EFFECT OF BLACK PEPPER (*Piper nigrum*) AS NATURAL FEED ADDITIVE ON THE PERFORMANCE OF JAPANESE QUAIL

By

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CERTIFICATE

VEERANKI SRI DIVYA, GVM / 14 - 007 has satisfactorily prosecuted the course of research and that the thesis entitled **"EFFECT OF BLACK PEPPER (*Piper nigrum*) AS NATURAL FEED ADDITIVE ON THE PERFORMANCE OF JAPANESE QUAIL"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by her for a degree of any University.

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CERTIFICATE

This is to certify that the thesis entitled **"EFFECT OF BLACK PEPPER (*Piper nigrum*) AS NATURAL FEED ADDITIVE ON THE PERFORMANCE OF JAPANESE QUAIL"** submitted in partial fulfillment of the requirements for the degree of MASTER OF VETERINARY SCIENCE for Sri Venkateswara Veterinary University, Tirupati is a record of the bonafide research work carried out by VERANKI SRI DIVYA, GVM/14-007 under our guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee. No part of the thesis has been submitted by the student for any other degree or diploma. The published part has been fully acknowledged. All the assistance and help received during the course of investigations have been duly acknowledged by the author of the thesis.

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Dr. V. Sri Divya . . ✍

DECLARATION

I, Dr. **VEERANKI SRI DIVYA** hereby declare that the thesis entitled **"EFFECT OF BLACK PEPPER (*Piper nigrum*) AS NATURAL FEED ADDITIVE ON THE PERFORMANCE OF JAPANESE QUAIL"** submitted to **SRI VENKATESWARA VETERINARY UNIVERSITY** for the Degree of **MASTER OF VETERINARY SCIENCE** is a result of original research work done by me. It is further declared that the thesis or any part thereof has not been published earlier in any manner.

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ABSTRACT

The present investigation is carried out to study the effect of inclusion of black pepper at varying levels on the growth performance, serum biochemical profile, carcass characteristics and nutrient utilization of Japanese quails. One hundred and fifty day old quail chicks distributed randomly to five dietary groups each with three replicates of ten quails and were offered diet containing 24% CP and 2900 kcal/kg ME. During the experiment, black pepper is included at 0.0% (T_1: Control), 0.25% (T_2), 0.50% (T_3), 0.75% (T_4) and 1.0% (T_5) level by marginal adjustment of other feed ingredients. All the rations were made iso-caloric and iso-nitrogenous.

Chemical analysis indicated that black pepper contained 12.87 % CP, 4.85% EE, 20.97% CF, 1.46% calcium and 0.8% phosphorous. Results indicated that body weight gain increased ($P<0.05$) while feed intake and feed consumed/kg gain decreased ($P<0.01$) with increased level of inclusion of black pepper from 0 to 1.0% in the diet

The serum biochemical profile of quails revealed that the total protein ($P<0.01$), albumin ($P<0.05$), creatinine ($P>0.05$), calcium and phosphorus ($P<0.01$) contents increased while serum glucose, triglycerides and total cholesterol levels decreased ($P<0.01$) with increase in the inclusion of black pepper from 0 to 1.0% in the diet. Further, the study indicated that the HDL cholesterol level increased ($P<0.01$) while LDL and VLDL cholesterol levels decreased ($P<0.01$) in serum with increased level of inclusion of black pepper from 0 to 1.0 % in the diet.

The digestibility of gross nutrients and fibre fractions increased ($P<0.01$) with increased level of inclusion of black pepper from 0 to 1.0% in the diet. Similarly, the percent nitrogen, calcium and phosphorus utilization also increased ($P<0.01$) with increase in the level of inclusion of black pepper from 0 to 1.0 % in the diet. Further, the study indicated that the carcass yield

(P<0.05), dressing percentage (P<0.01), ready to cook yield (P<0.05) and meat to bone ratio (P<0.01) increased with increased level of inclusion of black pepper from 0 to 1.0% in the diet. However, inclusion of black pepper at graded levels in the diet of quails had no effect (P>0.05) on percent weight of heart, liver, gizzard and giblet.

The feed cost/kg gain decreased by ₹ 2.19 (T_2), 2.20 (T_3), 3.67 (T_4) and 3.99 (T_5) in quails fed diets containing black pepper at varying levels as compared to the control.The present study indicated that black pepper can be incorporated up to 1.0% level as natural feed additive in the diet of quails without any adverse effect on production performance.

LIST OF ABBREVIATIONS

%	:	Percent
±	:	Plus or Minus
<	:	Lesser than
>	:	Greater than
/	:	Per
µ	:	micro
₹	:	Rupee
d	:	Day
dl	:	Deciliter
g	:	Grams
mg/dl	:	Milligrams per deciliter
mg	:	Milli gram
ml	:	Milli liter
ADF	:	Acid detergent fibre
ADL	:	Acid detergent lignin
AIA	:	Acid insoluble ash
AOAC	:	Association of Official Analytical Chemists
avg.b.wt	:	Average Body Weight
BW	:	Body weight
BWG	:	Body weight gain
Ca	:	Calcium
CF	:	Crude fibre
CP	:	Crude protein
CRD	:	Completely Randomized Design
BP	:	Black pepper
DORB	:	De-oiled rice bran
DM	:	Dry matter
DMB	:	Dry matter basis
EE	:	Ether extract
et al.	:	And others
FCR	:	Feed conversion ratio

FE	:	Feed efficiency
F : G	:	Feed: Gain
g	:	gram
GE	:	Gross energy
HDL-C	:	High density lipoprotein cholesterol
Kcal	:	Kilocalories
Kg	:	Kilogram
LDL-C	:	Low density lipoprotein cholesterol
LW	:	Live weight
LWG	:	Live weight gain
ME	:	Metabolizable energy
MEq	:	Milli-equivalent
NDF	:	Neutral detergent fibre
NFE	:	Nitrogen free extract
NRC	:	National Research Council
NS	:	Non-significant
OM	:	Organic matter
P	:	Phosphorus
PUFA	:	Poly Unsaturated Fatty Acid
Rpm	:	Rotations per minute
SBM	:	Soybean meal
SEM	:	Standard error mean
SS	:	Statistical significance
T	:	Treatment
TA	:	Total ash
VLDL-C	:	Very low density lipoprotein cholesterol
viz.,	:	Namely

CHAPTER - I

INTRODUCTION

Dietary antibiotics have an important role in animal production as growth and health promoters. However, feeding birds even on low concentration of antibiotics has been a long concern due to the development of antibiotic resistant bacteria. The residual effect of antibiotics in animal products has decreased their use in animal feed. The ban on the use of antibiotics has increased the need to have some alternative which could produce similar effects as that of antibiotics in animals. Phytogenic compounds such as essential oils and spices have been reported to exhibit growth promoting properties (Windisch *et al*., 2007). Considering the health hazard, the feed manufacturer and poultry farmers have been actively looking for efficacious alternatives. Black pepper could be considered as one such alternative growth promoting source.

Black pepper (*Piper nigrum*) is a well known medicinal plant that grows in nature and is mainly cultivated in tropical parts of the world. It has many therapeutic effects like, anti-ache effect (Moorthy *et al*., 2009), anti-oxidant and anti-bacterial effects (Gulcin, 2005). In addition, it has the ability to enhance secretions of gastric and pancreatic enzymes thereby improving digestibility (Orav *et* al., 2004). Generally, spices are reported to have positive effect on villi, nutrient absorption and digesta viscosity (Lee *et al*., 2004;

Sirinivasan, 2007). It is a good source of dietary protein for consumption by human and animals, and fatty acids which are predominantly lauric acid, myristic acid, palmitoleic acid, oleic, stearic acid and lignoceric acid. Black pepper was found to be rich in glutathione peroxidase and glucose-6-phosphate dehydrogenase. It is reported that the active principle, piperine present in black pepper has hepatoprotective, gastroprotective, anti-inflammatory properties and can dramatically increase absorption of selenium, vitamin B-complex, beta carotene, curcumin as well as other nutrients (Khalaf *et al.*, 2008). Piperine enhances the thermogenesis of lipid and accelerates energy metabolism in the body (Malini *et al.,* 1999).

Quail is considered to be a bird which has been bred and produced in India. It is considered a prolific meat and egg producer with dual production capacity and thus attained a valuable status in commercial enterprises. Quails are popular for their high protein (26%) and less fatty (3%) meat, which is also known for increasing the sexual instinct in humans (Shinde *et al*., 2014). Further, quail has unique qualities like hardiness and adaptability to diversified agro-climatic condition making it ideal for rural poultry production, thus creating employment opportunities.Thus, industrial breeding of quail has become a profitable and productive industry throughout the world (Shukuhmand, 2008).

Inconsistent results and inadequate data exist regarding the potential use of black pepper as growth promoter in poultry. Black pepper as an alternative to antibiotic growth promoters has been recommended for feeding broilers

(Mansoub, 2011; Ghaedi *et al*., 2013; Valiollahi *et al*., 2013) while some researchers reported no significant improvement in body weight gain and feed conversion (Puvaca *et al*., 2014). On the other hand, studies on the feeding value of black pepper in Japanese quails (Myandoab and Mansoub, 2011) are very limited. Hence, the present investigation has been proposed to study the effect of inclusion of black pepper at varying levels as a natural feed additive in Japanese quail with the following objectives:

1. To determine the optimum level of inclusion of black pepper in the diet of Japanese quails.
2. To study the effect of black pepper incorporation in the diet as natural feed additive on production performance of quails.
3. To evaluate the effect of dietary incorporation of black pepper on nutrient utilization in quails.
4. To study the carcass characteristics in quails fed diets containing black pepper.
5. To study the economics of black pepper inclusion in quail diets.

CHAPTER-II

REVIEW OF LITERATURE

Black pepper (*Piper nigrum*) is a flowering vine in the family Piperaceae, cultivated for its fruit, which is usually dried and used as a spice. Black pepper is cultivated mainly in tropical parts of the world. India is known as "The Home of the Black Pepper". Historically, black pepper was termed as "The Black Gold" because of its commercial, economical and trade value. Black pepper seeds are used extensively to prepare powders and extracts for inclusion into poultry diets as feed additives (Cardoso *et al*., 2012). The present research was conducted by incorporating black pepper in poultry rations and the effect of incorporation of black pepper in poultry rations on various parameters are reviewed in this chapter.

2.1 CHEMICAL COMPOSITION OF BLACK PEPPER

2.1.1 Proximate composition of black pepper

The DM, CP, EE, CF, TA and NFE content of black pepper ranged from 85.071 to 92.26, 11.67 to 12.87, 3.28 to 4.85, 18.72 to 20.97, 5.99 to 6.0 and 55.31 to 60.34, respectively. The calcium and phosphorous contents of black pepper powder ranged from 1.1 to 1.46 and 0.76 to 0.8, respectively (Table 1).

2.1.2 Cell wall constituents of black pepper

The NDF, ADF, hemi-cellulose, cellulose and lignin content of black pepper were 52.25, 39.5, 12.75, 26.34 and 1.5 %, respectively.

2.2 NUTRITIVE VALUE OF BLACK PEPPER

Black pepper is a spice which is reported to have antibacterial and antioxidant properties in addition to its ability to enhance secretions of gastric and pancreatic enzymes there by improving digestibility (Orav *et al.,* 2004; Srinivasan., 2007). Generally, spices are reported to have positive effect on villi, nutrient absorption and digestal viscosity (Lee *et al*., 2004; Srinivasan, 2007). Black pepper was richest in glutathione peroxidase and glucose-6-phosphate dehydrogenase. Both water extract and ethanol extract of black pepper exhibited strong antioxidant activity. Antimicrobial, larvicidal and anti-cancer activities of *Piper nigrum* have been reported (Khalaf *et al.,* 2008). Seed portion contains protein (11.56 %), carbohydrate (45.16%), crude fiber (16.66 %), fatty acids (12.02 %), which are predominantly lauric acid (26.93 %), myristic (8.26 %), palmitoleic (13.47 %), palmitic (12.24 %), oleic (17.82 %), stearic (17.28 %) and lignoceric acid (4.00 %) (Hossain *et al* ., 2014).

Table 1: Chemical composition (on %DMB) of black pepper

S.No	DM	CP	CF	EE	TA	NFE	Ca	P	Author
1	95.32	25.45	23.6	5.34	3.57	42.04	1.9		AI-jasass *et al.,* 2012.
2	90.8	8.5		20.3	7.7		0.4		Bouba *et al*., 2012
3		25.45	23.6	4.85					Mohammed *et al*., 2012
4	86.92	9.18	5.5	12.77	6.47	66.08			Emeka *et al*., 2013
5		9.0	18.60	8.06	5.09	59.25			Sruthi *et al.*, 2013
6	87.3	9.33	4.20	17.3	9.90	59.27	4.2	2.5	Bolanle *et al*., 2014
7	83.07	3.54	48.95	16.30	1.65	29.56	6.44	1.45	Ameh *et al*., 2016
8		9.29	26.80	22.80	2.75	38.36	0.30	0.07	Keswet *et al*., 2015
9	87.65	5.86	8.79	9.89	6.33	69.13	0.1	0.21	Balogun *et al.*, 2016

In addition, the bioactive molecule, piperine, present in pepper has major pharmacological impact on the neuromuscular system and can help in digestion (Ferreira *et al.,* 1999; Great., 2003). Among its chemical and biological activities, piperine exhibits anti microbial (Reddy *et al*., 2004), anti-inflammatory (Pradeep and Kuttan, 2004) and anti oxidant (Mittal and Gupta, 2000) properties. Piperine induces alterations in membrane dynamics and permeation characteristics, as well as synthesis of proteins associated with cyto skeletal function, resulting in an increase in the small intestine absorptive surface (Khajuriaet *et al*., 2002).

Piperine has been reported to have several pharmacological effects such as anti-diarrhoeal and hepato protective (Koul and Kapil, 1993; Bajad *et al*., 2001). Piperic acid has a high anti-hyper lipidemic activity (Han *et al*., 2008). Piperine is also an active ingredient which works to increase thermogenesis in the body (Bhutani *et a*l., 2009; Ahmad *et al*., 2012), increases digestion and energy metabolism in the body and increases the bio availability of certain drugs in the organism (Ahmad *et al.,* 2012) so as to improve immunity.

Properties include stimulation of digestive enzymes, saliva, hydrochloric acid and mucus; ability to improve the height of villi, reduction in crypt depth, anti-flatulence ability and anti-microbial activity according to Mathe *et al.*(1996); Jang *et al.* (2004); and Srinivasan *et al.* (2007). It has been found that *Piper nigrum* leaf extract inhibits the growth of pseudomonas aeruginosa describes the anti microbial activity of volatile oils of black pepper against *Bacillus subtilis*, *P.*

aeruginosa, *Aspergillus niger*, *Candida albicans* and *Saccharomyces cervisisae*. Investigation of Abou–Elkhair *et al*. (2014) showed that black pepper in broiler nutrition had influence on improved health status through increase of serum globulin concentration. Many researchers proved that an increase in body weight and decrease in feed efficiency when used this herbal plant in broiler diets (Greathead *et al*., 1999; Iqbal *et al*., 2011).

