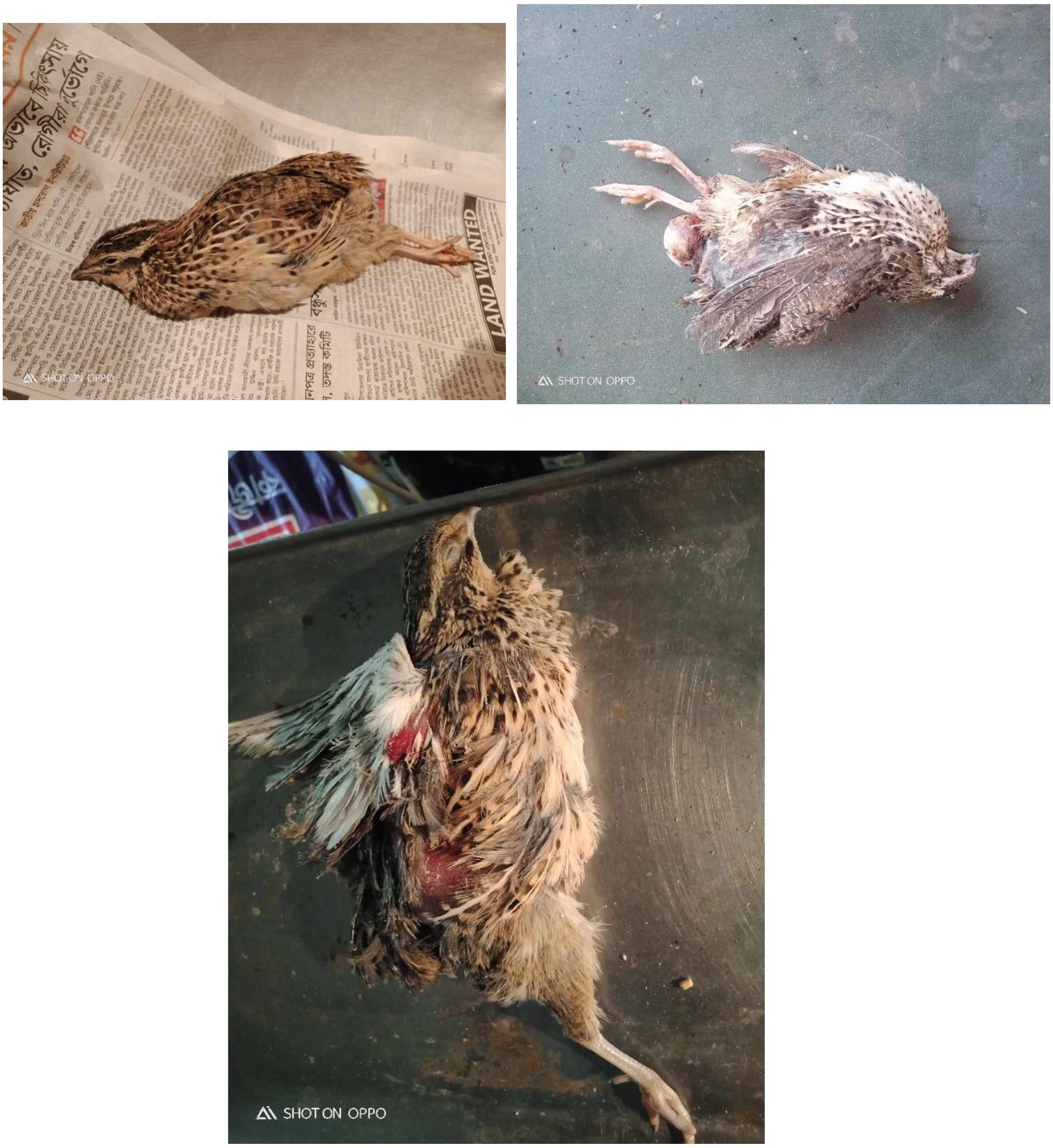

Figure 31 Some dead quails

3.9 Calculations

3.9.1 Live weight gain

The average body weight gain of each replication was calculated by deducting initial body weight from the final body weight of the birds.

Body weight gain = Final weight – Initial weight

3.9.2 Feed intake

Feed intake was calculated as the total feed consumption in a replication divided by number of birds in each replication.

$$\text{Feed intake (g/bird)} = \frac{\text{Total feed consumption in each replication}}{\text{Number of birds in each replication}}$$

3.9.3 Feed conversion ratio

Feed conversion ratio (FCR) was calculated as the total feed consumption divided by the weight of egg produced per week in each replication.

$$\text{FCR (Egg mass)} = \frac{\text{Feed intake per week(kg)}}{\text{Egg weight per week (kg)}}$$

$$\text{FCR (feed efficiency)} = \frac{\text{Feed intake per week(kg)}}{\text{Live weight gain per week (kg)}}$$

3.10 Uniformity

Initial uniformity weight of the birds was approximately 98.06%.

3.10.1 Flock Uniformity

r1= Average $_+$ (Average × 10) ÷ 100

r2= Average $_-$ (Average × 10) ÷ 100

So, flock uniformity = $\frac{\text{Flock number within r1 and r2}}{\text{Total flock number}} \times 100\%$

3.10.2 Egg uniformity

r1= Average $_+$ (Average × 10) ÷ 100

r2= Average $_-$ (Average × 10) ÷ 100

So, egg uniformity = $\frac{\text{Number of egg within r1 and r2}}{\text{Total number of egg}} \times 100\%$

3.11 Egg production

Daily egg production was recorded. Everyday eggs were collected in the evening & stored in the refrigerator until being sold. Birds matured sexually at 6^{th} week of age. At the age of 7^{th} week of age, birds were in production with full length.

Figure 32 Egg collection and measuring weight

Figure 33 Egg storage

3.12 Media preparation

First of all, the instrument were autoclaved at 121ºC for 15 minutes. SS (Salmonella-Shigella) agar was used for media preparation. According to the manufacturer the media was prepared and then poured to the petri dishes and waited for being frozen. Then the petri dishes were stored in the incubator at 37ºC over-night removing moisture.

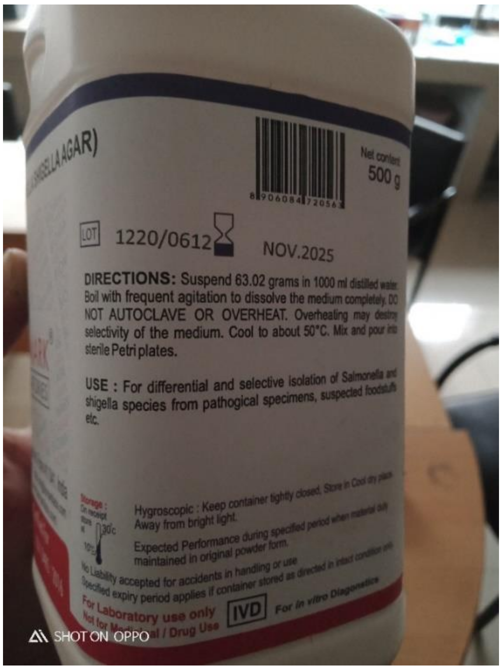

Figure 34 SS Agar

Figure 35 Agar mixing with hot Electric stirrer

Figure 36 Preparing media

3.13 Sample preparation

3.13.1 Sample collection

Sample was collected early in the morning just before doing dilution for media. Both faeces and egg sample were collected from every replication. Faeces sample was collected in the Eppendorf tube from each replication with separate twigs. After collecting sample, replication names e.g., T_1R_1, T_4R_3, T_2R_3, T_5R_2 & so on were written on the outside of each Eppendorf tube.

Figure 37 Sample collection in Eppendorf tube

3.13.2 Dilution:

The procedure for a ten-fold dilution of a sample to a dilution factor of 10^{-2} is as follows. I had used 10^{-1} for both faeces and egg.

1. The collected sample was taken in a test tube. Eight test tubes containing 9 ml of Phosphate Buffer Saline (PBS) solution in each, were taken.
2. A sterile pipette was used to measure sample and diluent.
3. Measured with the pipette, 1 ml of properly mixed sample was drawn.
4. Then the sample was added to the 1st tube to make the total volume of 10 ml (9ml diluent + 1ml sample). This provides an initial dilution of 10^{-1}.
5. The dilution was mixed completely by emptying and filling the pipette several times.
6. The pipette tip was ejected and a new pipette tip was fixed to the pipette.

Figure 38 Preparing dilation for bacterial growth

7. Again, 1ml of mixture was drawn from the 10^{-1} dilution and was poured into the 2nd tube. Then the 2nd tube had a total dilution factor of 10^{-2}.
8. After that, the repetition of same process was done for the remaining tubes, taking 1 ml from the former tube and affixing it to the next 9 ml diluents.
9. As two tubes were used, the final dilution for the bacteria would be 10^{-2} (1 in 100).

3.13.3 Incubation

After poring diluted sample in the media, it was incubated at 37ºC over-night for bacterial growth. The incubation period for salmonellosis is approximately 12–72 hours, but it can be longer. Salmonella gastroenteritis is characterized by the sudden onset of diarrhoea (sometimes blood-tinged), abdominal cramps, fever, and occasional nausea and vomiting. Illness usually lasts 4–7 days. (Adesiyun, *et al.*, 2014)

3.13.4 Colony counting

In the next day the incubated petri dishes were observed for colony count. Colonies were counted and recorded.