2.3 EFFECT OF INCLUSION OF BLACK PEPPER ON THE PERFORMANCE OF POULTRY

Harthi *et al*. (2002) studied the effects of feeding black pepper, hot pepper, canella and neomycin on the performance of broiler chicks at different doses individually in trial 1 and 2 or as a mixture without or with neomycin in trial 3 as compared to herbs and spices free diet and antibiotic (Neomycin) supplemented diet. Results revealed that 0.1 % of black pepper, 0.2 % or 0.3 % of hot pepper and 0.2 % of canella decreased total feed intake.There was an additive effect of 2.7 % of black pepper when added over neomycin on growth of broilers reared in battery ($P<0.07$) and a 3.1 % non-significant improvement in FCR during 18-42 d of age.

Myandoab *et al*. (2011) conducted an experiment to study the effect of using different levels of black pepper on the performance of Japanese quails. A total of 500 quails were divided in to 4 groups and 5 repetitions with 25 quails each *viz*., Control group (G1) did not receive any black pepper, 1% of black pepper (G2), 1.5% of black pepper (G3), 2% of black pepper (G4). Results revealed that using black pepper had significant effects on performance ($P<0.05$). Supplementation of Japanese quails with black pepper at 2% level has improved the daily feed intake and body weight gain and at 1.5% level has improved the FCR when compared to control.

An experiment was conducted to establish the effects of using different levels of black pepper on the performance of broiler chickens. The study was carried out with 225 broiler chickens in 5 treatment groups and 3 repetitions for each group with 15 chickens. Experimental groups include control group fed basal diet (T_1), basal diet supplemented with 1% probiotic (*L.acidophilus* and *L.casei*) (T_2), 0.5% of black pepper (T_3), 0.75% of black pepper (T_4) and 1% black pepper (T_5). The results indicated that using different level of black pepper had significant effects on performance ($P<0.05$). (Mansoub *et al*., 2011)

An experiment was conducted to study the efficiency of utilization of feed supplemented with mixture of Black pepper (BP) and Hot red pepper (Hrp) to broiler on its productive performance. A total of 300 day old unsexed chicks were divided in to five groups of 60 birds each and were allocated to five feeding treatments. Group 1 is considered as a control group, Group 2, 3, 4 and 5 involved the addition of 0.25, 0.5, 0.75 and 1% of a mixture of BP and Hrp respectively. Results indicated that broilers supplemented with black pepper at 0.75 and 1%

levels in the diet performed a highly significant ($P \leq 0.05$) average in live weight gain, feed consumption and feed conversion ratio. (AI-Kassie *et al*., 2012).

An experiment was conducted to study the effects of turmeric rhizome powder (TRP) and black pepper (BP) on production performance of male broiler chickens. A total of 288 day-old male chicks were obtained from a commercial hatchery. A 2*3 factorial experiment with two levels of dietary TRP (0 and 0.5 g/kg), and three levels of BP (0, 0.5 and 1 g/kg) was used in a completely randomized design. Each of the 6 assay mash diets was fed to 4 replicates of 12 chicks each for 21 days. Results indicated that Supplementation of diet with 1g BP significantly reduced FCR in the first week; however, this pronounced effect was not observed in the later weeks.The BWG and FI were not influenced by BP. (Akbarian *et al*., 2012).

Molla *et al.* (2012) studied the effects of using an extract of nishyinda leaves, black pepper and cinnamon on growth performance of broilers. A total of 20 day- old broiler chicks were divided in to 2 equal groups. Group A served as control while group B was supplemented with polyherbal extract @ 1 ml / litre in drinking water. Weekly body weight gain up to six weeks was measured. They reported that the polyherbal extract significantly ($P<0.05$) improved weight gain.

In another study, Nath *et al*. (2012) evaluated the influence of effect of using an extract of tulsi, black pepper and cloves on performance of broilers. A total of 20 day-old broiler chicks were randomly divided in to two equal groups A and B. Group A served as control without any supplement while group B was supplemented with Tulsi, black pepper and Clove extract @ 1 ml / litre in drinking water. Results indicated that supplementation of TBC extract played a vital role in gaining body weight in the treatment group B which gained significantly ($P<0.01$) higher body weight in comparision to control group A.

Ghaedi *et al.* (2013) investigated the effect of using virginiamycin, black pepper extract on the productive performance of broiler chicks. A total of 240 one day Ross 308 broiler chicks were divided in to 4 groups of 20 birds each and assigned to 3 treatment diets. Chicks were fed by $T_{1,}$ basal diet as control; $T_{2,}$ basal diet with virginiamycin powder 200 (g/ton); $T_{3,}$ basal diet with black pepper extract in their water 2 (mg/ml). Results revealed that broiler chicks fed on diet with black pepper extract in their water at 2 mg/ml level has significantly improved ($P<0.05$) the body weights and feed to gain ratios when compared to control.

An experiment was conducted to study the effects of red pepper, black pepper and their mixture powder on productive performance of broiler chicks. A total of 320 one day old Ross 308 chicks were fed by basal diet as control, 0.02 % red pepper (T_1), 0.02 % black pepper (T_2) and 0.01 % red pepper + 0.01 % black pepper powders (T_3). Results revealed that chicks fed with red pepper, black pepper and mixed powders had higher feed intake, body weight gain and lower ($P<0.05$) FCR (Shahverdi *et al.*, 2013).

Tripathi *et al*. (2013) conducted an experiment to determine the effect of using ajwain, hot red pepper and black pepper on the performance of Japanese quail. A total of one hundred twenty broiler Japanese quails were divided in complete randomized block design, into control (C) and three treatment groups (3 each) viz: $T_{1,}$ T_2 and T_3 with 30 birds in each group. The diet offered to control group was without feed additive while in treatment groups 0.5% ajwain, 0.5% hot red pepper and 0.25 % black pepper respectively were provided to the birds through the basal diet. The results indicated that Japanese quails supplemented with black pepper at 0.25 % level has significantly improved the FCR and body weight gain when compared to control.

In another study, Valliollahi *et al.* (*2013*) investigated the effects of feeding ginger, black pepper on the production performance of broiler chicks. A total 240 one day broiler chicks were randomly divided in to four groups of 15 birds each and assigned to 4 treatment diets. Chicks were fed by basal diet as control diet, 2% ginger (T_1), 2% black pepper (T_2) and 1% ginger + 1% black pepper powders (T_3). Results revealed that broilers supplemented with black pepper at 2% level and mixture powders of black pepper and ginger at 1% and 1% levels respectively, significantly increased ($P<0.05$) the feed intake and body weight in comparision to control.

Abou-Elkhair *et al.* (2014) conducted an experiment to study the effect of black pepper, turmeric powder and coriander seeds on the performance of broiler chickens. A total of two hundred and ten one day old cob 500 broiler chicks were randomly assigned to 7 treatments, each treatment comprised 3 replicates with 10 birds each. The groups were allocated as basal diet with no supplement (control), basal diet +0.5% black pepper (T_1), basal diet +0.5 % turmeric powder (T_2), basal diet + 2 % coriander seeds T_3), basal diet + mixture of black pepper and turmeric powder (T_4), basal diet + mixture of black pepper and coriander seeds (T_5) and basal diet + mixture of black pepper, turmeric powder and coriander seeds (T_6). Results revealed that higher values of final body weight gain ($P<0.01$) during the whole period of 5 weeks were observed in broilers on $T_{1,}$ $T_{3,}$ T_5 and T_6 compared with chickens in the control group.

Faham *et al*. (2014) studied the effect of using biostrong, lactobac, pungent substances (black pepper, red pepper and ginger) and zinc bacitracin on the performance of broilers. One hundred and fifty unsexed one day old age Hubbard chicks were weighed and randomly allocated for four dietary treatment groups. The 1st group was fed with basal diet (control), while the 2nd, (T_1), 3rd (T_2), 4th (T_3) and 5th (T_4) groups were fed the basal diets supplemented with biostrong, probiotic, pungent substances (ginger 166g/ton, black pepper 167g/ton and red pepper 167 g/ton) and zinc bacteriacin at the level of 150 g, 1000 g, 500g and 500g/ton, respectively. The results revealed that broilers fed on diet with pungent substances significantly decreased ($P\leq0.05$) the feed intake and increased the feed conversion at 0-18 days.

Later, Mohamed *et al*. (2014) studied the effects of using mixture powder levels of red pepper and black pepper as natural feed additives on productive performance in broiler chicks. A total of 160 one-day old, unsexed broiler chicks were randomly divided in to four experimental groups. Each group was further subdivided into five replicates at the rate of eight chicks per pen in complete randomized design.The birds were fed on two basal diets (starter and finisher diet). The first group (A) fed on basal diet with out feed additives (control diet).The other groups (B), (C) and (D) were fed on basal diets supplemented with different mixture powder levels of 0.4% (0.2% red pepper + 0.2% black pepper), 0.6% (0.3% red pepper +0.3% black pepper) and 1% (0.5% red pepper +0.5% black pepper) respectively. The experimental diets were fed for 6-weeks duration. It was concluded that, broilers fed on diet with 1% mixture powder of red and black peppers had significantly ($P<0.05$) heaviest body weight gain, higher feed intake, and best feed conversion ratio.

In another study, Puvaca *et al*. (2014) investigated the effect of using black pepper, hot red pepper and garlic on the performance of broilers. A total of 1200 hybrid line Hubbard broiler chickens were divided in to eight treatments consisting of four replicates in each treatment. Control treatment (T1) was fed with standard commercial mixtures based on corn meal and soybean meal. Experimental groups were fed with same commercial mixtures with addition of spice herbs as following: garlic 0.5 (T2) and 1.0 g/100 g (T3), black pepper 0.5

(T4) and 1.0 g/100 g (T5), hot red pepper 0.5 (T6) and 1.0 g/100 g (T7) and mixture of garlic, black pepper and hot red pepper (1:1:1) in total of 0.5 g/100g (T8). The results indicated that addition of black pepper at levels of 0.5 and 1 % led to a statistically significant ($P<0.05$) lower body weight compared to other experimental treatments, but with out significant differences ($P>0.05$) compared to control treatment.

Valliollahi *et al.* (2014) conducted a study to evaluate the effect of feeding diets containing ginger and black pepper on the performance of broiler chicks. A total 240 one day broiler chicks were randomly divided in to four treatments. Each treatment was further divided in to four replicates. Chicks were fed a basal diet as control group (T_1), basal diet with 0.02% ginger powder (T_2), basal diet with 0.02 % black pepper powder (T_3) and basal diet with 0.01% ginger + 0.01% black pepper powder (T_4). Results revealed that feed intake, body weight gain and total body weight increased significantly in T_3 and T_4 in comparision to control diets ($P<0.05$).

An experiment was conducted to study the effect of piperine as a phytogenic feed additive on quails performance and egg quality.The experiment used a completely randomized design with five treatments and four replications and used ten quails with one week of age in each replication.The piperine was added to the diets at concentrations of 0 (T0), 15 (T1), 30 (T2), 45 (T3) and 60 mg/kg body weight (T4) for 8 consecutive weeks.The results indicated that addition of 60 mg/kg body weight (T4) of piperine significantly (P<0.05) reduced feed consumption, egg production and egg mass as compared to the other treatments.(Hilmi *et al.,* 2014).

Recently, Ndelekwute *et al.* (2015) conducted an experiment to study the effect of different levels of ground black pepper on the growth performance. One hundred and fifty day old chicks of Arbor-acre strain were divided into five dietary groups having three replication with 10 in each.There were five dietary treatments each containing 0, 0.25, 0.50, 0.75 and 1.0 % black pepper (BP) fed at both starter and finisher phases. The results revealed that at the starter phase 0.25 and 0.5% BP improved live weight significantly (P<0.05). Feed intake was significantly (P<0.05) reduced at 1.0% BP. There were no significant differences (P>0.05) in daily gain and feed:gain ratio. At the finisher phase, final live weight was improved by 0.25% (P<0.05). Final live weight, feed intake, weight gain and feed:gain ratio were negatively affected by 0.75 and 1.0 % BP.

2.4 EFFECT OF INCLUSION OF BLACK PEPPER ON SERUM BIOCHEMICAL PROFILE

Myandoab *et al.* (2011) studied the effects of using black pepper at levels 0, 1, 1.5 and 2 % on blood constituents of japanese quails and reported that use of black pepper at 1, 1.5 and 2 % levels as feed additive resulted in reduced concentration of serum total cholesterol and triglycerides compared to the control group ($P<0.05$). But there is no effect on total protein and albumin.

Mansoub *et al*. (2011) studied the effect of using different levels of black pepper on certain blood biochemical parameters in broiler chickens and concluded that black pepper at 0.5, 0.75 and 1% levels have beneficial effect on cholesterol metabolism that resulted in reduced serum cholesterol and triglyceride concentrations and increased HDL cholesterol concentration in broilers.

Akbarian *et al.*(2012) evaluated the influence of dietary supplementation of turmeric rhizome at levels 0 and 0.5 g / kg and black pepper at levels 0, 0.5 and 1 g / kg on cholesterol metabolism and reported that their interaction did not have a significant effect ($P>0.05$) on LDL, HDL and cholesterol in serum of chicks.

AI-Kassie *et al*. (2012) studied the efficiency of utilization of feed supplemented with mixture of Black pepper (BP) and Hot red pepper (Hrp) to broiler on its blood plasma and reported that feeding of mixture of black and hot red pepper powders at 0.75 and 1% levels in the diet decreased significantly ($P<0.05$) the total cholesterol concentration in broilers.

Shahverdi *et al*. (2013) evaluated the influence of dietary supplementation of red pepper, black pepper and their mixtures on serum biochemical profile of broiler chicks and reported that supplementation of 0.02 % black pepper and mixture of 0.01 % red pepper + 0.01 % black pepper resulted in decrease in the concentrations of blood calcium and H/L ratio ($P<0.05$).

Valiollahi *et al*. (2013) studied the effects of using 2 % ginger (T1), 2 % black pepper (T2) and 1 % ginger + 1 % black pepper powders (T_3) on certain blood biochemical parameters in broiler chicks and reported that feeding of mixture of ginger and black pepper at 1% and 1% levels in the diet decreased significantly (P<0.05) the tryglyceride content but they increased the amount of blood cholesterol significantly in broiler chicks.

Abou-Eikhair *et al.* (2014) evaluated the influence of dietary supplementation of black pepper, turmeric powder and coriander seeds on certain blood biochemical parameters in broiler chicks and reported that feeding of black pepper at level of 0.5 % and turmeric powder at level of 0.5 % mixtures showed significantly higher concentrations of serum total protein (P<0.05) and Serum globulin concentration (P<0.01).

Faham *et al.* (2014) studied the effect of using biostrong (T_1), lactobac (T_2)$_)$, pungent substances (black pepper, red pepper and ginger-T_3) and zinc bacteriacin (T_4) on certain blood bio chemical parameters of broilers and reported that broilers fed with pungent substances (ginger 166gm/ton, black pepper 167gm/ton and red pepper 167 gm/ton) recorded the best ratios and lowest cholesterol concentrations than control.