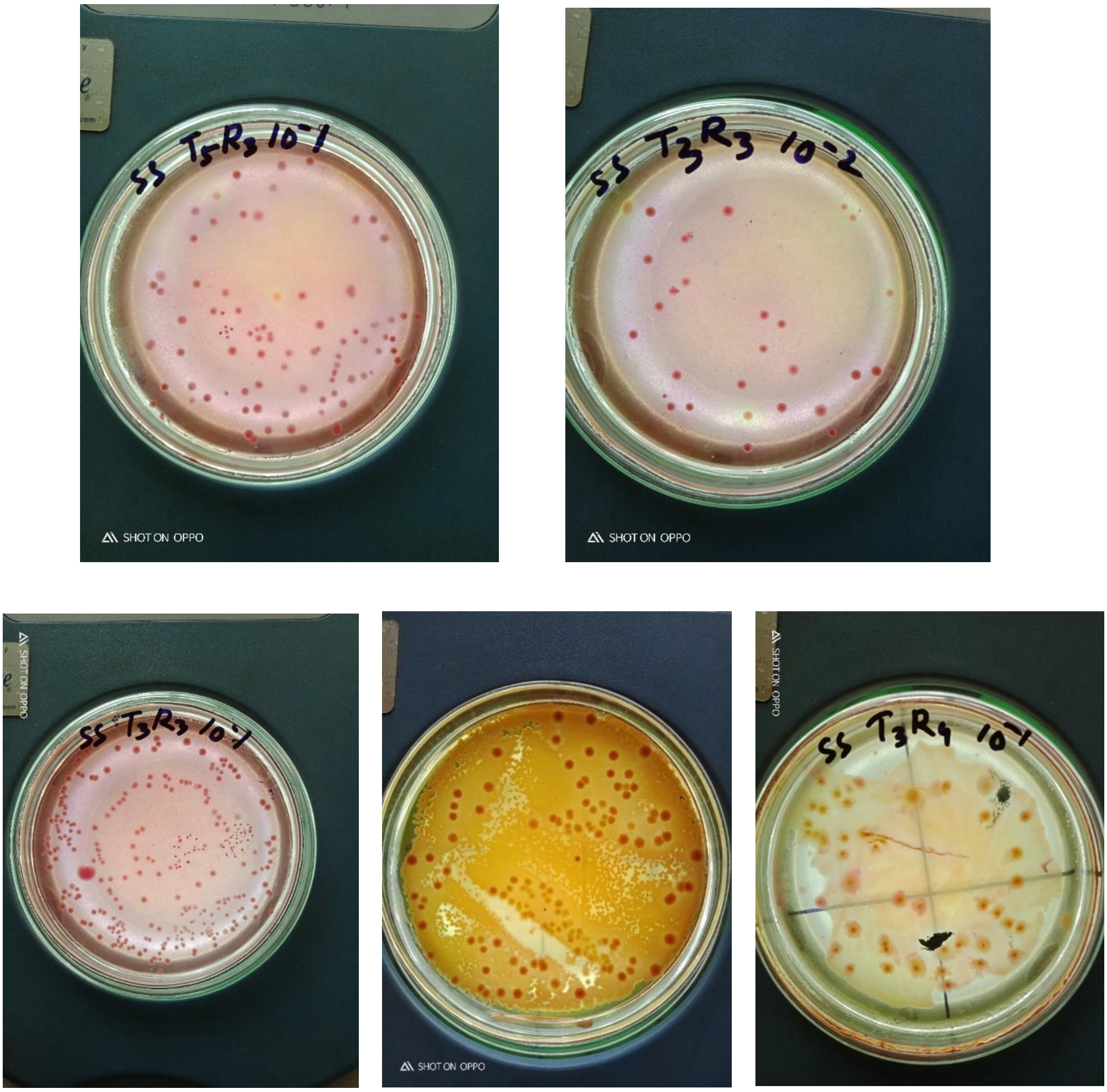

Figure 39 Microbial growth (Salmonella)

3.14 Statistical Analysis

By using SPSS software, all the statistical analyses of the study were calculated. All data were evaluated with a one-way analysis of variance (with the diet as the fixed factor) using the post-hoc Newman-Keuls test, and ($p<0.05$) was considered to be statistically significant.

CHAPTER IV

RESULT AND DISCUSSION

Calculation of different parameters were done to assess the successfulness of quail production which compare results from different treatment groups. The performance of layer quails is measured through different factors was as below:

- The level of feed consumption
- The achievement of body weight
- Feed Conversion Ratio
- Survivability rate
- Egg production and quality
- Measurement and assessment of these factors reflect the maintenance and production performance of quails.

4.1 Feed consumption

The results of the present study showed that the feed intake of quails (n=1) was not significantly affected by the level of feed supplementation with neem and moringa leaf extract. However, the weekly feed intake of quails as a function of the level of feed supplementation with neem and moringa leaf extract has been irregular (Table 9). At the end of 8^{th} week of age the treatment T_3 (0.25% neem + 0.25% moringa– 1.2%) induced relatively higher intake (193.13±0.06 g) over other treatments followed by T_2 (Antibiotic) (196.75±0.047 kg) whereas the lowest feed consumption (183.44±0.05 g) was observed from the feed supplementation treatment of T_5 (Neem + moringa – 0.75%).

At 9^{th} week, the highest feed consumption (215.13±0.07 g) was recorded from T_3 (0.25% neem + 0.25% moringa– 1.2%) treatment and at 10^{th} and 11^{th} week of age the highest food intake (190.38±0.06 and 198.25±0.05 g, respectively) also recorded from T_3 and T_1 respectively, whereas the lowest feed consumption at 9^{th}, 10^{th} and 11^{th} week of age (199±0.06, 182.33±0.13 and 185.78±0.13 g, respectively) was recorded from T_4 (0.25% neem + 0.25% moringa – 1.0%), T_5 (0.25% neem + 0.25% moringa– 0.75%) and T_5, respectively. The increased feed intake might be due to hunger increasing and digestion stimulating, antibacterial and hepato-protective

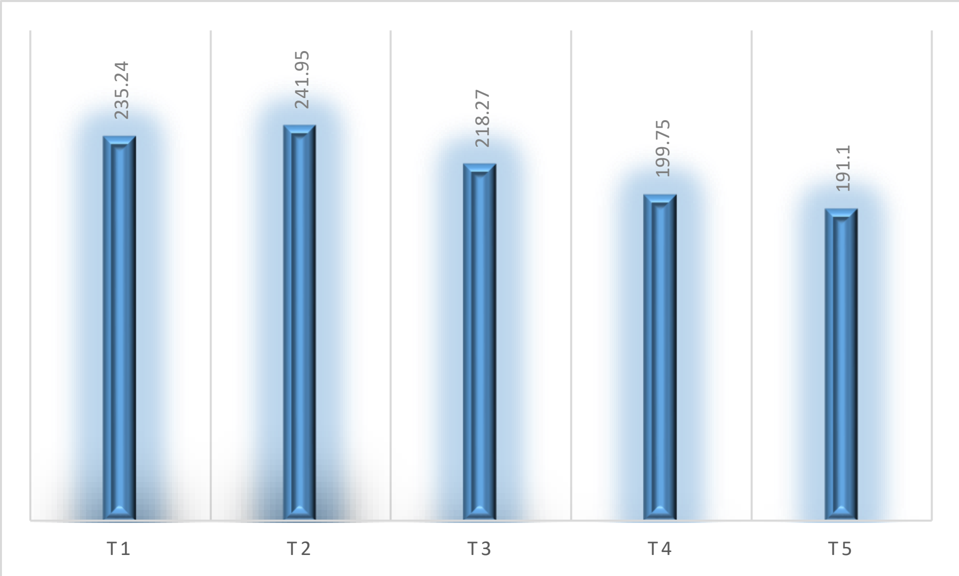

Figure 40 Feed Consumption at Final age

properties of neem and moringa, which might have helped the quails to improve their gut health. At the age of 13th, 14th and 15th week feed consumption was significant ($p<0.05$) (Table 9). It was observed that at 12th, 13th, 14th and 15th week of age, birds of T_2 (Antibiotic) fed the highest (234.59 ± 15.28, 230.28 ± 11.43^a, 236.13 ± 13.46^a and 241.95 ± 5.92^a, respectively). At the age of 14th and 15th week, birds of T_5 fed the lowest amount of feed (191.18 ± 19.16^b and 191.10 ± 16.97^b respectively) compared to other treatment groups. Again, at the age of 12th and 13th week, birds of T_5 fed (203.94 ± 6.44 and 203.78 ± 13.07^b respectively) less than the T_1 (control) fed (215.49 ± 8.75 and 208.05 ± 2.08^{ab} respectively). Similar findings were observed in previous studies (Nnenna & Okey, 2013 and Vasanthakumar, *et al.*, 2013) who observed non-significant feed consumption in the birds fed with Neem and Tulsi supplemented diet. Similar findings were also observed in previous studies of the results of (Khatun, *et al.*, 2013, (Nath, *et al.*, 2012 and Hasan *et al.*, 2016) where higher feed intake was observed in Neem fed birds. Similar findings were found (Behnamifar *et al.*, 2015) thet caraway extract feeding with water supplement fed lowest amount compared with the control group. But from Haque, (2018) somr different findings were observed that birds supplied with 2% neem and nyshinda leaf fed the highest amount of feed at the final week of age compared to the other teatment groups.