Puvaca *et al*. (2014) studied the effect of dietary supplementation of black pepper, hot red pepper and garlic on blood biochemical parameters of broilers and reported that the supplementation of black pepper at 0.5 and 1.0g /100g levels resulted in decrease in the concentrations of cholesterol, triglyceride and glucose in plasma ($P<0.05$).

Valliollahi *et al.* (2014) studied the effect of using ginger and black pepper on certain blood biochemical parameters of broiler chicks and reported that supplementation of 0.02% black pepper in the diet decreased ($P<0.05$) significantly the total cholesterol and triglyceride concentration and increased phosphorous concentration ($P<0.05$).

2.5 EFFECT OF INCORPORATION OF BLACK PEPPER ON NUTRIENT DIGESTIBILITY

Puvaca *et al.* (2014) studied the effects of inclusion of black pepper on crude fat digestibility in broilers and reported that supplementation of black pepper at 0.5 g / 100 g corn-soy diet increased significantly ($P<0.05$) the digestibility of crude fat compared to the conrol.

Ndelekwute *et al.* (2015) studied the effects of dietary supplementation of black pepper at varying levels on nutrient digestibility of broilers and results indicated that supplementation of black pepper at 0.25, 0.5, 0.75 and 1% levels significantly influenced ($P<0.05$) the digestibility of dry matter, crude protein and ether extract when compared to the control. While, Digestibility of nitrogen free extract and metabolizable energy utilization were significantly ($P<0.05$) reduced by black pepper at 1 % but were improved at 0.25 and 0.5 %.

2.6 EFFECT OF INCORPORATION OF BLACK PEPPER ON CARCASS CHARACTERISTICS

Myandoab *et al.* (2011) evaluated the effects of black pepper at levels 0, 1, 1.5 and 2 % on the visceral organs of japanese quails and reported that supplementing the diet with black pepper at 2% level in quails has significantly increased ($P<0.05$) the percent of breast and gizzard compared to the control.

Mansoub *et al.*(2011) studied the effect of using different levels of black pepper on the visceral organs of broiler chickens and reported that supplementing the diet with black pepper at 1% level in broiler chickens has significantly increased ($P<0.05$) the percent of liver compared to the control.

Al-Kassie *et al*. (2012) studied the effect of using different levels of hot red pepper and black pepper on blood parameters of broilers and reported that, the inclusion of mixture of hot red pepper and black pepper at levels 0.75 and 1% in the diets improved significantly ($P<0.05$) the dressing percentage of broilers and no significant difference ($P>0.05$) was reported on the percentage of edible giblets liver, heart and gizzard.

Nath *et al*. (2012) studied the effect of using an extract of tulsi, black pepper and cloves extract @ 1 ml / litre (TBC) in drinking water on carcass characteristics of broilers and reported that supplementation of TBC extract @ 1 ml / litre had no significant effect (P>0.05) on dressing percentages, relative gizzard weights and relative spleen weight.

In another study, Ghaedi *et al*. (2013) investigated the effect of using virginiamycin, black pepper extract on carcass characteristics of broilers and found that, chicks fed on basal diet using black pepper extract in their water at 2 mg / ml, significantly (P<0.05) produced higher carcass yeild percentage and significantly reduced (P<0.05) abdominal fat percentage when compared to control.

Sahverdi *et al.* (2013) studied the effects of using red pepper at level (0.02 %), black pepper at level (0.02 %) and mixture at levels (0.01 +0.01 %) on carcass characteristics in broiler chicks and reported that supplementation of 0.02 % Black pepper and mixture of both in broilers has significantly (P<0.05) increased the percent of liver and drum stick and reduced abdominal fat (P<0.05) compared to control group.

Valiollahi *et al.* (2013) investigated the effects of feeding ginger, black pepper on the visceral organs of broiler chickens and reported that supplementing the diet with mixture of ginger and black pepper at 1 % and 1 % levels, there were no significant differences in heart percentage and reduced abdominal fat percentage statistically ($P<0.05$). The group fed with 2 % black pepper in broiler chickens had no significant effect ($P>0.05$) on the percent weight of breast compared to control group.

Faham *et al*. (2014) studied the effect of using biostrong (T_1), lactobac (T_2), pungent substances black pepper, red pepper and ginger-T_3 and zinc bacitracin (T_4) on carcass characters of broilers and reported that broilers fed with pungent substances at 500 g / ton level, (ginger 166 g / ton, black pepper 167 g / ton and red pepper 167 g/ ton) had no significant effect ($P>0.05$) on all characteristics except for total edible parts.

Mohamed *et al.* (2014) studied the effects of using mixture powder levels of red pepper and black pepper as natural feed additives on the dressing percentage of broiler chicks and reported that broilers fed on diet with powder of 0.5 % of red pepper + 0.5% black pepper mixture yeilded significantly ($P<0.05$) highest hot and cold dressing percentages and highest commercial cuts percentages (breast, drumstick and thigh) and significantly ($P<0.05$) decreased the abdominal fat percentage and increased the liver and gizzard percentages compared to the control diet.

Puvaca *et al*. (2014) studied the effect of using black pepper, hot red pepper and garlic on dressing percentage of broilers and reported that broilers supplemented with black pepper in the diet at 1% level has significantly increased ($P<0.05$) the dressing and commercial cuts percentages (breast, drumstick and thigh) and had no significant effect on percentages of edible giblets (liver, heart and gizzard) ($P>0.05$).

Valliollahi *et al.* (2014) studied the effect of using ginger and black pepper on carcass characters of broiler chicks and reported that feeding broiler chicks with 0.02% black pepper in the diet has significantly decreased ($P<0.05$) the weight of liver, while the weight of drumstick and gizzard increased significantly ($P<0.05$) when compared to the control.

2.7 EFFECT OF INCORPORATION OF BLACK PEPPER ON COST ECONOMICS

Faham *et al.* (2014) studied the effect of using biostrong (T_1), lactobac (T_2), pungent substances (black pepper, red pepper and ginger-T_3) and zinc bacteriacin (T_4) on cost economics. Results indicated that during the period from 1 to 32 days of age, chicks fed diet supplemented with pungent substances had the best economical and relative efficiency values.

Mohamed *et al.(*2014) studied the effect of using red pepper and black pepper on cost economics and reported that Supplementation of basal broiler diet with mixture of red pepper and black pepper resulted in improved economical efficiency. Further, they reported that the best economical and relative economical efficiencies were recorded for broilers fed basal diet supplemented with 1 %

mixture powder level (0.5 % red pepper +0.5 % black pepper) showed highest profitability ratio (1.81) compared to control group.

CHAPTER III

MATERIALS AND METHODS

The present experiment was planned to study the effect of inclusion of black pepper seed as natural feed additive on the performance of Japanese quails. The research work was carried out in the Poultry Experimental Unit attached to the Instructional Livestock Farm Complex, N.T.R College of Veterinary Science, Gannavaram (A.P).

3.1 PROCUREMENT OF FEED INGREDIENTS

Black pepper, de-oiled rice bran, maize, soybean meal, fish meal, mineral mixture (Polchem hygiene laboratories) and salt were procured from the local market and were ground and mixed in the Feed Mixing Plant attached to Department of Animal Nutrition.

3.2 CHEMICAL ANALYSIS

The chemical composition of black pepper and other feed ingredients used in the experiment and the experimental diets were determined as per AOAC (2007), while the calcium and phosphorus contents were estimated as per Talapatra *et al*. (1940).The cell-wall constituents of black pepper seed and other feed ingredients used in the experiment and experimental diets were estimated as per Van Soest *et al.* (1991). Hemi-cellulose was calculated as the difference between NDF and ADF.

3.3 EXPERIMENTAL DESIGN

The experiment was carried out for 5 weeks in a completely randomized design (CRD) experiment. During the experiment, black pepper seed was ground and is included at 0% (T_1; Control), 0.25% (T_2), 0.50% (T_3), 0.75% (T_4) and 1.0% (T_5) levels in iso-caloric and iso-nitrogenous broiler quail diets. The broiler quail diets were formulated according to NRC, 1994 specifications. One hundred and fifty day-old Japanese quail chicks were weighed individually (avg. b. wt. 7.42 ± 0.38 g), wing banded and randomly divided into 5 equal groups of 3 replicates each with 10 chicks / replicate. Each group of quail was allotted to one of the dietary treatments at random. The data for growth rate and feed intake were recorded at weekly intervals.

3.4 MANAGEMENT AND FEEDING

All the chicks were housed in battery brooders all through the experiment. Feed and water were provided *ad libitum*. The chicks were fed on respective experimental diets *ad libitum* from 0 to 5 weeks. The feed offered and feed leftover was weighed daily, to quantify the feed utilized. B-complex vitamins and antibiotics were offered in water for 3 days during first week.

3.5 NUTRIENT UTILIZATION

3.5.1 Digestibility of nutrients

During the last 3 days of the trial, faeces were collected from all the 5 treatments of 3 replicates which were previously fasted for 12 hours to empty the birds gut to mark the beginning and end of faeces collection. The faeces collected were oven-dried for a period of 18 hours at a temperature of about 105^0C and weighed daily. At the end of the collection period, the faecal samples collected from each treatment per day were pooled, ground and thoroughly mixed to obtain a homogenous mixture. Samples of faeces were subjected to proximate analysis according to standard methods outlined (AOAC, 2007) and the results obtained were used to calculate the apparent digestibility using the following formula.

$$\text{Apparent digestibility coefficient} = \frac{\text{Nutrient in feed} - \text{Nutrient in faeces}}{\text{Nutrient in feed}} \times 100$$

3.5.2 Nitrogen utilization in rations

Total excreta were collected at the end of experiment for 3 days in each group and the dry matter content of excreta was estimated.Total excreta for each group was pooled, dried and stored for further analysis. Feed intake for 3 days was calculated for each group.Total nitrogen in feeds and excreta were determined using Kjeldahl method (AOAC, 2007) and the nitrogen utilization was calculated as follows:

$$\text{N utilization (\%)} = \frac{(\text{N intake} - \text{N excretion})}{\text{N intake}} \times 100$$

3.6 CARCASS CHARACTERISTICS

At the end of study period (5th week), two birds per replicate and thus a total 6 birds per treatment were randomly selected, weighed and slaughtered. The data on dressing percentage, carcass yield, meat bone ratio, ready-to-cook yield and percent weights of heart, liver and gizzard were recorded. At the time of slaughter, blood samples were collected from each bird to determine serum cholesterol, proteins, triglycerides, glucose, creatinine, calcium and phosphorous.

3.7 SERUM BIOCHEMISTRY

3.7.1 Estimation of Serum cholesterol

Cholesterol was estimated using diagnostic kit (M/s. Span Diagnostics Private Limited) by enzymatic method of Allian (1974) for *in vitro* estimation.

Principle

Cholesterol esterase hydrolyzes cholesterol ester and the free cholesterol thus formed is oxidized by the cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide.The hydrogen peroxide so formed reacts with 4-amino antipyrine and phenol in the presence of peroxides (POD) to produce red coloured quinoneimine dye. The intensity of colour produced is proportional to the cholesterol concentration which is measured colorimetrically at 505 nm.

Procedure

	Blank (B)	**Standard (S)**	**Test (T)**
Serum	-	-	10 µl
Cholesterol Standard	-	10 µl	-
Cholesterol reagent	1000 µl	1000 µl	1000 µl

Three test tubes were labeled as blank (B), standard (S) and test (T). 1 ml of cholesterol reagent was added to all the three test tubes. 10 µl of cholesterol standard and 10 µl serum were added to test tubes labeled as standard and test, respectively. Tubes were mixed well and incubated at 37°C for 10 minutes. Absorbance of test (T), standard (S) against blank (B) were recorded at 505 nm

Calculation

$$\text{Cholesterol (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of standard}} \times 200$$

3.7.2 Estimation of HDL-C, LDL-C and VLDL-C

HDL-C was estimated using diagnostic kit (M/s. Span Diagnostics Private

Limited) and following the PEG precipitation and enzymatic method of Weibs and Smith (1985).

Principle

Low and very low-density lipoproteins are precipitated by a solution containing PEG 6000, leaving behind the HDL in solution. HDL cholesterol is estimated in the supernatant by a series of enzymatic reactions which are initiated by the oxidation of cholesterol to cholestenone (Cholest-4-en-3-one) by cholesterol oxidase, accompanied by the formation of hydrogen peroxide. In a second reaction catalyzed by peroxidase 4-amino antipyrine, phenol reacts with hydrogen peroxide to form pink coloured quinoneimine. Absorbance at 505 nm is directly proportional to HDL cholesterol concentration.

STEP-A

HDL-Cholesterol separation

The precipitating reagent 0.2 ml and serum 0.2 ml were mixed well and allowed to stand at room temperature for 10 min, and centrifuged at 2000 rpm for 15 min, to get clear supernatant.

STEP-B

HDL-Cholesterol estimation

	Blank (B)	**Standard (S)**	**Test (T)**
Supernatant from Step A	-	-	100 µl
HDL-Cholesterol Standard	-	100 µl	-
Cholesterol reagent	1000 µl	1000 µl	1000 µl

Three test tubes were taken and labeled as blank (B), standard (S) and test (T). In second and third test tubes, 100µl of HDL-standard and 100 µl of supernatant from step A were taken, respectively. Cholesterol reagent, 1000 µl was added to all the three test tubes.

The tubes were mixed well and the absorbance of test (T) and standard (S) against blank (B) are recorded at 505 nm.

Calculation

$$\text{HDL-Cholesterol (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of standard}} \times 50 \times 2$$

$$\text{LDL-Cholesterol} = \text{Total Cholesterol} - \left(\frac{\text{Triglycerides}}{5}\right) - \text{HDL Cholesterol}$$

VLDL-Cholesterol = Total Cholesterol – (HDL-C + LDL-C)

3.7.3 Estimation of Triglycerides

Triglycerides were estimated by using diagnostic kit (M/s. Span Diagnostics Private Limited) using the enzymatic method of Gowan (1983).

Principle

Triglycerides from serum are hydrolyzed by lipoprotein lipase (LPL) to produce glycerol and free fatty acid (FFA).In presence of glycerol kinase (GK), Adenosine Triphosphate phosphorylates glycerol to produce Glycerol 3-Phosphate which is further oxidized by Glycerol 3-Phosphate oxidase (GPO) to produce Dihydroxy Acetone Phosphate (DAP) and H_2O_2. In presence of peroxidase hydrogen peroxide couples with 4-Aminoantipyrine and 4-Chlorphenol to produce red coloured quinoneimine dye. Absorbance of coloured dye is measured at 505 nm. The intensity of colour produced is directly proportional to the concentration of triglycerides in the sample.