Table 9 Effects of different level water supplementation on feed consumption in Japanese quail at different week of age (n=1)

Treatments	Feed consumption of quails (g) (n=1)							
	8th week (Age)	9th week (age)	10th week (age)	11th week (age)	12th week(age)	13th week(age)	14th week(age)	15th week(age)
T_1	190.87±0.07	212.75±0.05	189.38±0.05	198.25±0.05	215.49 ± 8.75	208.05 ± 2.08^{ab}	229.22 ± 8.59^{a}	235.24 ± 4.24^{a}
T_2	196.75±0.05	214.63±0.05	189.88±0.06	190.38±0.07	234.59 ± 15.28	230.28 ± 11.43^{a}	236.13 ± 13.46^{a}	241.95 ± 5.92^{a}
T_3	193.13±0.06	215.13±0.07	190.38±0.06	189.38±0.03	212.86 ± 18.57	221.17 ± 3.95^{ab}	226.47 ± 4.82^{ab}	218.27 ± 4.46^{ab}
T_4	185.63±0.02	199±0.06	190.25±0.02	198.25±0.03	199.61 ± 10.86	178.73 ± 2.86^{c}	205.17 ± 5.53^{ab}	199.75 ± 9.76^{b}
T_5	183.44±0.05	201±0.09	182.33±0.13	185.78±0.13	203.94 ± 6.44	203.78 ± 13.07^{b}	191.18 ± 19.16^{b}	191.10 ± 16.97^{b}
Mean ± SE	194.5^{NS}±0.05	213.75^{NS}±0.06	193^{NS} ±0.07	197^{NS} ±0.07	213.30 ^{NS}± 5.78	208.40* ± 5.16	217.64* ± 6.02	217.26* ± 5.89

T_1 = Control, T_2 = Antibiotic, T_3 = 0.25% neem + 0.25% moringa– 1.2%, T_4 = 0.25% neem + 0.25% moringa– 1.0%, T_5 = 0.25% neem + 0.25% moringa– 0.75%, NS= Non-significance. (*)= Significance, $^{a, b}$: Means in the same row with different letters show significant differences (P<0.05)

4.2 Live weight

The overall mean live weight of different groups showed that there was significant difference (P<0.05) among different treatments of supplementation group at different weeks of age (Table 10). At the end of 8th week of age, body weight of T_5 (0.25% neem + 0.25% moringa– 0.75%) group (n=1) showed the highest result (176.87^a±5.60 g) followed by T_2 (Antibiotic) (170.63^b±1.80 g) whereas T_1 (control) treatment group showed the lowest body weight (164.75^d±2.60 g) which showed non-significant difference with T_4 (0.25% neem + 0.25% moringa– 1.0%). Similarly, at 9th, 10th and 11th week of age, the maximum body weight (n=1) was recorded from T_5 (0.25% neem + 0.25% moringa– 0.75%) (188.88^a±9.00, 176.87^a±7.70 and 175^a±8.20 g, respectively). On the other hand, at 9th week of age the lowest body weight (173.13^d±1.30 g) was achieved from T_1 (control) while at 10th and 11th week of age, the lowest body weight (162^e±6.20 and 163.25^d±6.30 g, respectively) was recorded from T_2 (Antibiotic). At the 12th, 13th, 14th and 15th week of age some different observations were found. Increase of weight was non-significant at the age of 15th week. In the T5, quail's weight was the lowest (175.79 ± 5.28 g) compared with the other groups. Quails of T2 (Antibiotic) and T3 had increased weight (184.75 ± 0.77 g and 184.56 ± 4.20 g, respectively) more than other groups. The result of the present study was similar with the findings of (Muhammad, *et al*, 2015 and Arshad, *et al*., 2021) who found significant variation on weekly live weight of quail using herb supplementation in diet. In the (Haque, 2018) similar findings were observed that the author found significant (P<0.01) result compared with the other treatment groups.

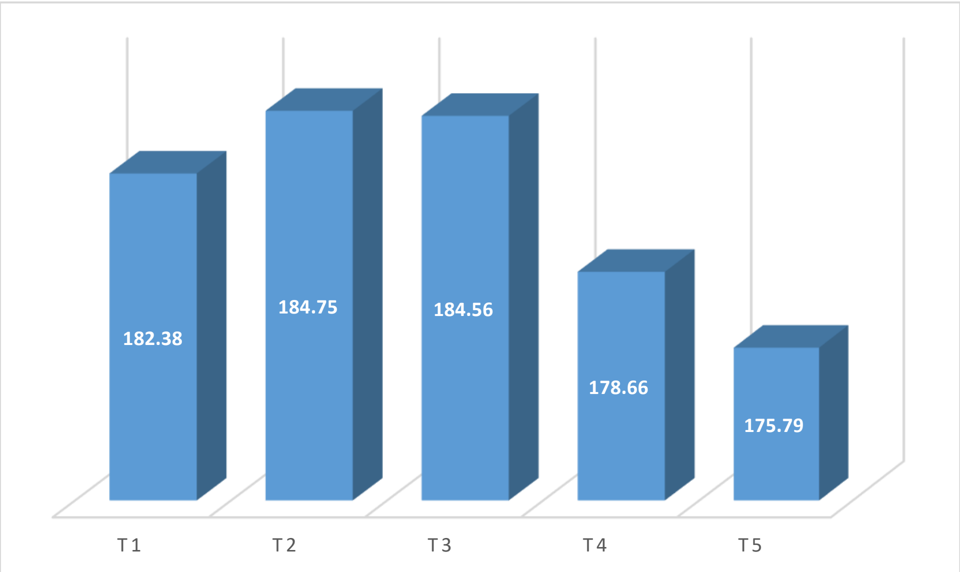

Figure 41 Live weight (gm) at 15th week of age

Table 10 Effects of different level water supplementation on live weight gain at different week of age (n=1)

Treatments	Live weight of quails (g) (n=1)				
	8^{th} week(age)	9^{th} week(age)	10^{th} week(age)	11^{th} week(age)	15^{th} week(age)
T_1	164.75^{d}±2.6	173.13^{d}±1.3	165.75^{d}±1.9	167.13^{c}±1.4	182.38 ± 5.16
T_2	170.63^{b}±1.80	175.88^{c}±5.5	162^{e}±6.2	163.25^{d}±6.3	184.75 ± 0.77
T_3	166.25^{c}±3.00	178.5^{b}±4.5	168.5^{c}±5.2	168.63^{b}±3.7	184.56 ± 4.20
T_4	165.25^{d}±1.90	177.75^{b}±2.8	170.5^{b}±1.9	169.75^{b}±4.2	178.66 ± 5.07
T_5	176.87^{a}±5.60	188.88^{a}±9.0	175.88^{a}±7.7	175^{a}±8.2	175.79 ± 5.28
Mean ± SE	168.75^{*}±3.40	178.75^{*}±5.3	168.75^{*}±4.5	168.63^{*}±5.0	181.23 ± 1.94

T_1 = Control, T_2 = Antibiotic, T_3 = 0.25% neem + 0.25% moringa– 1.2%, T_4 = 0.25% neem + 0.25% moringa– 1.0%, T_5 = 0.25% neem + 0.25% moringa– 0.75%

4.3 Egg production (number)

Egg production by number at 9^{th}, 10^{th} and 11^{th} week of age was significantly influenced ($P<0.05$) due to different supplementation treatments to Japanese quail's diet (Table 11). Results indicated that the treatment T_5 (0.25% neem + 0.25% moringa– 0.75%) showed over all best performance on egg production in number compared to other feed supplementation treatment whereas the treatment T_4 (0.25% neem + 0.25% moringa– 1.0%) showed least performance. At 8^{th}, 9^{th}, 10^{th} and 11^{th} week of age, the highest egg production in number (6.78^{a}±2.869, 5.94^{a}±2.217, 5.03^{a}±3.425 and 6.22^{a}±2.287, respectively) was recorded from T_5 (0.25% neem + 0.25% moringa– 0.75%) whereas the lowest egg production in number (5.78^{c}±3.425, 4.88^{c}±4.378, 4.50^{b}±3.674 and 4.81^{c}±2.748, respectively) was given by the treatment T_4 (0.25% neem + 0.25% moringa– 1.0%). At the age of 10^{th} week, egg production of T_5 (0.25% neem + 0.25% moringa– 0.75%), T_1 (control), T_2 (Antibiotic) and T_3 (0.25% neem + 0.25% moringa– 1.2%) showed significantly similar results among them which was also at