Procedure

	Blank (B)	**Standard (S)**	**Test (T)**
Serum	-	-	10 µl
Triglyceride Standard	-	10 µl	-
Triglyceride mono reagent	1000 µl	1000 µl	1000 µl

Three test tubes were taken labeled as blank (B), standard (S) and test (T).

1000 µl of enzyme reagent was taken in all 3 test tubes. 10 µl of triglyceride standard, 10 µl of serum were added to test tubes labeled standard and test, respectively. Absorbance of standard (S) and test (T) were recorded against blank (B) at 505 nm.

Calculation

$$\text{Triglycerides (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of standard}} \times 200$$

3.7.4 Estimation of total proteins in serum

Serum total proteins were estimated by using diagnostic kit (M/s. Span Diagnostics Private Limited) following modified Biuret method

Principle

Peptide bonds of proteins react with cupric ions in alkaline medium to give a violet coloured chelate, the absorbance of which is measured at 578 nm. The absorbance of final colour is proportional to the total protein concentration in the sample.

Procedure

	Blank (B)	Standard (S)	Test (T)
Serum	-	-	10 µl
Protein Standard	-	10 µl	-
Biuret reagent	1000 µl	1000 µl	1000 µl

Test tubes were taken and labeled as blank (B), standard (S) and test (T). One ml of biuret reagent was added in all the test tubes. 0.01 ml of standard and 0.01 ml of serum were added to the test tubes labeled as standard and test, respectively.Tubes were mixed well and incubated at 37°C for 5 minutes. Absorbance of standard (S) and test (T) against blank (B) were measured at 578 nm.

Calculation

$$\text{Total protein (g/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of standard}} \times 6.5$$

3.7.5 Estimation of serum albumin

The serum albumin was estimated by using diagnostic kit (M/s. Span Diagnostics Private Limited) using the bromocresol green (BCG), end Point assay method.

Principle

At pH 3.68, albumin binds with bromocresol green (BCG) in a buffered medium to produce a green coloured complex. The absorbance of final colour is measured at 630 nm. The intensity of this colour is proportional to the albumin concentration in the sample.

Procedure

	Blank (B)	**Standard (S)**	**Test (T)**
Serum	-	-	10 µl
Albumin Standard	-	10 µl	-
Albumin reagent	1000 µl	1000 µl	1000 µl

Three test tubes were labeled as blank (B), standard (S) and test (T). 1 ml of BCG reagent was added in all three test tubes. 0.01 ml of standard and 0.01 ml of serum were added to test tubes labeled as standard (S) and test (T), respectively.The tubes were mixed well and the absorbance of standard (S) and test (T) against blank (B) were measured at 630 nm.

Calculation

$$\text{Albumin (g/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of standard}} \times 4$$

Globulins = Total Protein – Albumin

$$\text{A/G ratio} = \frac{\text{Albumin}}{\text{Globulins}}$$

3.7.6 Estimation of serum calcium

The serum calcium was estimated by using diagnostic kit (M/s. Span Diagnostics Private Limited).

Principle

In alkaline solution, calcium binds with metal complexing dye O-Cresolphththalein Complex one (OCPC) to form a Bluish - Purple complex, which is measured at 578 nm. The intensity of colour formed is proportional to calcium concentration in the Sample. Hydroxyquinoline will act as a masking agent and eliminate the interference of magnesium.

Procedure

	Blank (B)	**Standard (S)**	**Test (T)**
Serum	-	-	20 µl
Calcium Standard	-	20 µl	-
Working calcium reagent	1000 µL	1000 µL	1000 µL

Three test tubes were labeled as blank (B), standard (S) and test (T). 1 ml of working calcium reagent was added in all tubes. 0.02 ml of standard and 0.02 ml of serum were added to test tubes labeled standard (S) and test (T), respectively. Test tubes were mixed well and the absorbance of standard (S) and test (T) against blank (B) were measured at 578 nm.

Calculation

$$\text{Calcium (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of standard}} \times 10$$

3.7.7 Estimation of serum Phosphorous

The serum Phosphorous was estimated by using diagnostic kit (M/s. Span Diagnostics Private Limited).

Principle

In acidic medium, inorganic phosphorous reacts with ammonium molybdate to form phosphomolybdate complex. This colourless phosphomolybdate complex is measured at 340 nm and is directly proportional to the concentration of inorganic phosphorous in the sample.

Procedure

	Blank (B)	Standard (S)	Test (T)
Serum	-	-	10 µl
Phosphorous Standard	-	10 µl	-
Molybdate reagent	1000 µL	1000 µL	1000 µL

Three test tubes were labeled as blank (B), standard (S) and test (T). 1 ml of working molybdate reagent was added in all tubes. 0.01 ml of phosphorous standard and 0.01 ml of serum were added to test tubes labeled standard (S) and test (T), respectively. Test tubes were mixed well and the absorbance of standard (S) and test (T) against blank (B) were measured at 340 nm.

Calculation

$$\text{Inorganic Phosphorous (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of standard}} \times 5$$

3.7.8 Estimation of serum glucose

The serum glucose was estimated by using diagnostic kit (M/s. Span Diagnostics Private Limited).

Principle

Glucose oxidase (GOD) oxidises glucose to gluconic acid and hydrogen peroxide. In presence of enzyme peroxidase, released hydrogen peroxide is coupled with phenol and 4-aminoantipyrine (4-AAP) to form coloured quinoneimine dye. Absorbance of coloured dye is measured at 505 nm and is directly proportional to Glucose concentration in the sample.

Procedure

	Blank (B)	**Standard (S)**	**Test (T)**
Serum	-	-	20 µl
Glucose Standard	-	20 µl	-
Working Glucose Reagent	1500 µL	1500 µL	1500 µL
Mix well. Incubate at 37^0C for 10 minutes or at room temperature (15 – 30^0C) for 30 minutes.			
Purified water	1500 µL	1500 µL	1500 µL

Three test tubes were labeled as blank (B), standard (S) and test (T). In each test tube 1.5 ml of Working Glucose reagent was added. 0.02 ml of Glucose standard and 0.02 ml of serum were added to test tubes labeled standard (S) and test (T), respectively. The tubes were mixed well and incubated at 37^0C for 10 minutes. In each test tube 1.5 ml of purified water was added and the absorbance of standard (S) and test (T) against blank (B) were measured at 490-550 nm.

Calculation

Absorbance of Test

Glucose (mg/dl) = --------------------------------------- x 100

Absorbance of standard

3.7.9 Estimation of serum creatinine

The serum creatinine was estimated by using diagnostic kit (M/s. Span Diagnostics Private Limited) by following Jeffe's method.

Principle

Creatinine reacts with alkaline picrate to produce an orange-yellow colour (the Jeffe's reaction). Specificity of the assay has been improved by the introduction of an initial rate method. The absorbance of the orange-yellow colour formed is directly proportional to creatinine concentration and is measured colorimetrically at 500-520 nm.

Procedure

	Blank (B)	**Standard (S)**	**Test (T)**
Distilled Water	1000 µl	-	-
Serum	-	-	100 µl
Creatinine Standard	-	100 µl	-
Working Creatinine Reagent	-	1000 µl	1000 µl

Three test tubes were labeled as blank (B), standard (S) and test (T). In blank (B) test tube 1 ml of distilled water was added. In standard (S) and test (T) test tubes 1 ml of Working Creatinine reagent was added. 0.1 ml of Creatinine standard (concentration 2 mg/dl) and 0.1 ml of serum were added to test tubes labeled standard (S) and test (T), respectively. The tubes were mixed well and read initial absorbance (A_1) 20 seconds after mixing and final absorbance (A_2) 80 seconds after mixing against blank at 505 nm.

Calculation

$$\Delta A = A_2 - A_1$$

$$\text{Creatinine (mg/dl)} = \frac{\Delta A \text{ of Test}}{\Delta A \text{ of Standard}} \times \text{Concentration of standard}$$

3.8 COST ECONOMICS

The relative economy of rearing quails up to 5 weeks of age by incorporating black pepper seed at varying levels in the diets was calculated based on the actual feed cost at the prevailing market.

3.9 STATISTICAL ANALYSIS

Statistical analysis of the data was carried out according to the procedures suggested by Snedecor and Cochran (1993).

CHAPTER - IV

RESULTS

The results on various aspects pertaining to chemical composition, digestibility of nutrients, growth performance, serum biochemical profile and carcass characteristics of quails fed diets containing black pepper at graded levels are presented in this chapter.

4.1 CHEMICAL COMPOSITION OF FEED INGREDIENTS

4.1.1 Proximate composition of feed ingredients

The chemical composition of black pepper and other feed ingredients used in the present study are shown in Table 2. The percent DM, OM, CP, EE, CF, NFE, TA and AIA of black pepper powder were 92.26, 94.0, 12.87, 4.85, 20.97, 55.31, 6.0 and 0.25, respectively. The per cent calcium and phosphorous content of black pepper were 1.46 and 0.80, respectively.

The per cent DM, OM, CP, EE, CF, NFE, TA and AIA content of maize, de-oiled rice bran, soybean meal and fish meal were 92.03, 93.01, 91.09 and 86.94; 97.96, 81.75, 88.37 and 79.77; 9.12, 13.24, 46.02 and 50.57; 1.81, 1.08, 1.08 and 8.02; 2.46, 6.15, 13.03 and 3.42; 84.57, 61.28, 28.24 and 17.66; 2.04, 18.25, 11.63 and 20.23; and 0.56, 3.54, 2.98 and 4.05, respectively. The per cent calcium and phosphorous content of maize, de-oiled rice bran, soybean meal and fish meal were 0.21 and 0.39; 0.41 and 0.74; 0.19 and 0.28; 4.98 and 3.14, respectively.

4.1.2 Cell wall constituents of feed ingredients

The per cent cell wall constituents of black pepper and other feed ingredients used in the present study are shown in Table 3. The per cent NDF, ADF, hemi-cellulose, cellulose, ADL and silica content of black pepper were 52.25, 39.5, 12.75, 26.34, 1.5 and 2.1, respectively. The per cent NDF, ADF, hemi-cellulose, cellulose, ADL and silica contents of maize, de-oiled rice bran, soybean meal and fish meal were 17.29, 32.79, 25.04 and 40.21; 8.26, 19.48, 17.65 and 21.05; 9.03, 13.31, 7.39 and 19.16; 4.05, 8.19, 8.74 and 18.05; 2.35, 7.35, 4.05 and 9.05; 0.62, 2.97, 2.51 and 6.24, respectively

Table 2: Chemical composition[*] (%) of feed ingredients

Nutrient	Maize	DORB	SBM	Fish meal	Black pepper
Dry matter	92.03	93.01	91.09	86.94	92.26
Organic matter	97.96	81.75	88.37	79.77	94.00
Crude protein	9.12	13.24	46.02	50.57	12.87
Ether extract	1.81	1.08	1.08	8.02	4.85
Crude fibre	2.46	6.15	13.03	3.42	20.97
NFE	84.57	61.28	28.24	17.76	55.31
Total Ash	2.04	18.25	11.63	20.23	6.0
AIA	0.56	3.54	2.98	4.05	0.25
Calcium (%)	0.21	0.41	0.19	4.98	1.46
Phosphorus (%)	0.39	0.74	0.28	3.14	0.80

*On dry matter basis except for DM.

Table 3: Cell-wall constituents* (%) of feed ingredients

Constituent	Maize	DORB	SBM	Fish meal	Black pepper
NDF	17.29	32.79	25.04	40.21	52.25
ADF	8.26	19.48	17.65	21.05	39.5
Hemi-cellulose	9.03	13.31	7.39	19.16	12.75
Cellulose	4.05	8.19	8.74	18.05	26.34
ADL	2.35	7.35	4.05	9.05	1.5
Silica	0.62	2.97	2.51	6.24	2.1

*On dry matter basis.

4.2 INGREDIENT COMPOSITION OF QUAIL DIETS

Five diets were formulated (Table 4) incorporating black pepper at 0 (T_1), 0.25 (T_2), 0.50 (T_3), 0.75 (T_4) and 1.0 (T_5) per cent levels by marginal adjustment of other ingredients. Mineral mixture with salt was included up to 0.4 per cent level in all the diets. Feed additives like Hyblend AB_2D_3K (150 g / 100 kg), trace mineral mix (150 g/100 kg) and mycosorb (100 g/100 kg) were included in all the diets. The feed cost (₹) per kg of diet was 26.16 (T_1), 27.50 (T_2), 28.85 (T_3), 30.17 (T_4) and 31.52 (T_5), respectively.

Table 4: Ingredient composition (%) of quail diets

Constituent	T_1	T_2	T_3	T_4	T_5	Cost/kg (Rs)
Maize	47.5	47.5	47.5	47.0	47.0	16.00
DORB	7.5	7.25	7.0	7.25	7.0	13.50
Soybean meal	36.5	36.5	36.5	36.5	36.5	38.00
Fish meal	5.00	5.00	5.00	5.00	5.00	30.00
Black pepper powder	0.00	0.25	0.50	0.75	1.00	550
Oil	0.50	0.50	0.50	0.50	0.50	20.00
DCP	1.00	1.00	1.00	1.00	1.00	26.00
Shell grit	1.00	1.00	1.00	1.00	1.00	0.00
Salt	0.25	0.25	0.25	0.25	0.25	3.00
Trace min mix	0.15	0.15	0.15	0.15	0.15	240.0
Feed additives	0.60	0.60	0.60	0.60	0.60	
Total	100	100	100	100	100	
Feed cost/100 kg (Rs)	2616	2750	2885	3017	3152	

4.3 CHEMICAL COMPOSITION OF QUAIL DIETS

4.3.1 Proximate composition of quail diets

The proximate composition of quail diets is presented in Table 5. The diets were iso-caloric and iso-nitrogenous with ME (kcal/kg) and CP (%) content of 2900 K cal and 24.0 per cent, respectively. The EE, CF, TA and AIA contents were within the range of 2.16 to 3.98; 8.13 to 12.83; 9.05 to 10.16; and 2.32 to 2.72 per cent, respectively.

Calcium and phosphorous content of diets were within the range of 0.87 to 0.91and 0.75 to 0.80 per cent, respectively. The protein: energy ratio is about 1:120 in all the treatments.

4.3.2 Cell-wall constituents of quail diets

The cell-wall constituents of quail diets are presented in table 6. The NDF, ADF, hemi-cellulose, cellulose, lignin and silica of the diets ranged from 55.0 to 61.80, 19.22 to 23.16, 35.78 to 38.64, 4.23 to 7.9, 8.43 to 12.34 and 1.43 to 2.00 per cent, respectively.

Table 5: Chemical composition* (%) of quail diets

Nutrient	T_1	T_2	T_3	T_4	T_5
Dry matter	93.12	95.5	95.69	95.70	95.95
Organic matter	90.95	90.79	90.37	90.06	89.84
Crude protein	23.98	23.99	23.99	24.00	24.00
Ether extract	2.16	2.63	2.73	3.20	3.98
Crude fibre	8.13	8.48	9.30	10.31	12.83
NFE	56.68	55.69	54.35	52.55	49.03
Total Ash	9.05	9.21	9.63	9.94	10.16
AIA	2.32	2.38	2.65	2.67	2.72
ME kcal/kg (calculated)	2900	2902	2905	2902	2905
Proten: Energy ratio	1:120	1:120	1:120	1:120	1:120
Calcium (%)	0.87	0.88	0.89	0.90	0.91
Phosphorus (%)	0.75	0.76	0.77	0.79	0.80

*On dry matter basis except for DM.