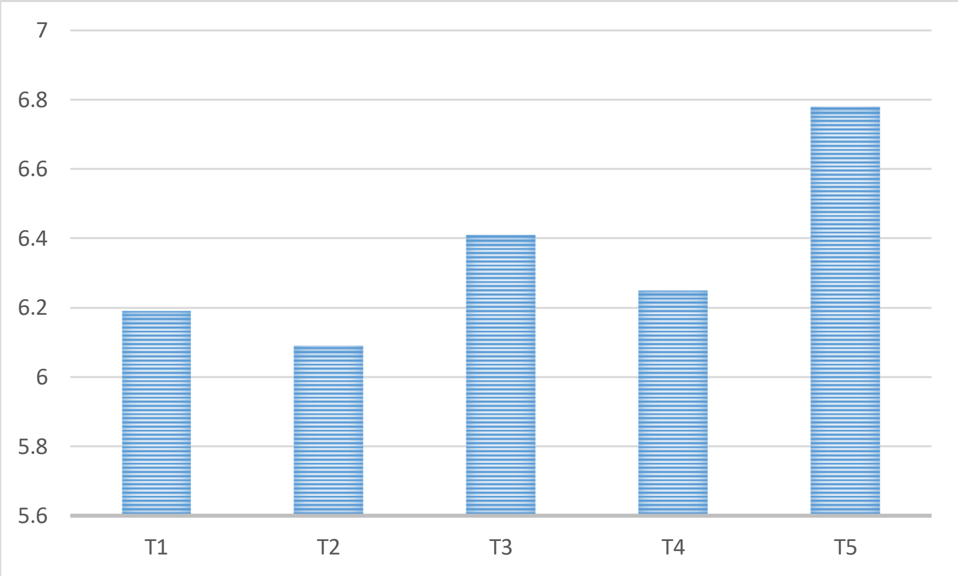

Figure 42 Egg production (number) at 15th week of age

11th week of age between T_5 (0.25% neem + 0.25% moringa– 0.75%) and T_1 (control).

At 12th, 13th, 14th and 15th week of age T5 also showed the highest result (6.19 ± 1.56, 6.72 ± 1.80[a], 6.63±2.12[a] and 6.78 ± 3.01 respectively) compared to that of T1 (control) and other treatment groups. T1, T2, T3 and T4 showed significant result at the 13th week of age. Bur at the 15th week of age all results were non-significant differences. Similar results were found by Ashour, *et al.*, (2020) who supplied *Moringa* leaf meal at 0.1% with diet. Abu & Akangbe, (2017) reported that 2% Moringa leaf meal increased the production performance and he found significant result in his study. These observations corroborate with the results of Tesfay, *et al.*, (2011) who studied the effect of *Moringa oleifera* leaf meal on egg laying rate of hens by exposing them to three experimental diets containing different amounts of *M. oleifera* (0%, 1% and 2%). Their results showed that diets supplemented with 1% induced high egg production in number compared to the others.

Table 11 Egg production (number) of Japanese quail as influenced by different water supplementation treatments in diet (n=1)

Treatments	Egg production (number) (n=1)							
	8th week (Age)	9th week (Age)	10th week (Age)	11th week (Age)	12th week	13th week	14th week	15th week
T_1	6.25^{b}±2.89	5.44^{b}±1.32	4.97^{a}±1.55	5.88^{ab}±0.71	5.63± 2.12	5.56^{b} ± 1.04	5.97^{b} ± 0.75	6.19 ± 1.19
T_2	6.25^{b}±2.27	5.59^{b}±2.18	4.84^{ab}±3.09	5.47^{b}±3.25	5.41 ± 0.95	5.63^{b} ± 2.27	5.66^{b} ± 1.25	6.09 ± 1.25
T_3	6.16^{bc}±2.46	5.47^{b}±1.70	4.78^{ab}±1.97	5.47^{b}±1.65	5.66 ± 2.56	5.78^{b} ± 1.49	6.19^{ab} ± 0.65	6.41 ± 1.32
T_4	5.78^{c}±3.43	4.88^{c}±4.38	4.50^{b}±3.67	4.81^{c}±2.75	5.50 ± 2.65	5.31^{b} ± 0.87	5.91^{b} ± 2.39	6.25 ± 2.12
T_5	6.78^{a}±2.87	5.94^{a}±2.22	5.03^{a}±3.43	6.22^{a}±2.29	6.19 ± 1.56	6.72^{a} ± 1.80	6.63^{a} ± 2.12	6.78 ± 3.01
Mean ± SE	6.24±2.87	5.46±2.56	4.83±2.93	5.57±2.39	5.68 ± 0.96	5.80 ± 1.09	6.07 ± 0.87	6.34 ± 0.88
Level sof significance	*	*	*	*	NS	*	*	NS

T_1 = Control, T_2 = Antibiotic, T_3 = 0.25% neem + 0.25% moringa– 1.2%, T_4 = 0.25% neem + 0.25% moringa– 1.0%, T_5 = 0.25% neem + 0.25% moringa– 0.75%, NS= Non-significance. (*)= Significance, $^{a,\,b}$: Means in the same row with different letters show significant differences ($P<0.05$).

4.4 Egg production (weight)

Different treatment groups of feed supplementation in Japanese quail's diet showed significant variation ($P<0.05$) on weekly egg weight of Japanese quail (n=1) (Table 12). Irregular variation was found among the supplementation treatments with the increase of duration. Results on egg week (n=1) of 8th week of age, T_5 (0.25% neem + 0.25% moringa– 0.75%) treatment gave highest egg weight ($10.55^a \pm 3.10$g) followed by T_1 (control) ($.625^b \pm 4.30$g) and T_2 (Antibiotic) ($9.73^b \pm 2.40$ g) whereas T_4 (0.25% neem + 0.25% moringa– 1.0%) treatment showed lowest egg weight ($9.00^d \pm 3.80$g) at the end of 8th week of age. Treatment T_1 (control) and T_2 (Antibiotic) showed non-significant variation among them at 8th week of age performance. Similarly, at the end of 9th, 10th & 11th week of age, the highest egg weight ($9.60^a \pm 2.50$, $8.00^a \pm 3.80$ and $10.21^a \pm 2.60$g, respectively) was achieved by T_5 (0.25% neem + 0.25% moringa– 0.75%) supplementation whereas the lowest ($7.88^d \pm 4.90$, $7.13^c \pm 4.10$ and $8.20^d \pm 4.10$ g) was registered from the treatment T_4 (0.25% neem + 0.25% moringa– 1.0%).

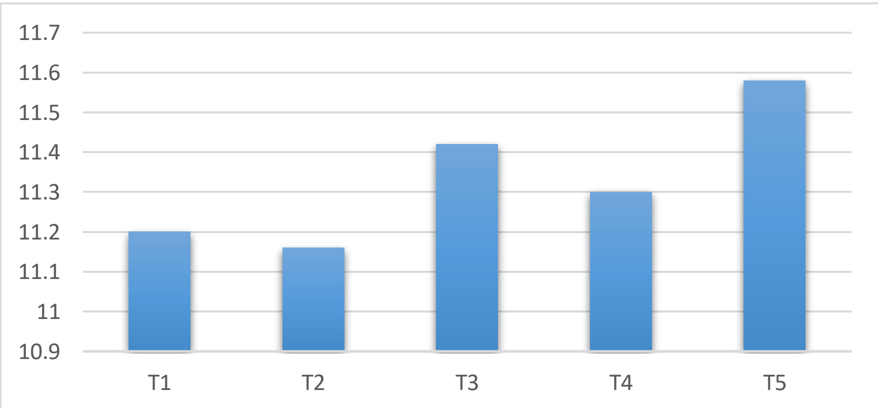

Figure 43 Egg production with significance weight at final age

At the 12th, 13th, 14th and 15th week maximum egg weight (11.58 ± 0.07^a g) was observed in the T5 whereas the minimum egg weight (10.60 ± 0.27g) was found in the T2 (Antibiotic). Results of 12th and 13th non-significant but egg weights of 14th and 15th week of age were significantly differ among the treatment groups. Similar result was also observed by Tesfay *et al.*, (2011) who found the effect of *M. oleifera* leaf meal on egg laying rate of hens by exposing them to three experimental diets containing different amounts of *M. oleifera* (0%, 1% and 2%). Their results showed that diets supplemented with 1% induced high egg production and the heaviest eggs compared to the others. (El-Attrouny & Iraqi, 2021)

Table 12 Effects of different levels of supplementation in water of Japanese quail on weekly egg production in weight basis (n=1)

Treatments	Egg weight of quails (g) (n=1)							
	8th week (age)	9th week(age)	10th week(age)	11th week(age)	12th week	13th week	14th week	15th week
T_1	9.63 ±4.30[b]	8.77 ±1.50[c]	7.90 ±1.80[a]	9.38 ±0.80[b]	10.70 ± 0.25	10.75 ± 0.06	10.98 ± 0.04[c]	11.20 ± 0.05[b]
T_2	9.73 ±2.40[b]	9.04±2.50[b]	7.70 ±3.40[b]	9.00 ±3.70[c]	10.60 ± 0.27	10.85 ± 0.14	11.11 ± 0.08[bc]	11.16 ± 0.07[b]
T_3	9.57 ±2.70[bc]	8.84 ±1.90[c]	7.61 ±2.20[b]	8.98 ±1.90[c]	10.91 ± 0.15	10.98 ± 0.38	11.34 ± 0.13[ab]	11.42 ± 0.10[ab]
T_4	9.00 ±3.80[d]	7.88 ±4.90[d]	7.13 ±4.10[c]	8.20 ±4.10[d]	10.83 ± 0.36	10.78 ± 0.30	11.20 ± 0.12[bc]	11.30 ± 0.11[b]
T_5	10.55 ±3.10[a]	9.60 ±2.50[a]	8.00 ±3.80[a]	10.21 ±2.60[a]	11.16 ± 0.19	11.30 ± 0.17	11.55 ± 0.09[a]	11.58 ± 0.07[a]
Mean ± SE	9.70 ±3.50	8.82 ±2.90	7.66 ±3.30	9.16 ±2.90	10.84 ± 0.11	10.93 ± 0.11	11.24 ± 0.06	11.33 ± 0.05
Level of significance	*	*	*	*	NS	NS	*	*

T_1 = Control, T_2 = Antibiotic, T_3 = 0.25% neem + 0.25% moringa– 1.2%, T_4 = 0.25% neem + 0.25% moringa– 1.0%, T_5 = 0.25% neem + 0.25% moringa– 0.75%, NS= Non-significance. (*)= Significance, [a, b]: Means in the same row with different letters show significant differences ($P<0.05$).