Table 6: Cell-wall constituents* (%) of quail diets

Constituent	T_1	T_2	T_3	T_4	T_5
NDF	55.00	56.70	58.63	60.72	61.80
ADF	19.22	20.30	21.18	22.36	23.16
Hemi-cellulose	35.78	36.40	37.45	38.36	38.64
Cellulose	4.23	4.75	4.82	6.56	7.9
ADL	8.43	10.26	11.45	12.23	12.34
Silica	1.43	1.46	1.56	1.76	2.00

*On dry matter basis.

4.4 EFFECT OF DIETARY INCLUSION OF BLACK PEPPER ON GROWTH PERFORMANCE OF JAPANESE QUAILS

4.4.1 Effect of black pepper on body weight gain

Body weight gains during the experimental period are given in Table 7. During the experimental period (0-5 weeks), the body weight gains ranged from 187.73 to 205.36 g among different treatments. The body weight gains increased linearly from T_1 to T_5 with increased level of inclusion of black pepper from 0 to 1.0% in the diet and the differences between the treatments were significant ($P<0.05$). However, no significant difference ($P>0.05$) was observed among quails fed diets containing black pepper at 0 and 0.25 per cent levels.

4.4.2 Effect of black pepper on feed intake

Feed intakes of quails during the experimental period are given in Table 7. During the experimental period (0-5 weeks), feed intake ranged from 639.62 to 726.08 g among different treatments. The feed intake decreased linearly from T_1 to T_5 with increased level of inclusion of black pepper from 0 to 1.0% in the diet and the differences between treatments were significant ($P<0.01$). The feed intake was significantly lower ($P<0.01$) in quails fed diets containing 1.0 % black pepper as compared to those fed diets containing black pepper powder at 0, 0.25, 0.5 and 0.75 % level.

4.4.3 Effect of black pepper on feed conversion ratio

Feed conversion ratio of quails during the experimental period is given in Table 7. The feed consumed / kg gain during the experimental period (0-5 weeks) ranged from 3.11 to 3.90 among different treatments. Feed consumed / kg gain decreased linearly from T_1 to T_5 with increased level of inclusion of black pepper powder from 0 to 1.0 % in the diet and the differences between the treatments were significant ($P<0.01$). However, no significant difference ($P>0.05$) was observed among quail fed diets containing black pepper powder at 0.25 and 0.5% and 0.75 and 1.0 % levels .

Table 7: Effect of dietary inclusion of black pepper at varying levels on body weight gain (g), feed intake (g) and feed conversion ratio

Treatment	B. Wt gain (g)	Feed intake (g)	Feed Conversion Ratio
T_1	$187.73^{a} \pm 3.57$	$726.08^{e} \pm 7.07$	$3.90^{c} \pm 0.07$
T_2	$195.04^{ab} \pm 3.52$	$701.11^{d} \pm 3.37$	$3.63^{b} \pm 0.07$
T_3	$198.53^{b} \pm 3.48$	$686.09^{c} \pm 3.45$	$3.46^{b} \pm 0.07$
T_4	$202.36^{b} \pm 3.41$	$659.60^{b} \pm 3.73$	$3.26^{a} \pm 0.06$
T_5	$205.36^{b} \pm 3.70$	$639.62^{a} \pm 1.15$	$3.11^{a} \pm 0.07$
SEM	1.63	3.31	0.04
SS	*	**	**

Values in column bearing different super scripts differ significantly *(P<0.05), ** (P<0.01)

4.5 EFFECT OF DIETARY INCLUSION OF BLACK PEPPER ON SERUM BIOCHEMICAL PROFILE OF JAPANESE QUAILS

4.5.1 Effect of black pepper on total serum protein

The serum total protein content of quails fed experimental diets is given in Table 8. The serum total protein content ranged from 2.41 to 3.44 g/dl among different treatments. The serum total protein content increased significantly (P<0.01) with increased level of inclusion of black pepper from 0 to 1.0 % level in the diet. However, no significant difference (P>0.05) in serum total protein content was observed among quails fed diets containing black pepper at 0 and 0.25%, 0.50 and 0.75%, and 0.75 and 1.0 % levels.

4.5.2 Effect of black pepper on serum albumin

The serum albumin content of quails fed experimental diets is given in Table 8. The serum albumin content ranged from 1.48 to 2.04 g/dl among different treatments. The serum albumin content increased significantly (P<0.05) with increase in the level of inclusion of black pepper from 0 to 1.0 % in the diet. However, there is no significant difference (P>0.05) in serum albumin content among quails fed diets containing black pepper at 0 and 0.25%, 0 and 0.5%, 0.25 and 0.5%, 0.5 and 0.75%, 0.5 and 1.0%, and 0.75 and 1.0% levels.

4.5.3 Effect of black pepper on serum globulin

The serum globulin content of quails fed experimental diets is given in Table 8. The serum globulin content of different treatments ranged from 0.92 to 1.39 g/dl. The serum globulin content increased linearly from T_1 to T_5 with increased level of inclusion of black pepper from 0 to 1.0 % level in the diet but the differences between the treatments were not significant (P>0.05).

4.5.4 Effect of black pepper on serum glucose

The serum glucose content of quails fed experimental diets is given in Table 9. The serum glucose content of different treatments ranged from 199.31 to 243.09 mg/dl. The serum glucose content decreased linearly from T_1 to T_5 with increased level of inclusion of black pepper from 0 to 1.0% level in the diet and the differences between the treatments were statistically significant (P<0.01).

4.5.5 Effect of black pepper on serum triglyceride

The serum triglyceride content of quails fed experimental diets is given in Table 9. The serum triglyceride content ranged from 147.08 to 170.69 mg/dl among different treatments. The serum triglyceride content decreased linearly from T_1 to T_5 with increased level of inclusion of black pepper from 0 to 1.0% level in the diet and the differences between the treatments were statistically significant (P<0.01).

Table 8: Effect of dietary inclusion of black pepper at varying levels on serum total protein, albumin and globulins (g/dl)

Treatment	Total protein	Albumin	Globulin
T_1	$2.41^{a} \pm 0.08$	$1.48^{a} \pm 0.13$	0.92 ± 0.13
T_2	$2.62^{a} \pm 0.14$	$1.50^{a} \pm 0.13$	1.12 ± 0.16
T_3	$2.97^{b} \pm 0.03$	$1.76^{ab} \pm 0.21$	1.21 ± 0.23
T_4	$3.28^{bc} \pm 0.17$	$2.01^{b} \pm 0.07$	1.27 ± 0.17
T_5	$3.44^{c} \pm 0.05$	$2.04^{b} \pm 0.12$	1.39 ± 0.12
SEM	0.08	0.07	0.07
SS	**	*	NS

Values in column bearing different super scripts differ significantly *($P<0.05$), ** ($P<0.01$).

4.5.6 Effect of black pepper on serum total cholesterol

The serum total cholesterol content of quails fed experimental diets is given in Table 9.The serum total cholesterol content ranged from 161.91 to 190.26 mg/dl among different treatments. Increased level of inclusion of black pepper from 0 to 1.0 % in the diet decreased significantly ($P<0.01$) the serum total cholesterol content in quails. The serum total cholesterol content was significantly ($P<0.01$) lower in T_5 and higher in T_1 group of quails as compared to other treatments.

4.5.7 Effect of black pepper on HDL Cholesterol

The values of HDL Cholesterol are shown in Table 10. The serum HDL Cholesterol content ranged from 96.81 to 102.20 mg/dl among different treatments. The serum HDL Cholesterol content increased linearly from T_1 to T_5 with increased level of inclusion of black pepper from 0 to 1.0% level in the diet and the differences between the treatments were statistically significant ($P<0.01$). However, there is no-significant difference ($P>0.05$) in serum HDL Cholesterol content among quails fed diets containing black pepper at 0.25 and 0.5%, and 0.5 and 0.75% levels.

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Table 9: Effect of dietary inclusion of black pepper at varying levels on serum glucose, triglycerides and total cholesterol (mg/dl)

Treatment	Glucose	Triglycerides	Total cholesterol
T_1	243.09^e ± 0.79	170.69^e ± 0.05	190.26^e ± 01
T_2	235.56^d ± 0.97	164.60^d ± 0.08	188.06^d ± 0.5
T_3	223.53^c ± 0.90	159.22^c ± 0.16	179.69^c ± 0.4
T_4	211.78^b ± 0.99	151.11^b ± 0.20	170.01^b ± 0.1
T_5	199.31^a ± 0.45	147.08^a ± 0.26	161.91^a ± 0.4
SEM	2.95	1.60	2.0
SS	**	**	**

Values in column bearing different super scripts differ significantly **(P<0.01)

4.5.8 Effect of black pepper on LDL Cholesterol

The values of LDL Cholesterol are shown in Table 10. The serum LDL Cholesterol content ranged from 30.29 to 59.30 mg/dl among different treatments. Increased level of inclusion of black pepper from 0 to 1.0 % in the diet decreased significantly ($P<0.01$) the serum LDL cholesterol content in quails. The serum LDL cholesterol content was significantly ($P<0.01$) lower in T_5 and higher in T_1 group of quails as compared to other treatments.

4.5.9 Effect of black pepper on VLDL Cholesterol

The values of VLDL Cholesterol are shown in Table 10. The serum VLDL Cholesterol content ranged from 29.41 to 34.13 mg/dl among different treatments. The serum VLDL Cholesterol content decreased linearly from T_1 to T_5 with increased level of inclusion of black pepper from 0 to 1.0% level in the diet and the differences between the treatments were statistically significant ($P<0.01$).

4.5.10 Effect of black pepper on Serum Creatinine

The serum creatinine content of quails fed experimental diets is presented in Table11. The serum creatinine content ranged from 1.31 to 2.11 mg/dl among different treatments. The serum creatinine content increased linearly from T_1 to T_5 with increased level of inclusion of black pepper from 0 to 1.0 % level in the diet but the differences between the treatments were not significant ($P>0.05$).

Table 10: Effect of dietary inclusion of black pepper at varying levels on different cholesterol components (mg / dl)

Treatment	HDL-C	LDL-C	VLDL-C
T_1	$96.81^{a} \pm 0.04$	$59.30^{e} \pm 0.12$	$34.13^{e} \pm 0.01$
T_2	$98.47^{b} \pm 0.65$	$56.66^{d} \pm 0.18$	$32.92^{d} \pm 0.01$
T_3	$99.56^{bc} \pm 0.10$	$48.28^{c} \pm 0.46$	$31.84^{c} \pm 0.03$
T_4	$100.27^{c} \pm 0.46$	$39.52^{b} \pm 0.54$	$30.22^{b} \pm 0.04$
T_5	$102.20^{d} \pm 0.61$	$30.29^{a} \pm 0.49$	$29.41^{a} \pm 0.05$
SEM	0.38	2.0	0.32
SS	**	**	**

Values in column bearing different super scripts differ significantly **(P<0.01).

4.5.11 Effect of black pepper on Serum Calcium

The serum calcium content of quails fed experimental diets is presented in Table11. The serum calcium content ranged from 6.71to 8.12 mg/dl among different treatments. The serum calcium content increased significantly (P<0.01) with increased level of inclusion of black pepper from 0 to 1% in the diet. However, no significant difference (P>0.05) was observed among quails fed diets containing black pepper at 0.5 and 0.75 % levels.

4.5.12Effect of black pepper on serum phosphorous

The serum phosphorous content of quails fed experimental diets is presented in Table 11. The serum phosphorous content ranged from 3.16 to 4.01 mg/dl among different treatments.The serum phosphorous content increased significantly (P<0.01) with increased level of inclusion of black pepper from 0 to 1% in the diet. However, no significant difference (P>0.05) was observed among quails fed diets containing black pepper at 0.25 and 0.5%, and 0.5 and 0.75% levels.

Table 11: Effect of dietary inclusion of black pepper at varying levels on serum creatinine, calcium and phosphorous (mg / dl)

Treatment	Creatinine	Calcium	Phosphorous
T_1	1.31 ± 0.20	6.71^{a} ± 0.06	3.16^{a} ± 0.02
T_2	1.33 ± 0.09	7.30^{b} ± 0.12	3.52^{b} ± 0.06
T_3	1.70 ± 0.16	7.63^{c} ± 0.11	3.58^{bc} ± 0.01
T_4	1.72 ± 0.57	7.72^{c} ± 0.06	3.67^{c} ± 0.00
T_5	2.11 ± 0.29	8.12^{d} ± 0.13	4.01^{d} ± 0.05
SEM	0.14	0.09	0.05
SS	NS	**	**

Values in column bearing different super scripts differ significantly ** ($P<0.01$)

4.6 EFFECT OF DIETARY INCLUSION OF BLACK PEPPER ON DIGESTIBILITY OF NUTRIENTS

4.6.1 Effect of black pepper on digestibility of proximate constituents

The effect of dietary inclusion of black pepper at varying levels in quail diets on digestibility of proximate constituents is given in Table 12.The digestibility coefficients of dry matter ranged from 65.23 to 69.16%, organic matter from 59.68 to 64.91%, crude protein from 55.58 to 61.86%, crude fibre from 47.06 to 52.83%, ether extract from 49.87 to 62.07% and nitrogen free extract from 50.00 to 55.07%. The digestibility coefficients of DM, OM, CP, CF, EE and NFE increased linearly from T_1 to T_5, with increased level of inclusion of black pepper from 0 to 1.0 % in the diet and the differences between different treatments were statistically significant ($P<0.01$).

4.6.2 Effect of black pepper on digestibility of cell wall constituents

The digestibility of cell wall constituents is presented in Table13. The digestibility coefficients of neutral detergent fibre ranged from 45.00 to 52.29, acid detergent fibre from 48.86 to 52.18, hemi-cellulose from 42.91 to 50.05 and cellulose from 39.92 to 47.96 per cent. The digestibility of NDF, hemi-cellulose, cellulose ($P<0.01$) and ADF($P<0.05$) increased with increased level of inclusion of black pepper from 0 to 1.0 % level in the diet and the differences between different treatments were statistically significant ($P<0.01$).

Table 12: Effect of dietary inclusion of black pepper at varying levels on digestibility of proximate constituents

Treatment	**Dry matter**	**Organic matter**	**Crude protein**	**Crude fibre**	**Ether extract**	**NFE**
T_1	$65.23^{a} \pm 0.08$	$59.68^{a} \pm 0.33$	$55.58^{a} \pm 0.67$	$47.06^{a} \pm 0.22$	$49.87^{a} \pm 2.33$	$50.00^{a} \pm 0.45$
T_2	$66.26^{b} \pm 0.40$	$61.10^{b} \pm 0.20$	$57.86^{b} \pm 0.18$	$49.98^{b} \pm 1.5$	$50.82^{b} \pm 2.19$	$51.66^{b} \pm 0.11$
T_3	$67.27^{c} \pm 0.55$	$62.24^{c} \pm 0.10$	$58.04^{c} \pm 0.29$	$50.71^{bc} \pm 1.31$	$55.58^{b} \pm 0.49$	$52.97^{c} \pm 0.16$
T_4	$68.93^{d} \pm 0.38$	$63.42^{d} \pm 0.09$	$60.36^{d} \pm 0.42$	$51.63^{bc} \pm 1.91$	$60.39^{c} \pm 2.12$	$54.10^{d} \pm 0.19$
T_5	$69.16^{e} \pm 0.90$	$64.91^{e} \pm 0.24$	$61.86^{e} \pm 0.33$	$52.83^{d} \pm 0.39$	$62.07^{d} \pm 1.15$	$55.07^{d} \pm 0.16$
SEM	0.49	0.25	0.61	0.90	2.45	0.24
SS	**	**	**	**	**	**

Values in column bearing different super scripts differ significantly **(P<0.01)

Table 13: Effect of dietary inclusion of black pepper at varying levels on digestibility of cell wall constituents

Treatment	NDF	ADF	Hemi cellulose	Cellulose
T_1	$45.00^a \pm 0.24$	$48.86^a \pm 1.27$	$42.91^a \pm 0.18$	$39.92^a \pm 0.54$
T_2	$46.21^a \pm 0.28$	$50.41^{ab} \pm 0.74$	$45.38^b \pm 0.05$	$42.78^b \pm 0.12$
T_3	$47.21^b \pm 0.57$	$50.52^b \pm 1.58$	$47.40^c \pm 0.08$	$44.89^c \pm 0.28$
T_4	$51.5^c \pm 1.16$	$51.40^c \pm 0.25$	$49.80^d \pm 0.28$	$45.50^c \pm 0.24$
T_5	$52.29^c \pm 0.49$	$52.18^c \pm 2.06$	$50.05^d \pm 0.24$	$47.96^d \pm 0.21$
SEM	0.65	0.71	0.74	0.76
SS	**	*	**	**

Values in column bearing different super scripts differ significantly *(P<0.05) **(P<0.01)

4.7 EFFECT OF DIETARY INCLUSION OF BLACK PEPPER ON NUTRIENT BALANCES

The data pertaining to nitrogen, calcium and phosphorous utilization in quails fed diets containing black pepper at varying levels is given in Table 14.

4.7.1 Effect of black pepper on nitrogen utilization

The per cent nitrogen utilization in quails during the experimental period is presented in Table 14. The nitrogen utilization ranged from 55.41 to 61.55 per cent among different treatments. The per cent nitrogen utilization increased linearly from T_1 to T_5 with increase in the level of inclusion of black pepper from 0 to 1.0 % in the diet and the differences between treatments were significant ($P<0.01$). However, no significant difference ($P>0.05$) was observed among quails fed diets containing black pepper at 0.5 and 0.75% levels.

4.7.2 Effect of black pepper on calcium utilization

The per cent calcium utilization in quails during the experimental period is presented in Table 14. The calcium utilization ranged from 57.72 to 64.92 per cent among different treatments.The per cent calcium utilization increased ($P<0.01$) with increase in the level of inclusion of black pepper from 0 to 1.0 % in the diet and the differences between treatments were significant ($P<0.01$). However, no significant difference ($P>0.05$) was observed among quails fed diets containing black pepper powder at 0.5 and 0.75% levels.

4.7.3 Effect of black pepper on phosphorous utilization

The per cent phosphorous utilization in quails during the experimental period is presented in Table14. The phosphorous utilization ranged from 40.13 to 44.45 per cent among different treatments. The per cent phosphorous utilization increased linearly from T_1 to T_5 with increase in the level of inclusion of black pepper from 0 to 1.0 % in the diet and the differences between treatments were significant (P<0.01). However, no significant differences (P>0.05) were observed among quails fed diets containing black pepper at 0 and 0.25%, 0.25 and 0.5%, 0.25 and 0.75 and 0.5 and 0.75% levels.

4.8 EFFECT OF DIETARY INCLUSION OF BLACK PEPPER ON CARCASS CHARACTERISTICS

The carcass yield (g), dressing percentage, ready to cook yield (%) and meat to bone ratio in quails fed diets containing black pepper at varying levels are presented in Table 15. The carcass yield ranged from 118.33 to 129.67 g among different treatments. The carcass yield increased significantly (P<0.05) with increase in the level of inclusion of black pepper from 0 to 1.0 % in the diet. However, the carcass yield did not differ significantly (P>0.05) between quails fed diets containing black pepper at 0 and 0.25 %, 0.25 and 0.5%, 0.25 and 0.75%, 0.25 and 1%, 0.5 and 0.75%, 0.5 and 1.0% and, 0.75 and 1.0 % levels.

Table 14: Effect of dietary inclusion of black pepper at varying levels on percent nitrogen, calcium and phosphorous utilization

Treatment	Nitrogen	Calcium	Phosphorous
T_1	$55.41^{a} \pm 0.6$	$57.72^{a} \pm 0.1$	$40.13^{a} \pm 0.79$
T_2	$55.72^{b} \pm 0.19$	$58.00^{b} \pm 0.4$	$40.25^{ab} \pm 0.96$
T_3	$58.23^{c} \pm 0.20$	$60.75^{c} \pm 0.5$	$41.99^{b} \pm 0.97$
T_4	$59.33^{c} \pm 0.08$	$62.47^{c} \pm 0.4$	$42.03^{b} \pm 0.40$
T_5	$61.55^{d} \pm 0.05$	$64.92^{d} \pm 0.1$	$44.45^{c} \pm 0.98$
SEM	1.60	2.0	2.95
SS	**	**	**

Values in column bearing different superscripts differ significantly**(P<0.01)

The dressing percentage ranged from 58.16 to 60.79 per cent among different treatments. The dressing percentage increased with inclusion of black pepper from 0 to 1.0% level in the diet and the differences between treatments were significant ($P<0.01$). However, the dressing percentage did not differ significantly ($P>0.05$) between quails fed diets containing black pepper at 0.25 and 0.5%, 0.25 and 0.75%, 0.25 and 1.0%, 0.5 and 0.75%, 0.5 and 1.0% and, 0.75 and 1.0% levels.

The ready to cook yield ranged from 109.17 to 120.67 g among different treatments. The ready to cook yield increased with inclusion of black pepper from 0 to 1.0% level in the diet and the differences between treatments were significant ($P<0.05$). However, the ready to cook yield did not differ significantly ($P>0.05$) between quails fed diets containing black pepper at 0 and 0.25%, 0.25 and 0.5%, 0.5 and 0.75%, 0.5 and 1.0% and, 0.75 and 1.0% levels.

The meat to bone ratio ranged from 3.67 to 5.45 among different treatments. The meat to bone ratio was significantly ($P<0.01$) higher in T_5 group of quails as compared to other treatments. The meat to bone ratio increased with inclusion of black pepper from 0 to 1.0% level in the diet and the differences between treatments were statistically significant ($P<0.01$).

Table 15: Effect of dietary inclusion of black pepper at varying levels on carcass yield, dressing per cent, ready to cook yield and meat to bone ratio

Treatment	Carcass yield (g)	Dressing %	Ready to cook yield	Meat: Bone ratio
T_1	$118.33^{a} \pm 2.7$	$58.16^{a} \pm 1.15$	$109.17^{a} \pm 1.6$	$3.67^{a} \pm 0.02$
T_2	$123.83^{ab} \pm 2.3$	$59.59^{b} \pm 0.92$	$113.33^{ab} \pm 2.1$	$4.19^{b} \pm 0.00$
T_3	$126.17^{b} \pm 2.8$	$60.30^{b} \pm 1.6$	$115.50^{bc} \pm 2.1$	$4.61^{c} \pm 0.01$
T_4	$129.17^{b} \pm 2.4$	$60.55^{b} \pm 2.8$	$119.33^{c} \pm 1.5$	$5.20^{d} \pm 0.00$
T_5	$129.67^{b} \pm 1.3$	$60.79^{b} \pm 1.0$	$120.67^{c} \pm 1.8$	$5.45^{e} \pm 0.02$
SEM	1.25	1.14	1.09	0.12
SS	*	**	*	**

Values in column bearing different super scripts differ significantly *(P<0.05) **(P<0.05)

The percent weight of heart, liver, gizzard and giblet in quails fed diets containing black pepper at varying levels are presented in Table 16. The per cent weight of heart, liver, gizzard and giblet in quails fed on different diets ranged from 1.5 to 1.90, 2.83 to 4.0, 3.83 to 4.76 and 9.33 to 9.49, respectively. The per cent weight of heart, gizzard and giblets increased while that of liver decreased with inclusion of black pepper from 0 to 1% level in the diet but the differences between treatments were not statistically significant (P>0.05).

4.9 EFFECT OF DIETARY INCLUSION OF BLACK PEPPER ON COST ECONOMICS

The feed cost/kg gain in quails fed diets containing black pepper powder at varying levels is presented in table 17. The feed cost/ kg gain ranged from ₹ 98.02 to 102.02 among different treatments. The feed cost/ kg gain decreased linearly from T_1 to T_5 with increased level of inclusion of black pepper from 0 to 1.0 % in the diet. However, the differences between treatments were not statistically significant (P>0.05).

Table 16: Effect of dietary inclusion of black pepper at varying levels on per cent heart, liver, gizzard and giblet

Treatment	Heart (%)	Liver (%)	Gizzard (%)	Giblet (%)
T_1	1.50 ± 0.22	4.00 ± 0.40	3.83 ± 0.30	9.33 ± 0.30
T_2	1.52 ± 0.22	3.83 ± 0.30	4.00 ± 0.02	9.35 ± 0.20
T_3	1.54 ± 0.22	3.67 ± 0.40	4.17 ± 0.30	9.38 ± 0.30
T_4	1.67 ± 0.21	3.49 ± 0.20	4.33 ± 0.20	9.39 ± 0.30
T_5	1.90 ± 0.16	2.83 ± 0.40	4.76 ± 0.20	9.49 ± 0.80
SEM	0.09	0.11	0.11	0.2
SS	NS	NS	NS	NS

NS: Non-significant

Table 17: Effect of dietary inclusion of black pepper at varying levels on feed cost per kg gain (₹)

Treatment	0-5 weeks
T_1	102.02 ± 1.80
T_2	99.825 ± 1.90
T_3	99.82 ± 1.80
T_4	98.35 ± 2.08
T_5	98.03 ± 0.86
SEM	0.86
SS	NS

NS: Non-significant

CHAPTER - V

DISCUSSION

The chemical composition of black pepper and the effect of inclusion of black pepper at varying levels in the diet on the growth performance, serum biochemical profile, digestibility of nutrients and carcass characteristics of quails are discussed in conjunction with the available literature.

5.1 CHEMICAL COMPOSITION OF BLACK PEPPER

5.1.1 Proximate composition of black pepper

The chemical composition of black pepper used in the present study was 92.26, 94.0, 12.87, 4.85, 20.97, 55.31, 6.0, 0.25, 1.46 and 0.80 per cent for DM, OM, CP, EE, CF, NFE, TA, AIA, Ca and P, respectively (Table 2). The CP content of black pepper in the present study was estimated as 12.87 % which was higher than the values reported by Bolanle *et al*. (2014), Sruthi *et al.* (2013), Emeka *et al*. (2013) and Bouba *et al*. (2012) who reported the CP value of black pepper powder as 9.3, 9.0, 9.18 and 8.5 per cent, respectively and lower than the value reported by Mohammed *et al*. (2012) who reported the CP content as 25.45 per cent.

The EE content (4.85%) of black pepper obtained in the present study corroborated with value reported earlier (Mohammed *et al.*, 2012). However, Bolanle *et al*. (2014) and Balogun *et al*. (2016) reported higher EE values in black pepper as compared to EE value observed in the present study.

The crude fibre content (20.97%) of black pepper obtained in the present study corroborrated with the findings of Sruthi *et al.* (2013) who reported the CF content in black pepper powder as 18. 6 %. On the other hand, Mohammed *et al.* (2012) reported higher CF content of 23.6% while Bolanle *et al*. (2014) and Balogun *et al*. (2016) reported lower CF content ranging from 4.2 to 8.79 % in black pepper when compared to the CF content observed in the present study.

The NFE content of black pepper in the present study was estimated as 55.31 %. In contrast, Sruthi *et al.* (2013) and Bolanle *et al.* (2014) reported higher NFE content while Balogun *et al.* (2016) reported lower NFE content in black pepper as compared to that observed in the present study.

In the present study, the total ash content of black pepper was reported as 6.0 %. The total ash content of black pepper obtained in the present study corroborated with findings of Emeka *et al*. (2013) and Balogun *et al.* (2016). However, Bolanle *et al*. (2014) and Bouba *et al*. (2012) reported higher TA content while Sruthi *et al.* (2013) reported lower TA content in black pepper as compared to the value observed in the present study.

The calcium content (1.46 %) of black pepper observed in the present study was comparable with the values reported earlier (AI-jasass *et al.,* 2012). On the other hand, Bolanle *et al*. (2014) reported higher calcium content while Bouba *et al*. (2012) and Balogun *et al.* (2016) reported lower lower calcium content in black pepper as compared to that reported in this study. Further, the phosphorous content of black pepper in the present study was estimated as 0.80%. In contrast, Bolanle *et al*. (2014) reported higher while Balogun *et al.* (2016) reported lower phosphorous content in black pepper as compared to the present findings.

5.1.2 Cell wall constituents of black pepper

The per cent NDF, ADF, hemi-cellulose, cellulose, ADL and silica content of black pepper powder were 52.25, 39.5, 12.75, 26.34, 1.5 and 2.1, respectively.

These differences in chemical composition of black pepper observed in the present study when compared to those values reported earlier may be attributed to variation in the strain, climatic conditions, cultivation practices and processing methods adopted etc.

5.2 INGREDIENT COMPOSITION OF QUAIL DIETS

The quail diets were formulated based on the recommendations of NRC (1994). Maize, DORB, soybean meal and fish meal were used in formulating diets, as these are the most commonly used and available ingredients in this region.

Black pepper was ground and incorporated in powder form up to 1.0 % level after reviewing the literature. El-Tazi *et al.* (2014) studied the effect of inclusion of black pepper as phytobiotic growth promoter in commercial broiler chicken at 0, 0.5, 0.75 and 1.0 % level in the diet and reported that broilers can be fed up to 1.0 % level without any adverse effects. Similarly, AI-kassie *et al*. (2011) fed broiler chicken with diets containing black pepper powder at 0.5, 0.75 and 1 % level and reported that black pepper can be fed up to 1 % level in the diet of broiler chicken without any deleterious effects. On the other hand, Shahverdi *et al*. (2013) studied the effect of inclusion of black pepper up to 0.02 % level in the diet of broiler chicks and reported that 0.02 % black pepper can be fed in the diet of broiler chicken without any adverse effects. In another experiment, Ghaedi *et al.* (2013) studied the effect of supplementation of black pepper extract and virginiamycin powder as phytogenic feed additive growth promoter in Japanese quail and reported that 2 mg black pepper extract / ml water can be applied as an alternative to antibiotic to maintain growth performance. Based on the results obtained by different workers, in the present study black pepper was incorporated up to 1.0 % in quail diets.