4.5 Feed conversion ratio (FCR)

Regarding, feed conversion ratio (FCR) at weekly performance, statistical analysis showed a significant difference ($P<0.05$) at 10^{th} and 11^{th} week of age in the FCR of the treatment groups but at 8^{th} and 9^{th} week of age, non-significant variation ($P>0.05$) was found (Table 13). However, at 8^{th} and 9^{th} week of age, the highest FCR (3.025±0.298 and 3.725±0.342, respectively) was noted in T_4 (0.25% neem + 0.25% moringa– 1.0%) and the lowest FCR (2.825±0.189 and 3.402±0.166, respectively) was recorded in T_5 (0.25% neem + 0.25% moringa– 0.75%) treatment. It was also observed that the highest FCR (3.925^a±0.388) was in T_4 (0.25% neem + 0.25% moringa– 1.0%) treatment that was significantly similar to T_1 (control), T_2 (Antibiotic) and T_3 (0.25% neem + 0.25% moringa– 1.2%) treatments whereas the lowest FCR (3.425^b±0.189) at 10^{th} week of age was recorded from T_5 (0.25% neem + 0.25% moringa– 0.75%). Similarly, at 11^{th} week of age, the highest FCR (3.525^a±0.269) was in T_4 (0.25% neem + 0.25% moringa– 1.0%) treatment whereas the lowest FCR (2.900^b±0.108) at 11^{th} week was recorded from T_5 (0.25% neem + 0.25% moringa– 0.75%) that was significantly similar to T_1 (control), T_2 (Antibiotic) and T_3 (0.25% neem + 0.25% moringa– 1.2%) treatments. At the age of 12^{th}, 13^{th}, 14 and 15^{th} week of age the FCR was significant ($p<0.05$) among the treatments. The lowest FCR (2.52 ± 0.03^d) was found in the T5 at the 15^{th} week of age. However, T5 at the age of 12^{th}, 13^{th}, 14 and 15^{th} week showed the lowest FCR (2.98 ± 0.05^c, 2.72 ± 0.03^c, 2.58 ± 0.05^c and 2.52 ± 0.03^d respectively) compared to that of other treatments. Result showed the highest FCR in the T2 at the age of 12^{th}, 13^{th}, 14 and 15^{th} week (3.45 ± 0.03^a, 3.28 ± 0.03^a, 3.18 ± 0.03^a and 3.18 ± 0.03^a respectively). It might be due to add extra protein concentrate the result was significant. Because Kouatcho, *et al*., (2020) found a non-significant result in his study. They also found significant variation among treatments with moringa supplementation regarding feed conversion ratio. Behnamifar *et al*., (2015) found non-significant ($p>0.05$) FCR in his study which was regarding with thyme, caraway and garlic extract. Similar result was recorded by Al-Ashoor *et al.*, (2020) that significant differences were found in FCR among the treatment groups. Similar result was observed by Mossa, *et al*., (2019) where significant differences were found among the treatment groups regarding FCR mass.

Table 13 Effects of different levels of supplementation in water of Japanese quail on feed conversion ratio (FCR) on the basis of egg mass

Treatments	Feed conversion ratio (FCR) (Egg mass) (n=1)							
	8th week(age)	9th week(age)	10th week(age)	11th week(age)	12th week(age)	13th week(age)	14th week(age)	15th week(age)
T_1	2.88±0.14	3.45±0.13	$3.73±0.29^{ab}$	$3.00±0.14^{b}$	$3.18 ± 0.03^{b}$	$3.10 ± 0.04^{b}$	$3.18 ± 0.03^{a}$	$3.15 ± 0.03^{a}$
T_2	2.90±0.11	3.43±0.17	$3.58±0.17^{ab}$	$3.03±0.17^{b}$	$3.45 ± 0.03^{a}$	$3.28 ± 0.03^{a}$	$3.18 ± 0.03^{a}$	$3.18 ± 0.03^{a}$
T_3	2.90±0.14	3.53±0.23	$3.60±0.18^{ab}$	$3.00±0.09^{b}$	$3.10 ± 0.00^{b}$	$3.18 ± 0.05^{ab}$	$3.07 ± 0.03^{ab}$	$2.82 ± 0.03^{b}$
T_4	3.03±0.30	3.73±0.34	$3.93±0.39^{a}$	$3.53±0.27^{a}$	$3.10 ± 0.04^{b}$	$2.88 ± 0.09^{c}$	$2.97 ± 0.08^{b}$	$2.73 ± 0.03^{c}$
T_5	2.83±0.19	3.40±0.17	$3.43±0.19^{b}$	$2.90±0.11^{b}$	$2.98 ± 0.05^{c}$	$2.72 ± 0.03^{c}$	$2.58 ± 0.05^{c}$	$2.52 ± 0.03^{d}$
Mean ± SE	2.91 ±0.19	3.51 ±0.19	3.65 ±0.24	3.09 ±0.14	3.16 ± 0.04	3.03 ± 0.05	3.00 ± 0.05	2.88 ± 0.06
Level of significance	NS	NS	*	*	*	*	*	*

T_1 = Control, T_2 = Antibiotic, T_3 = 0.25% neem + 0.25% moringa– 1.2%, T_4 = 0.25% neem + 0.25% moringa– 1.0%, T_5 = 0.25% neem + 0.25% moringa– 0.75%, NS= Non-significance. (*)= Significance, $^{a,\,b}$: Means in the same row with different letters show significant differences ($P<0.05$).

4.6 Percent hen day egg production (HDEP)

Percent hen day egg production (HDEP) was significant ($P<0.05$) due to different supplementation treatments at different duration (Table 14). Results revealed that the treatment group T_5 (0.25% neem + 0.25% moringa– 0.75%) showed the highest %HDEP ($91.60^a±2.90$, $80.35^a±2.80$, $73.55^a±3.80$ and $86.90^a±3.30$ percent at 8th, 9th, 10th and 11th week of age, respectively) whereas the lowest %HDEP ($82.57^c±6.10$, $69.65^b±7.80$, $64.28^d±6.60$ and $68.77^b±5.00$ percent at 8th, 9th, 10th and 11th week of age, respectively) was recorded from T_4 (0.25% neem + 0.25% moringa– 1.0%) supplementation treatment. It was also observed that at 9th and 11th week treatment T_1 (control), T_2 (Antibiotic) and T_3 (0.25% neem + 0.25% moringa– 1.2%) showed non-significant variation with T_5 (0.25% neem + 0.25% moringa– 0.75%).

At the age of 12th, 13th and 14th week of age almost similar non-significant differences were found among the treatment groups. But at the 15th week of age %HDEP was found significant. The highest %HDEP (91.58 ± 2.92) and the lowest (64.28 ± 6.56) were found in T_5 (0.25% neem + 0.25% moringa– 0.75%) and T_4 (0.25% neem + 0.25% moringa– 1.0%), respectively. Tesfay *et al.*, (2011) also found similar result with the present study and observed experimental diets containing different amounts of *M. oleifera* (0%, 1% and 2%). showed significant variation on egg production and diets supplemented with 1% induced high egg production and the heaviest eggs compared to the others. Similar result was found by Al-Ashoor *et al.*, (2020) which was significant differences among the treatment groups.

Table 14 Effects of different levels of supplementation in water of Japanese quail on percent HDEP (Hen day egg production)

Treatments	% HDEP (Hen day egg production) (n=1)							
	8th week(age)	9th week(age)	10th week(age)	11th week(age)	12th week(age)	13th week(age)	14th week(age)	15th week(age)
T_1	88.85^b±5.50	79.28^a±4.60	67.85^c±4.80	83.85^a±1.50	81.25 ± 2.08	80.35 ± 2.82	73.53 ± 3.86	86.92^a ± 3.25
T_2	89.28^b±4.10	80.62^a±3.80	71.18^b±4.00	83.10^a±4.10	89.28 ± 4.06	80.62 ± 3.76	71.18 ± 4.02	83.10^a ± 4.05
T_3	87.95^b±4.40	78.13^a±3.00	68.30^c±3.50	83.60^a±3.90	87.95 ± 4.40	78.12 ± 3.04	68.30 ± 3.53	83.61^a ± 3.91
T_4	82.57^c±6.10	69.65^b±7.80	64.28^d±6.60	68.77^b±5.00	82.58 ± 6.13	69.64 ± 7.82	64.28 ± 6.56	68.75^b ± 4.97
T_5	91.60^a±2.90	80.35^a±2.80	73.55^a±3.80	86.90^a±3.30	88.84 ± 5.51	79.26 ± 4.60	67.86 ± 4.82	83.84^a ± 1.46
Mean±SE	88.10 ±4.50	77.60 ±4.10	69.00 ±4.19	81.20 ±3.44	88.05 ± 2.00	77.60 ± 2.11	69.03 ± 1.10	91.58 ± 2.92
Level of significance	*	*	*	*	NS	NS	NS	*

T_1 = Control, T_2 = Antibiotic, T_3 = 0.25% neem + 0.25% moringa– 1.2%, T_4 = 0.25% neem + 0.25% moringa– 1.0%, T_5 = 0.25% neem + 0.25% moringa– 0.75%, NS= Non-significance. (*)= Significance, $^{a, b}$: Means in the same row with different letters show significant differences ($P<0.05$).

4.7 Weekly Growth (gram) Performance

Non-significant variation (P>0.05) was recorded on weekly growth performance of Japanese egg lying quail (n=1) observed at 8th, 9th, 10th and 11th week of age due to the effect of different levels of supplementation to quail's diet (Table 15). However, it was observed that at 8th week of age, reduced (negative) growth performance (-0.17±0.010g) was found from previous week but all were showed increased growth performance (positive) and the highest positive growth performance (7.34±0.009g) was in T_1 (control) treatment. At 9th week of age, all treatments showed positive growth performance and the highest (12.41±0.018g) was observed from T_4 (0.25% neem + 0.25% moringa– 1.0%) whereas the lowest growth performance (5.31±0.043g) was recorded from T_2 (Antibiotic) regarding body weight of 8th week of age. At 10th week of age, all treatments gave negative growth performance i.e. body weight was decreased compared to body weight of previous week (9th week of age). The maximum growth performance (12.41±0.018g) was recorded from T_4 (0.25% neem + 0.25% moringa– 1.0%) whereas the minimum (0.13±0.018g) was found from T_3 (0.25% neem + 0.25% moringa– 1.2%). Similarly, at 11th week of age, the maximum growth performance (1.25±0.011g) was found in T_2 (Antibiotic) treatment whereas the minimum (0.13±0.018g) was recorded in T_3 (0.25% neem + 0.25% moringa– 1.2%) treatment. T4 and T5 showed negative growth performance (-0.81±0.027 g and -1.72±0.014 g respectively).

At the age of 12th, 13th and 14th week of age T5 showed the maximum growth performance (22.00 ± 1.08^{a}, 21.00 ± 1.23, 22.75 ± 0.25^{a} and 24.75 ± 1.38^{ab} g, respectively) compared to that of the other treatment groups. 12th, 14th and 15th week of age results showed the significant differences whereas at 13th week of age the result showed non-significant difference. In the entire result T2 (Antibiotic) showed the maximum growth performance (30.00 ± 1.78^{a} g) whereas T4 showed the minimum growth performance (15.25 ± 2.63^{b} g). Significant result was found by Riyed & Majid *et al.*, (2019) for 2.5g/kg marjoram leaf powder which showed the highest growth performance. Non-significant result was found by Çakir, *et al.*, (2008) where the highest growth performance was observed with Avilamycin antibiotic.

Table 15 Weekly growth performance of Japanese quail at different duration influenced by different levels of supplementation in water

Treatments	Weekly growth performance (g)							
	8^{th} week(age)	9^{th} week(age)	10^{th} week(age)	11^{th} week(age)	12^{th} week	13^{th} week	14^{th} week	15^{th} week
T_1	7.34±0.01	8.34±0.02	-7.29±0.01	1.21±0.01	21.00 ± 0.41^{ab}	18.25 ± 2.02	19.50 ± 1.19^{ab}	14.50 ± 1.94^{c}
T_2	4.75±0.01	5.31±0.04	-13.9±0.04	1.25±0.01	17.25 ± 2.29^{ab}	16.25 ± 1.18	17.25 ± 2.29^{b}	30.00 ± 1.78^{a}
T_3	4.41±0.02	12.22±0.03	-9.97±0.02	0.13±0.02	$17.50\pm 1.66a^{b}$	19.00 ± 2.35	22.00 ± 1.23^{a}	24.25 ± 4.01^{ab}
T_4	2.75±0.01	12.41±0.02	-7.18±0.02	-0.81±0.03	15.25 ± 2.63^{b}	16.00 ± 2.61	20.00 ± 1.47^{ab}	22.25 ± 1.25^{b}
T_5	-0.17±0.01	10.64±0.04	-10.6±0.02	-1.72±0.01	22.00 ± 1.08^{a}	21.00 ± 1.23	22.75 ± 0.25^{a}	24.75 ± 1.38^{ab}
Mean±SE	3.75±0.01	10.0±0.03	10.13±0.03	0.0±0.02	18.60 ± 0.92	18.10 ± 0.89	20.30 ± 0.73	23.15 ± 1.47
Level of significance	NS	NS	NS	NS	*	NS	*	*

T_1 = Control, T_2 = Antibiotic, T_3 = 0.25% neem + 0.25% moringa– 1.2%, T_4 = 0.25% neem + 0.25% moringa– 1.0%, T_5 = 0.25% neem + 0.25% moringa– 0.75%, NS= Non-significance. (*)= Significance, [a, b]: Means in the same row with different letters show significant differences ($P<0.05$).

4.8 Survivability

Different supplementation treatment showed significant variation on survivability (Table 16). Results indicated that the treatment T_4 (0.25% neem + 0.25% moringa– 1.0%) showed the maximum survivability ($100.0^a \pm 00$ percent) which was significantly similar to the treatment T_1 (control) and T_3 (0.25% neem + 0.25% moringa– 1.2%) whereas the minimum survivability ($94.00^b \pm 3.50$ percent) was recorded from the treatment T_2 (Antibiotic) which was significantly same with T_5 (0.25% neem + 0.25% moringa– 0.75%). Similar result was also observed by the findings of Dey *et al.*, (2017).

At the next 4 weeks (12^{th}, 13^{th}, 14^{th} and 15^{th} week of age), there was not found any death case. In this time period the survivability was 100% and mortality was 0%. It was done possible only by proper monitoring considering feed supply, water supply, proper biosecurity maintenance, proper lighting management, proper hyegene management etc.

4.9 Microbial load (Salmonella population; CFU/gm)

Different supplementation treatment on feed diet of Japanese quail showed significant variation on faces contains (Table 16). The treatment T_5 (0.25% neem + 0.25% moringa– 0.75%) showed the highest *Salmonella* sp. population contains (8.22 ± 0.32^{ab}) followed by T_4 (7.05 ± 0.98^{ab}) whereas the lowest (4.15 ± 0.41^c) was recorded from the treatment T_3 (0.25% neem + 0.25% moringa– 1.2%).

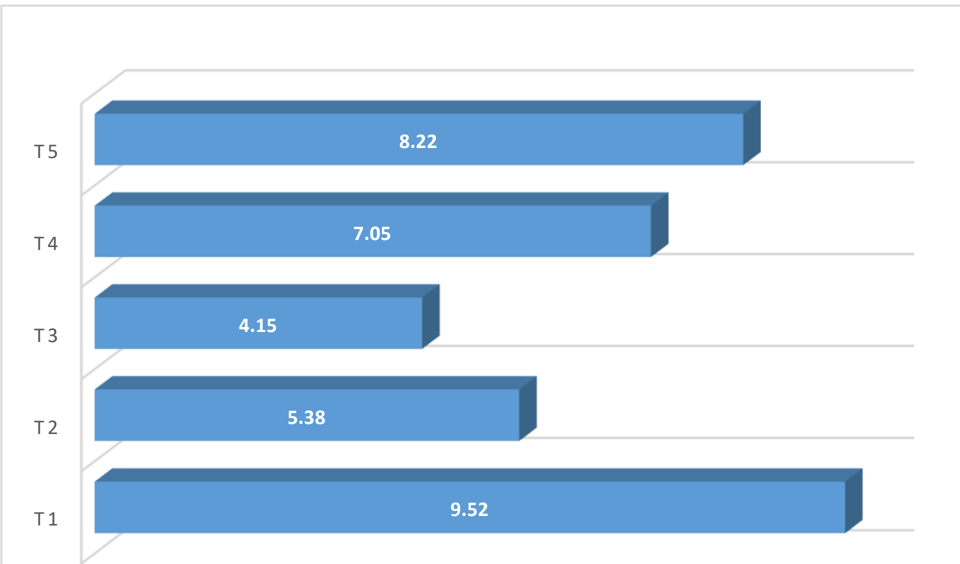

Figure 44 Significant Microbial load in faeces

Similar result was found by Hassan, *et al.*, (2020) where guava, sage, rhamnus, mulberry and olive leaves extract inhibited the growth of different microbial organism including *Salmonella enteritidis*. Pliego, *et al*., (2022) found that some medicinal plants such as green tea, nettle, pennyroyal, yarrow, and alfalfa in the form of seed, powder, and extract has a huge potentiality to reduce microbial growth and improve the immune system of the body.