5.3 CHEMICAL COMPOSITION OF QUAIL DIETS

5.3.1 Proximate composition of quail diets

The chemical composition of diets formulated by incorporating black pepper at varying levels and fed to Japanese quails in the present study was shown in Table 5. The diets were iso-nitrogenous and iso-caloric with a protein: energy ratio of 1:120. Iso-nitrogenous and iso-caloric diets were formulated using maize, DORB, soybean meal, fish meal and black pepper powder as per NRC (1994) specifications. Animal fat was added to the diets to make up the energy deficit. The study revealed that the DM, EE, CF, TA and AIA contents increased while OM and NFE contents decreased with increased level of inclusion of black pepper from 0 to 1.0% in the diet. This may be attributed to the inclusion of black pepper in the diet by marginal adjustment in the levels of maize and DORB (Table 4).

5.3.2 Cell wall constituents of quail diets

The study indicated that the cell wall constituents also did not follow a particular trend (Table 6). The study revealed that the NDF, ADF, hemi-cellulose, cellulose, ADL and silica contents increased with increased level of inclusion of black pepper from 0 to 1.0% in the diet which may be attributed to the inclusion of black pepper in the diet by marginal adjustment in the levels of maize and DORB (Table 4).

5.4 EFFECT OF DIETARY INCLUSION OF BLACK PEPPER ON GROWTH PERFORMANCE OF JAPANESE QUAILS

5.4.1 Effect of black pepper on body weight gain

During the experimental period, the body weight gain was significantly higher (P<0.05) in quails fed diets containing black pepper as compared to those fed control diet (Table 7). However, no significant difference (P>0.05) was observed in quails fed diets containing black pepper at 0.25 % as compared to the control. These results corroborated with the findings of Al-Kassie *et al*. (2011) who reported that supplementation of black pepper upto 1% level has improved significantly (P<0.05) the live body weight in broilers. The increased body weight gains observed in quails may be attributed to the stimulating effect of black pepper on the digestive system thus leading to improved body weight gain. Further, the level of black pepper used reflects the high activity of piperazine citrate included in the diet which may have affected the flow of digestive juices across the stomach (Al-Kassie *et al*., 2011). Similarly, El-Tazi *et al*. (2014) reported increased body weight gain in broilers upon feeding black pepper upto 1.0% level in the diet. In contradiction to the present findings, Ndelekwute *et al*. (2015) reported that final live weight gain was improved (P<0.05) in broilers fed black pepper at 0.25% while there was a negative effect when fed at 0.75 and 1.0% level. On the other hand, Puvaca *et al*. (2014) reported that feeding black pepper upto 1.0% level in the diet had no effect (P>0.05) on body weight gain in broilers. Further, several researchers reported significant (P<0.05) increase in live weight in broilers upon feeding black pepper at lower levels (Shahverdi *et al.,* 2013; Tripathi *et al.,* 2013; Valiollahi *et al.,* 2014).

5.4.2 Effect of black pepper on feed intake

The feed intake (g) during the experimental period decreased significantly (P<0.01) with increased level of inclusion of black pepper up to 1.0 % level in the diet of quails as compared to those fed control diet (Table 7). It is reported that herbal additives inhibit the growth and colonization of various pathogenic microorganisms including E. coli in the intestinal tract of chicken due to their antimicrobial activity (Galib *et al*., 2010). When the number of harmful bacteria in the intestinal tract is low, more nutrients are absorbed by the birds. As a result, the birds gain higher weight with lower feed consumption. Another reason for decreased feed intake with the addition of black pepper in the diet might be due to strong and spicy flavour resulting in the reduced palatability of the feed (Windisch *et al*., 2008). These results are in line with the findings of Ndelekwute *et al*. (2015) who reported significant decrease (P<0.05) in feed intake with inclusion of black pepper upto 1.0 % level in the diet of broilers. Similarly, decreased feed intakes upon feeding black pepper in the diet were also reported earlier (Ghaedi *et al*., 2013; Tripathi *et al*., 2013). In contrast, increased feed intake upon feeding diets containing black pepper were reported by earlier workers (El-Tazi *et al.,* 2014; Shahverdi *et al*., 2013; Al-Kassie *et al*., 2011). On the other hand, Valiollahi *et al.* (2014), Akbarian *et al.* (2012) and Moorthy *et al.* (2009) reported that feeding of black pepper in the diet of broilers had no effect ($P>0.05$) on feed intake.

5.4.3 Effect of black pepper on feed conversion ratio

Inclusion of black pepper at varying levels from 0 to 1.0% in the diet of quails had significant effect ($P<0.01$) on feed conversion ratio. The study indicated that the feed consumed / kg gain decreased significantly ($P<0.01$) with increased level of inclusion of black pepper up to 1.0% level in the diet of quails as compared to those fed control (Table 7).The improved feed conversion ratio observed in quails fed diets containing black pepper in the diet may be attributed to the presence of piperazine citrate which probably enhances the secretion of digestive juices in the stomach leading to greater efficiency in utilization of feed resulting in enhanced growth (Al-Kassie *et al*., 2011). These results corroborated with the findings of Al-Kassie *et al.* (2011) in broilers who reported improved FCR upon feeding black pepper upto 1.0% in the diet. Similarly, several researchers reported improved FCR upon feeding black pepper in the diet (El-Tazi *et al*., 2014; Valiollahi *et al.,* 2014; Ghaedi *et al.,* 2013; Shahverdi *et al*., 2013; Tripathi *et al*., 2013; Myandoab and Mansoub, 2011; Mansoub, 2011). In contrast, Ndelekwute *et al*. (2015) reported poor feed conversion ratio in broilers upon feeding black pepper in the diet.

5.5 EFFECT OF DIETARY INCLUSION OF BLACK PEPPER ON SERUM BIOCHEMICAL PROFILE OF JAPANESE QUAILS

5.5.1 Effect of black pepper on serum total protein

The serum total protein content (mg/dl) increased significantly (P<0.01) with increased level of inclusion of black pepper up to 1.0% level in the diet of quails (Table 8). In contrast to the findings of the present study, Myandoab and Mansoub (2011) reported that feeding of black pepper upto 2.0% in the diet had no effect (P>0.05) on serum total protein content in Japanese quails.

5.5.2 Effect of black pepper on serum albumin

The present study indicated a significant increase (P<0.05) in serum albumin content (mg/dl) with increased level of inclusion of black pepper from 0 to 1.0% in the diet of quails (Table 8). In contradiction to the present findings, Myandoab and Mansoub (2011) reported that feeding of black pepper upto 2.0% in the diet had no effect (P>0.05) on serum albumin content in Japanese quails. Similarly, Mansoub (2011) reported that feeding of black pepper upto 1.0% in the diet had no effect (P>0.05) on serum albumin content in broilers.

5.5.3 Effect of black pepper on serum globulin

Inclusion of black pepper upto to 1.0% level in the diet of quails had no effect (P>0.05) on serum globulin content (mg/dl) (Table 8). These results are very much in line with the findings of Mansoub (2011), who reported that feeding of black pepper upto 1.0% in the diet had no effect (P>0.05) on serum globulin content in broilers.

Higher levels of serum protein, albumin and globulin (which are protein products) observed in quails fed varying levels of black pepper in the diet can be related to increased nutrient absorption including protein and its presence in the blood serum. Further, it is reported that black pepper has anti-microbial effects and contain anti-oxidants that decrease lipid oxidation which might have enhanced the immune system resulting in increased concentration of serum parameters related to immunity *viz.* total protein, albumin and globulin levels.

5.5.4 Effect of black pepper on serum glucose

The serum glucose content (mg/dl) decreased significantly (P<0.01) with increased level of inclusion of black pepper from 0 to 1.0% in the diet of quails (Table 9). These results corroborated with the findings of Shahverdi *et al*. (2013) in broilers who reported that feeding black pepper at 0.02% in the diet resulted in decreased (P<0.05) serum glucose content as compared to the control. On the otherhand, Myandoab and Mansoub (2011) reported that inclusion of black pepper upto 2.0% in the diet had no effect (P>0.05) on serum glucose content of Japanese quails.

5.5.5 Effect of black pepper on serum triglycerides

The serum triglyceride content (mg/dl) decreased significantly (P<0.01) with increased level of inclusion of black pepper from 0 to 1.0% in the diet of quails (Table 9). The decreased serum triglyceride content observed in the present study upon feeding black pepper in the diet may be attributed to the high fibre content in herbs (20.97% in black pepper) which might have caused increased excretion of bile resulting in decreased triglyceride content. Corroborating the results of the present study, Akbarian *et a l.* (2012) reported that supplementation of black pepper at 1.0 % in the diet resulted in significant decrease (P<0.05) in serum triglyceride content of broilers. Similarly, decreased serum triglyceride content upon feeding black pepper in the diet were also reported earlier (Valiollahi *et al*., 2014; Ghaedi *et al*., 2013; Shahverdi *et al*., 2013; Mansoub, 2011; Myandoab and Mansoub, 2011).

5.5.6 Effect of black pepper on serum total cholesterol

The serum total cholesterol content (mg/ dl) decreased significantly (P<0.01) with inclusion of black pepper irrespective of level in the diet of quails (Table 9).The lowered serum cholesterol content in quails fed diets supplemented with black pepper at varying levels may be attributed to the presence of carvacrol and tymol, which might have effect on cholesterol and decrease this harmful parameters in blood (Al-kassie *et al.,* 2011). These results are in agreement with the findings of Al-kassie *et al.* (2012) who reported that supplementation of black pepper powder at 1.0 % in the diet had reduced significantly (P>0.05) the serum total cholesterol content of broilers. Similar findings were also reported earlier (Ghaedi *et al*., 2013; Shahverdi *et al*., 2013; Mansoub, 2011; Myandoab and Mansoub, 2011). However, Akbarian *et al.* (2012) reported that supplementation of black pepper upto 1.0 % in the diet had no effect (P>0.05) on serum total cholesterol content of broilers.

5.5.7 Effect of black pepper on HDL Cholesterol

The serum HDL cholesterol content (mg/dl) increased significantly (P<0.01) with increased level of inclusion of black pepper from 0 to 1.0% in the diet of quails (Table 10). The increased serum HDL cholesterol content observed in quails upon feeding black pepper in the diet might be attributed to the significant hypo-lipidaemic effect resulting in lowering the total cholesterol levels and triglycerides and thereby increasing the levels of high density lipoproteins (Lin *et al*., 2003). In corroboration with the findings of the present study, Mansoub (2011) reported that feeding of black pepper at 1.0% in the diet has increased (P<0.05) the serum HDL cholesterol concentration in broilers. Similarly, Ghaede *et al*. (2013) also reported that inclusion of black pepper as phytogenic feed additive resulted in increased ($P<0.05$) serum HDL cholesterol concentration in broilers. However, Akbarian *et al.* (2012) reported that feeding black pepper up to 1.0% in the diet had no effect ($P>0.05$) on HDL-Cholesterol level in broilers.

5.5.8 Effect of black pepper on LDL Cholesterol

The serum LDL cholesterol content (mg/dl) decreased significantly ($P<0.01$) with increased level of black pepper from 0 to 1.0% in the diet of quails (Table 10).The decreased LDL cholesterol content upon feeding black pepper in the diet might be attributed to the antioxidant properties of black pepper which prevents per oxidation of fatty tissues and decreases the lipogenic enzymes activity and contribute to reducing re-synthesis of fatty acids in the liver and subsequently reducing the blood LDL-C level. These results are in line with the findings of Ghaede *et al*. (2013) who reported that inclusion of black pepper at 2 mg/ml in water resulted in decreased ($P<0.01$) LDL cholesterol levels in broilers. On the otherhand, Akbarian *et al.* (2012) and Mansoub (2011) reported that feeding black pepper in the diet had no effect ($P>0.05$) on LDL-Cholesterol level in broilers.

5.5.9 Effect of black pepper on VLDL Cholesterol

The serum VLDL cholesterol content (mg/dl) decreased significantly ($P<0.01$) with increased level of black pepper from 0 to 1.0% in the diet of quails

(Table 10). This may be attributed to the hypo-lipidaemic effects of black pepper that enhanced hepatic bile acid synthesis and increased the degradation of cholesterol to faecal bile acids and neutral sterols which might have resulted in significant decrease (P<0.01) in VLDL cholesterol concentration in quails.

5.5.10 Effect of black pepper on serum creatinine

The serum creatinine content in quails fed diets containing varying levels of black pepper powder ranged from 1.31 to 2.11 mg / dl (Table 11). The study indicated that inclusion of black pepper upto 1.0% level in the diet had no effect (P>0.05) on serum creatinine content of quails (Table 11). These results are in line with the findings of Al-Shuwaili *et al.* (2015) who reported that feeding cinnamon powder in the diet had no effect (P>0.05) on serum creatinine content in turkeys.

5.5.11 Effect of black pepper on serum calcium

The serum calcium content in quails fed diets containing varying levels of black pepper ranged from 6.71 to 8.12 mg/dl (Table 11). The serum calcium content (mg / dl) increased significantly (P<0.01) with increased level of inclusion of black pepper from 0 to 1.0% level in the diet of quails. Similar to the findings observed in the present study, Vamsidhar (2015) also reported increased (P<0.01) serum calcium content in quails fed diets containing fenugreek seed up to 2.0% level. Higher levels of serum calcium observed in quails fed varying levels of black pepper powder in the diet can be related to the increase of nutrient absorption and its presence in the blood serum. In contrast to the present findings, Valiollahi *et al.* (2014) reported that supplementation of black pepper at 0.02 % in the diet had decreased significantly (P<0.05) the serum calcium content of broilers.

5.5.12 Effect of black pepper on serum phosphorous

The serum phosphorous content in quails fed diets containing varying levels of black pepper ranged from 3.16 to 4.01 mg/dl (Table 11). The study indicated that serum phosphorous content increased significantly (P<0.01) with increased level of inclusion of black pepper from 0 to 1.0 % in the diet. These results corroborated with the findings of Valiollahi *et al.* (2014) who reported that inclusion of black pepper powder at 0.02 % level in the diet of broilers resulted in increased (P<0.05) phosphorous levels in blood.

5.6 EFFECT OF DIETARY INCLUSION OF BLACK PEPPER ON DIGESTIBILITY OF NUTRIENTS

5.6.1 Effect of black pepper on digestibility of proximate constituents

The digestibility of DM, OM, CP, CF, EE and NFE increased significantly (P<0.01) with increased level of black pepper from 0 to 1% level in the diet (Table 12). The increase in the digestibility of gross nutrients observed in the present study may be attributed to the presence of black pepper in the diet. The result of the apparent digestibility of nutrients observed in the present study are in confirmity with the earlier report by Javed *et al*. (2009) who indicated that plant extracts at certain levels improved digestibility. Lee *et al*. (2004) and Windisch *et al.* (2007) also reported that spices and essential oils could be used to aid digestion in monogastric animals. Many reasons have been ascribed to this. Jang *et al.* (2004) attributed better nutrient digestion due to antimicrobial property of the essential oil in black pepper. Srinivasan *et al*. (2007) linked it to the ability of black pepper to induce saliva secretion, hydrochloric acid and mucus production. Platel and Srinivasan *et al*. (2000) attributed that the pungent properties of black pepper stimulated digestive enzymes activities. Similar to the findings of the present study, Ndelekwute *et al*. (2015) reported that inclusion of black pepper upto 1.0% in the diet of broiler chicks resulted in increased ($P<0.05$) DM, CP, EE and CF digestibility. Similarly, Puvaca *et al*. (2014) reported a significant increase ($P<0.05$) in EE digestibility (%) of broilers when fed black pepper at 0.5% level in the diet.

5.6.2 Effect of black pepper on digestibility of cell wall constituents

The digestibility of NDF, hemi-cellulose, cellulose ($P<0.01$) and ADF ($P<0.05$) increased significantly with inclusion of black pepper from 0 to 1% in the diet (Table 13). The increased digestibility of cell wall constituents observed in the present study may be attributed to the potential beneficial effect of black pepper on gastrointestinal tract micro-organisms and metabolites which reflected in improved digestibility of cell wall constituents. The results of the present study are very much in line with the findings of Vamsidhar (2015), who reported that the digestibility of cell wall constituents increased significantly ($P<0.01$) in quails with increased level of inclusion of fenugreek seed from 0 to 2.0% in the diet.

5.7 EFFECT OF DIETARY INCLUSION OF BLACK PEPPER ON NUTRIENT BALANCES

5.7.1 Effect of black pepper on nitrogen utilization

The per cent nitrogen utilization increased significantly ($P<0.01$) in quails with increasing level of black pepper from 0 to 1.0% in the diet (Table 14). This increase in per cent nitrogen utilization observed in the present study upon feeding quails with diets containing black pepper may be attributed to the essential oils which not only act as antibacterial and antioxidant, but also as stimulant of digestive enzymes in the intestinal mucosa which might have improved the utilization of nitrogen. Similarly, Vamsidhar (2015) reported increased ($P<0.01$) nitrogen utilization content in quails fed diets containing fenugreek seed up to 2.0% level.

5.7.2 Effect of black pepper on calcium utilization

The per cent calcium utilization increased significantly ($P<0.01$) in quails fed diets containing black pepper at varying levels as compared to the control (Table 14). This increase in per cent calcium utilization observed in the present study may be attributed to the increased digestibility of nutrients due to incorporation of black pepper in the diet. Similarly, increased ($P<0.01$) calcium utilization upon feeding fenugreek seed in the diet was also reported earlier (Vamsidhar, 2015).

5.7.3 Effect of black pepper on phosphorous utilization

The per cent phosphorous utilization was highest ($P<0.01$) in quails fed diets containing 1.0% black pepper as compared to the control (Table 14). This increase in per cent phosphorous utilization may be attributed to the increased digestibility of nutrients due to incorporation of black pepper in the diet. In line with the present findings, Vamsidhar (2015) also reported increased ($P<0.01$) phosphorous utilization upon feeding fenugreek seed in the diet.

Calcium and phosphorous absorption and metabolism are influenced by many factors such as the level, ratio of inclusion in the diet, vitamin D_3 and its derivatives, phytase and organic acids. Black pepper is a good source of vitamin B, C, E and D and they have essential oils that can help increase the absorption of vitamin D_3 and may be affective in calcium absorption (Ferreira *et al*., 1999).

5.8 EFFECT OF DIETARY INCLUSION OF BLACK PEPPER ON CARCASS CHARACTERISTICS

The carcass yield increased linearly from T_1 to T_5 with increased level of inclusion of black pepper from 0 to 1.0% in the diet (Table 15). The carcass yield (g) was significantly higher (P<0.05) in quails fed diets containing 1.0% black pepper in the diet as compared to those fed control diet. The increase in the carcass yield in quails observed in the present study may be attributed to the presence of essential oils in black pepper. These results corroborated with the findings of Ghaedi *et al*. (2013) who reported that carcass yield (%) was significantly higher (P<0.05) in quails fed black pepper in water at 2 mg/ml as compared to the control. Similarly, Shahverdi *et al*. (2013) also reported significant increase (P<0.05) in carcass per cent of broilers fed diets containing black pepper at 0.02% in the diet. On the otherhand, Myandoab and Mansoub (2011) reported that inclusion of black pepper at 2.0% in the diet of Japanese quails resulted in significant increase (P<0.05) in carcass per cent while there was no effect (P>0.05) at 1.0 and 1.5% level as compared to the control.

The dressing percentage increased significantly (P<0.01) in quails fed diets containing black pepper as compared to the control. In line with the findings of the present study, Al-Kassie *et al*. (2011) reported significant increase (P<0.05) in dressing per cent of broilers fed diets containing black pepper up to 1.0% level.

Similarly, increased dressing percentage in broilers upon feeding black pepper in the diet were also reported earlier (El-Tazi *et al*., 2014). However, Moorthy *et al*. (2009) reported that feeding black pepper powder at 0.2 % level in the diet had no effect (P>0.05) on dressed weight of broilers.

The ready to cook yield (g) was significantly higher (P<0.05) in quails fed diets containing black pepper at 1.0 % level as compared to the control (Table 15). However, inclusion of black pepper at 0.25% level had no effect (P>0.05) on ready to cook yield in quails. The increased ready to cook yield observed in quails in the present study may be attributed to the positive effect of black pepper in the diet. In contrast to the findings of the present study, Moorthy *et al*. (2009) reported that inclusion of black pepper as feed additive at 0.2% level in broiler diet had no effect (P>0.05) on ready to cook yield per cent.

The meat to bone ratio was significantly higher (P<0.05) in quails fed diets containing black pepper at 1.0 % level as compared to the control (Table 15). Similar findings were reported by Vamsidhar (2015) while feeding fenugreek seed up to 2.0% level in the diet of quails. The increased meat to bone ratio in quails observed in the present study may be attributed to the positive effects of black pepper in the diet.

Inclusion of black pepper upto 1.0% level in the diet had no effect (P>0.05) on per cent heart weight in quails (Table 16). These results corroborated with those of El-Tazi *et al*. (2014) who reported that incorporation of black pepper up to 1.0% in the diet had no effect (P>0.05) on heart weight (%) in broilers. Similarly, no effect (P>0.05) on heart weight (%) upon feeding black pepper in the diet were also reported earlier (Valiollahi *et al*., 2014; Shahverdi *et al*., 2013; Molla *et al*., 2012;Nath *et al*., 2012; Al-Kassie *et al*., 2011; Moorthy *et al*., 2009).

Inclusion of black pepper upto 1.0% level in the diet had no effect (P>0.05) on per cent liver weight in quails (Table 16). Similar to the present findings, some researchers reported that incorporation of black pepper in the diet had no effect (P>0.05) on per cent weight of liver (El-Tazi *et al*., 2014; Molla *et al*., 2012; Nath *et al*., 2012; Al-Kassie *et al*., 2011; and Moorthy *et al*., 2009). In contradiction to the present findings, Myandoab and Mansoub (2011) in quails, Shahverdi *et al*. (2013) and Ghaedi *et al*. (2013) in broilers reported that inclusion of black pepper in the diet resulted in significant increase (P<0.05) in per cent liver weight while Valiollahi *et al*. (2014) reported significant decrease (P<0.05) in per cent liver weight upon feeding black pepper.

Inclusion of black pepper upto 1.0% level in the diet had no effect (P>0.05) on per cent liver weight in quails (Table 16). These results are very much in line with the findings of El-Tazi *et al*. (2014) and Al-Kassie *et al*. (2011) who reported that incorporation of black pepper upto 1.0% in the diet had no effect (P>0.05) on per cent weight of gizzard in broilers. Similar findings were also reported by Ghaedi *et al*. (2013), Molla *et al*. (2012), Nath *et al*. (2012) and Moorthy *et al*.

(2009) in broilers. However, in contrast to the present findings, Myandoab and Mansoub (2011) in quails, Valiollahi *et al*. (2014) and Shahverdi *et al*. (2013) in broilers reported that inclusion of black pepper in the diet resulted in increased (P<0.05) percent weight of gizzard.

The per cent weight of giblets increased linearly from T_1 to T_5 with increased level of inclusion of black pepper from 0 to 1.0% in the diet (Table 16). However, the differences between treatments were not statistically significant (P>0.05).

5.9 EFFECT OF DIETARY INCLUSION OF BLACK PEPPER ON COST ECONOMICS

The feed cost / kg gain decreased with increased level of inclusion of black pepper in the diet of quails but the differences were not significant (P>0.05) (Table 17). The study indicated that the feed cost / kg gain decreased by ₹2.19 in T_2, ₹ 2.20 in T_3, ₹3.67 in T_4 and ₹3.99 in T_5 group of quails fed diets containing black pepper at varying levels as compared to the control. The decreased feed cost / kg gain observed in the present study may be attributed to the positive effect of black pepper inclusion in the diet resulting in increased body weight gains and decreased feed intake in quails (Table 7). Similar to the present findings, El-Tazi (2014) reported that inclusion of a mixture of red pepper and black pepper upto 1.0% level in broiler diets resulted in higher net profit / kg meat.

5.10 CONCLUSION

The present study indicated that inclusion of black pepper up to 1.0% in the diet had improved the performance of quails as evidenced from increased body weight gains, improved FCR and increased digestibility of nutrients. Further, the serum total cholesterol content decreased while the dressing percentage increased upon feeding black pepper in the diet. Thus, it is concluded that black pepper can be incorporated up to 1.0% level in the diet of quails without any adverse effects.

CHAPTER - VI

SUMMARY

Black pepper (*Piper nigrum*) is a well known medicinal plant that grows in nature and is mainly cultivated in tropical parts of the world. It is a good source of fatty acids which are predominantly lauric acid, myristic acid, palmitoleic acid, oleic, stearic acid and lignoceric acid. Generally, spices are reported to have positive effect on villi, nutrient absorption and digesta viscosity. On the other hand, antibiotics as growth promoters in poultry feed are posing serious health risks in humans, because of their residual effect in poultry meat and eggs. Hence, the present investigation was carried out to study the effect of inclusion of black pepper at varying levels in the diet on growth performance, carcass characteristics, serum biochemical profile and nutrient utilization of Japanese quails .

The results of present study indicated that the per cent DM, OM, CP, EE, CF, NFE, TA and AIA of black pepper were 92.26, 94.0, 12.87, 4.85, 20.97, 55.31, 6.0 and 0.25, respectively. The per cent NDF, ADF, hemi-cellulose, cellulose, ADL and silica content of black pepper were 52.25, 39.5, 12.75, 26.34, 1.50 and 2.10, respectively. The per cent calcium and phosphorous content of black pepper were 1.46 and 0.80, respectively. Five diets were formulated (Table 4) incorporating black pepper at 0.0% (T_1), 0.25% (T_2), 0.50% (T_3), 0.75% (T_4)

and 1.0% (T_5) by marginal adjustment of other ingredients. The feed cost (₹) per kg of diet was 26.16 (T_1), 27.50 (T_2), 28.85 (T_3), 30.17 (T_4) and 31.52 (T_5), respectively.

The body weight gain increased ($P<0.05$) with increased level of inclusion of black pepper from 0 to 1.0% in the diet. However, no significant difference ($P>0.05$) was observed between quails fed diets containing black pepper at 0.25 and 0.50% levels. The feed intake decreased significantly ($P<0.01$) in quails with increased level of inclusion of black pepper from 0 to 1.0% in the diet. Similarly, feed consumed / kg gain decreased ($P<0.01$) with increased level of inclusion of black pepper in the diet as compared to those fed control diet.

The serum biochemical profile of quails revealed that the serum total protein, calcium, phosphorous ($P<0.01$) and albumin ($P<0.05$) contents increased significantly while serum glucose, triglycerides and total cholesterol levels decreased ($P<0.01$) with increase in the level of inclusion of black pepper from 0 to 1.0% in the diet. Further, the study indicated that the HDL cholesterol level increased ($P<0.01$), while LDL and VLDL cholesterol levels decreased ($P<0.01$) in serum with increased level of inclusion of black pepper from 0 to 1.0% in the diet. However, incorporation of black pepper in the diet had no effect ($P>0.05$) on serum globulin and creatinine content of quails.

The digestibility of DM, OM, CP, EE, CF and NFE increased ($P<0.01$) with increased level of inclusion of black pepper from 0 to 1.0% level in the diet.

Similarly, the digestibility of NDF, cellulose, hemi-cellulose ($P<0.01$) and ADF ($P<0.05$) increased with increase in the level of inclusion of black pepper in the diet. Further, it is observed that the percent nitrogen, calcium and phosphorous utilization in quails increased significantly ($P<0.01$) with increase in the level of inclusion of black pepper from 0 to 1.0% level in the diet

The carcass yield ($P<0.05$), dressing percentage ($P<0.01$), ready to cook yield ($P<0.05$) and meat to bone ratio ($P<0.01$) increased significantly in quails fed diets containing black pepper at varying levels as compared to the control. On the other hand, the per cent weight of heart, gizard and giblets increased while per cent weight of liver decreased with increased level of inclusion of black pepper from 0 to 1.0% in the diet but the differences between treatments were not significant ($P>0.05$).

The feed cost / kg gain decreased linearly from T_1 to T_5 with increased level of inclusion of black pepper from 0 to 1.0% in the diet but the differences among treatments were not significant ($P>0.05$). The study indicated that the feed cost / kg gain decreased by ₹ 2.19 (T_2) , 2.20 (T_3), 3.67 (T_4) and 3.99 (T_5) in quails fed diets containing black pepper at varying levels as compared to the control.

Based on the results obtained from the present study the following conclusions are drawn.

1. The chemical composition indicated that black pepper is a good source of protein, ether extract, crude fibre and minerals for inclusion in quail diets.

2. Inclusion of black pepper up to 1.0 % level in the diet has improved the body weight gain and feed conversion ratio in quails.
3. Inclusion of black pepper up to 1.0% in the diet resulted in decreased serum glucose, triglyceride and total cholesterol content and improved the overall serum biochemical profile in quails.
4. Inclusion of black pepper up to 1.0% in the diet has increased the digestibility of gross nutrients and fibre fractions in quails.
5. Inclusion of black pepper up to 1.0 % in the diet has improved the carcass characteristics in quails.
6. Inclusion of black pepper up to 1.0% in the diet has decreased the feed cost per kg gain in quails.

Thus, the present study indicated that black pepper can be included up to 1.0% level as a natural feed additive in the diet of quails without any adverse effect on production performance.

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