Table 16 Effect of different levels of supplementation in fed diets of Japanese quail on survivability and microbial load (faces contains; CFU/gm)

Treatments	Survivability	Faeces ($\times 10^3$ CFU/g)
T_1	$97.00^{ab}\pm3.00$	9.52 ± 1.13^{bc}
T_2	$94.00^{b}\pm3.50$	5.38 ± 1.12^{a}
T_3	$97.00^{ab}\pm3.00$	4.15 ± 0.41^{c}
T_4	$100.0^{a}\pm00$	7.05 ± 0.98^{ab}
T_5	$94.50^{b}\pm3.30$	8.22 ± 0.32^{ab}
Mean±SE	96.5 ±3.51	6.86 ± 0.55
Significant level	*	*

T_1 = Control, T_2 = Antibiotic (Ciproflox), T_3 = 0.25% neem + 0.25% moringa– 1.2%, T_4 = 0.25% neem + 0.25% moringa– 1.0%, T_5 = 0.25% neem + 0.25% moringa– 0.75%, (*) = Significance

CHAPTER V

SUMMARY

The study was planned to determine the comparative efficacy of leaf powder of Neem (*Azadirachta indica*) and Moringa (*Moringa oleifera*) on the productive performance, health status and egg quality of commercial layer Japanese quail.

A total of 27 day-old 160 layer Japanese quail chicks were reared in Sher-e-Bangla Agricultural University Poultry Farm, Dhaka. Chicks were divided randomly into 5 experimental groups of 4 replications and each replication contains 8 chicks. These groups were allotted to five treatment designated as T_1 (control T_2 (Antibiotic; Ciproflox-100ml), T_3 (0.25% neem + 0.25% moringa– 1.2%), T_4 (0.25% neem + 0.25% moringa– 1.0%) and T_5 (0.25% neem + 0.25% moringa – 0.75%) treatment group.

The results showed that the weekly live body weight in 4^{th} week was significantly higher in T_5 (0.25% neem + 0.25% moringa– 0.75%) treatment group than control group (T_1). Final live weight was significantly higher in T_5 (Neem + moringa – 0.75%) ($1400^a \pm 8.20$ g) than control as well as other groups.Weekly feed consumption (FC) was non-significant in different group but total FC significantly higher in T_5 (Neem + moringa – 0.75%) than other treated group including control.

Weekly FCR was significantly lower in T_5 (Neem + moringa – 0.75%) group than T_1, T_2, and T_3 group in 8^{th} week to 15^{th} week whereas T_4 showed highest FCR at in 8^{th} week to 15^{th} week. In case of weekly growth performance, T_1 (control) treatment showed better performance than other treatments group.Survivability rate was higher (100%) in T_4 (Neem + moringa – 1.0%) group than others whereas T_2 (Antibiotic) group showed lowest performance ($94.00^b \pm 3.50$ percent).

In case of egg production by number and weight, T_5 (0.25% neem + 0.25% moringa– 0.75%) registered the maximum production at 4^{th} week to 7^{th} week compared to other treatment groups whereas T_4 (0.25% neem + 0.25% moringa– 1.0%) showed least performance. Regarding % HDEP (Hen day egg production), T_5 (0.25% neem + 0.25% moringa– 0.75%) gave higher result compared to others whereas T_4 (0.25% neem + 0.25% moringa– 1.0%) showed lowest results. Again, T_3 (0.25% neem + 0.25% moringa– 1.2%) showed lower *Salmonella* sp. population (faeces contains) and T_5 (0.25% neem + 0.25% moringa– 0.75%) showed higher result.

CHAPTER VI

CONCLUSION

The results of this experiment showed that mixture of 0.25% Neem (*Azadirachta indica*) and 0.25% Moringa (*Moringa oleifera*) leaf extract with drinking water up to 1.2% dietary levels can be used as growth promoters and alternative of antibiotic in the Japanese quail diets without serious adverse effects.

0.75% mixture of moringa and neem leaf extract can be increased production performance of the laying Japanese quails but not significantly affect the microbial loads in the faeces of laying Japanese quail.

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APPENDICES

Appendix 1. Weekly feed consumption (g/bird) of quail

Treatment	Replication	8th	9th	10th	11th	12th	13th	14th	15th
T1	R1	197.50	218.63	190.00	205.00	219.58	212.72	228.93	230,83
	R2	205.63	221.38	197.38	205.00	191.55	203.67	219.59	238.27
	R3	191.43	219.43	197.14	205.00	217.25	183.96	221.78	214.87
	R4	192.88	219.13	197.63	203.50	233.56	205.57	214.91	226.30
T2	R1	183.38	224.57	190.14	196.71	220.11	229.33	224.64	226.69
	R2	207.25	222.50	197.38	205.00	208.83	231.66	215.28	234.02
	R3	206.13	223.00	197.75	205.00	208.89	212.27	210.61	234.02
	R4	190.25	221.38	226.29	205.00	235.46	188.22	229.17	210.60
T3	R1	196.57	215.86	191.57	204.57	230.62	200.72	210.06	200.14
	R2	204.88	225.00	198.00	193.75	217.06	212.54	218.17	216.48
	R3	198.13	222.38	198.00	192.50	189.93	226.11	226.10	220.56
	R4	197.63	224.13	198.00	192.25	180.89	216.62	221.58	207.29
T4	R1	182.88	196.50	191.63	201.63	186.20	171.26	204.37	178.00
	R2	178.38	208.38	189.50	202.13	177.12	171.26	190.75	189.87
	R3	191.25	212.75	194.88	203.25	210.96	178.34	208.18	209.64
	R4	189.75	178.25	185.00	186.00	224.14	186.20	217.40	221.51
T5	R1	193.22	213.89	195.00	205.00	235.96	209.02	205.51	179.31
	R2	184.11	218.00	192.11	199.44	223.95	205.51	198.00	226.88
	R3	188.75	223.57	189.00	182.71	191.02	209.46	215.90	204.93
	R4	188.67	198.11	195.11	196.56	212.24	233.18	179.31	190.29

Appendix 2. Weekly egg weight (g/replication) of quail

Treatment	Replication	8^{th}	9th	10th	11^{th}	12^{th}	13^{th}	14^{th}	15^{th}
T1	R1	77.30	66.40	61.33	62.88	68.62	68.61	71.54	74.46
	R2	69.53	57.93	52.83	66.16	59.86	65.70	68.62	74.37
	R3	59.59	67.77	63.40	77.14	70.08	70.24	81.86	76.79
	R4	70.68	62.11	51.43	66.13	73.01	64.24	67.16	72.98
T2	R1	66.69	69.51	49.11	70.73	71.87	81.90	80.23	83.57
	R2	71.96	62.17	52.89	67.68	61.43	70.20	67.29	73.12
	R3	73.55	72.03	63.74	71.84	61.43	64.35	65.81	73.13
	R4	60.08	57.91	63.59	57.73	76.89	65.19	84.49	75.29
T3	R1	69.81	72.64	55.53	65.60	85.02	71.69	80.02	81.72
	R2	61.26	55.09	48.71	60.43	70.02	68.56	74.40	77.31
	R3	71.93	62.20	55.66	67.66	61.27	72.94	72.94	78.77
	R4	73.45	66.40	59.89	65.83	58.35	65.64	71.48	71.48
T4	R1	67.82	53.66	45.88	54.61	60.07	61.53	65.94	65.87
	R2	68.19	64.98	58.16	67.49	57.14	65.93	61.59	70.32
	R3	66.89	63.55	58.50	63.21	65.93	61.56	71.87	77.70
	R4	48.81	38.13	37.43	44.45	74.72	60.13	77.62	79.11
T5	R1	59.46	65.28	49.21	65.18	67.66	66.33	67.65	63.78
	R2	75.04	62.81	61.70	66.34	66.36	67.67	67.55	80.67
	R3	74.99	74.29	55.66	70.63	75.28	88.70	98.68	93.68
	R4	61.69	52.73	44.36	67.67	65.06	76.76	63.77	65.12

Appendix 3. Weekly feed conversion ratio of quail

Treatment	Replication	8^{th}	9^{th}	10^{th}	11^{th}	12^{th}	13^{th}	14^{th}	15^{th}
T1	R1	2.60	3.30	3.10	3.20	3.2	3.1	3.2	3.1
	R2	3.00	3.80	3.70	3.10	3.2	3.1	3.2	3.2
	R3	3.20	3.20	3.10	2.60	3.1	3	3.1	3.2
	R4	2.70	3.50	3.80	3.10	3.2	3.2	3.2	3.1
T2	R1	2.70	3.20	3.90	2.80	3.5	3.2	3.2	3.1
	R2	2.90	3.60	3.70	3.03	3.4	3.3	3.2	3.2
	R3	2.80	3.10	3.10	2.80	3.4	3.3	3.2	3.2
	R4	3.20	3.80	3.60	3.50	3.5	3.3	3.1	3.2
T3	R1	2.80	3.00	3.40	3.10	3.1	3.2	3	2.8
	R2	3.30	4.10	4.10	3.20	3.1	3.1	3.1	2.8
	R3	2.80	3.60	3.60	2.80	3.1	3.1	3.1	2.8
	R4	2.70	3.40	3.30	2.90	3.1	3.3	3.1	2.9
T4	R1	2.70	3.70	4.20	3.70	3.1	2.8	3.1	2.7
	R2	2.60	3.20	3.30	3.00	3.1	2.7	3.1	2.7
	R3	2.90	3.30	3.30	3.20	3.2	2.9	2.9	2.7
	R4	3.90	4.70	4.90	4.20	3	3.1	2.8	2.8
T5	R1	3.20	3.30	4.00	3.10	3.1	2.8	2.7	2.5
	R2	2.50	3.50	3.10	3.00	3	2.7	2.6	2.5
	R3	2.50	3.01	3.40	2.60	2.9	2.7	2.5	2.5
	R4	3.10	3.80	4.40	2.90	2.9	2.7	2.5	2.6

Appendix 4. Weekly HDEP (%) of quail

Treatment	Replication	8th	9th	10th	11th	12th	13th	14th	15th
T1	R1	100.00	83.93	78.57	87.50	100.00	83.93	78.57	87.50
	R2	91.07	73.21	67.86	82.14	91.07	73.21	67.86	82.14
	R3	87.76	85.71	81.63	95.92	87.76	85.71	81.63	95.92
	R4	87.50	78.57	66.07	82.14	87.50	78.57	66.07	82.14
T2	R1	87.50	79.63	63.27	87.76	87.50	79.63	63.27	87.76
	R2	94.64	78.57	67.86	83.93	94.64	78.57	67.86	83.93
	R3	96.43	91.07	82.14	89.29	96.43	91.07	82.14	89.29
	R4	78.57	73.21	71.43	71.43	78.57	73.21	71.43	71.43
T3	R1	80.36	80.36	62.50	81.63	80.36	80.36	62.50	81.63
	R2	80.36	69.64	62.50	75.00	80.36	69.64	62.50	75.00
	R3	94.64	78.57	71.43	83.93	94.64	78.57	71.43	83.93
	R4	96.43	83.93	76.79	93.88	96.43	83.93	76.79	93.88
T4	R1	89.29	67.86	58.93	67.86	89.29	67.86	58.93	67.86
	R2	89.29	82.14	75.00	73.21	89.29	82.14	75.00	73.21
	R3	87.50	80.36	75.00	78.57	87.50	80.36	75.00	78.57
	R4	64.23	48.21	48.21	55.36	64.23	48.21	48.21	55.36
T5	R1	77.78	82.54	63.49	80.95	77.78	82.54	63.49	80.95
	R2	98.41	79.37	79.37	82.54	98.41	79.37	79.37	82.54
	R3	98.21	88.46	71.43	87.76	98.2	88.46	71.43	87.76
	R4	80.95	66.67	57.14	84.13	80.95	66.67	57.14	84.13

Appendix 5. Effect of Different Treatment on *Salmonella* spp. Colony in faeces and whole egg sample

Treatment	Replication	No. of *Salmonella* spp. Colony (Average) in faeces	No. of *Salmonella* spp. Colony (Average) in whole egg
T1	R1	82	0
	R2	30	0
	R3	64	0
	R4	45	0
T2	R1	48	0
	R2	38	0
	R3	52	0
	R4	69	0
T3	R1	41	0
	R2	51	0
	R3	31	0
	R4	43	0
T4	R1	72	0
	R2	65	0
	R3	96	0
	R4	49	0
T5	R1	76	0
	R2	82	0
	R3	80	0
	R4	91	0

Appendix 6. Percent relative humidity (%RH) during experiment in November December, 2021

Age in weeks	Period (day)	Average relative humidity (%RH)				Weekly average %RH
		8.00 AM	12.00 AM	4.00 PM	8.00 PM	
4th	20.11.21 - 26.11.21	82	78	81	76	79.25
5th	27.11.21 - 03.12.21	77	75	74	72	74.50
6th	04.12.21 - 10.12.21	80	78	75	70	75.75
7th	11.12.21 - 17.12.21	76	72	68	71	71.75

Appendix 7. Average temperature (°C) during experiment in November-December, 2021

Age in weeks	Period (day)	Average temperature (°C)				Weekly average temp. (°C)
		8.00 AM	12.00 AM	4.00 PM	8.00 PM	
4th	20.11.21 - 26.11.21	24	27	26	23	25.00
5th	27.11.21 - 03.12.21	25	26	26	25	25.50
6th	04.12.21 - 10.12.21	23	25	27	24	24.75
7th	11.12.21 - 17.12.21	22	24	25	23	23.50

Appendix 8. Total cost of production, total income and BCR

Treatment	Total input cost (Tk.) (n=8)	Supplementation cost (Tk.) (n=8)	Total cost of production (Tk.)	Average survivability of 8 birds (8^{th} to 15^{th} week) (%)	Egg product-ion at 8^{th} to 15^{th} week	Egg selling price (Tk.)	Selling price of birds at the end of 15^{th} week (Tk.) regarding survivability	Total income (Tk.)	BCR
T_1	710	0.00	710	97.00	180	540	543.20	1083.20	1.53
T_2	710	40.00	750	94.00	177	531	526.40	1057.40	1.49
T_3	710	30.00	740	97.00	175	525	543.20	1068.20	1.50
T_4	710	20.00	730	100.00	160	480	560.00	1040.00	1.46
T_5	710	15.00	725	94.00	192	576	526.40	1102.40	1.55

Here,

- Structural cost of residence for 8 chicks = 100 Tk.
- Chick cost per unit replication = 8 × 35 = 280.00 Tk.
- Per quail feed/day = 25 g = 1.25 Tk. (According to 1sack feed = 50 kg = 2500 Tk.)
- So, feed cost for 8 quails for 4 weeks = 280 Tk.
- Feed cost at 15^{th} week of age = 280 Tk.
- Vaccine, medicine and treatment cost for 8 chicks at 15^{th} week of age = 50 Tk.

Miscellaneous cost for 8 quail of 8^{th} to 15^{th} week of age = 50 Tk.

- Feed supplementation cost at 8th to 15th week of age for -

  T_1 = 0.00 Tk.

  T_2 = 40.00 Tk.

  T_3 =30.00 Tk.

  T_4 = 20.00 Tk.

  T_5 = 15.00 Tk.

- Egg selling price = 3.00 Tk./piece
- Bird selling price at the end of 15th week = 70.00 Tk./piece

Appendix 9. Descriptive									
		No.	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
rep	1	4	2.50	1.291	.645	.45	4.55	1	4
	2	4	2.50	1.291	.645	.45	4.55	1	4
	3	4	2.50	1.291	.645	.45	4.55	1	4
	4	4	2.50	1.291	.645	.45	4.55	1	4
	5	4	2.50	1.291	.645	.45	4.55	1	4
	Total	20	2.50	1.147	.256	1.96	3.04	1	4
tw	1	4	10.70 ± 0.25	.498		9.90	11.49	10	11
	2	4	10.60 ± 0.27	.546		9.73	11.47	10	11
	3	4	10.91 ± 0.15	.304		10.43	11.39	11	11
	4	4	10.83 ± 0.36	.711		9.70	11.96	10	12
	5	4	11.16 ± 0.19	.374		10.57	11.76	11	12
	Total	20	10.84 ± 0.11	.492		10.61	11.07	10	12
thirw	1	4	10.75 ± 0.06	.120		10.56	10.94	11	11
	2	4	10.85 ± 0.14	.288		10.39	11.31	11	11
	3	4	10.98 ± 0.38	.757		9.78	12.18	10	12
	4	4	10.78 ± 0.30	.598		9.83	11.73	10	11
	5	4	11.30 ± 0.17	.348		10.75	11.86	11	12
	Total	20	10.93 ± 0.11	.473		10.71	11.15	10	12

		No.	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
fouw	1	4	10.98 ± 0.04	.076		10.86	11.10	11	11
	2	4	11.11 ± 0.08	.159		10.86	11.37	11	11
	3	4	11.34 ± 0.13	.253		10.94	11.74	11	12
	4	4	11.20 ± 0.12	.246		10.81	11.59	11	12
	5	4	11.55 ± 0.09	.173		11.27	11.82	11	12
	Total	20	11.24 ± 0.06	.264		11.11	11.36	11	12
fifw	1	4	11.20 ± 0.05	.105		11.03	11.37	11	11
	2	4	11.16 ± 0.07	.145		10.93	11.39	11	11
	3	4	11.42 ± 0.10	.190		11.12	11.72	11	12
	4	4	11.30 ± 0.11	.223		10.95	11.66	11	11
	5	4	11.58 ± 0.07	.139		11.36	11.81	11	12
	Total	20	11.33 ± 0.05	.216		11.23	11.43	11	12
ave	1	4	10.91 ± 0.08	.154		10.66	11.15	11	11
	2	4	10.93 ± 0.13	.250		10.53	11.33	11	11
	3	4	11.16 ± 0.08	.159		10.91	11.42	11	11
	4	4	11.03 ± 0.16	.324		10.51	11.54	11	11
	5	4	11.40 ± 0.11	.213		11.06	11.74	11	12
	Total	20	11.09 ± 0.06	.275		10.96	11.21	11	12