Effect of Saffron (*Crocus sativus* L.) petals as feed additive on performance of broiler chicken

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Thesis

Submitted to

Faculty of Veterinary Sciences & Animal Husbandry
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Dedicated

To My Beloved Parents

And my Advisor

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Faculty of Veterinary Sciences & Animal Husbandry
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Certificate - I

This is to certify that the thesis entitled**"Effect of Saffron (*Crocus sativus*L.) petals as feed additive on Performance of Broiler Chicken"** submitted in partial fulfillment of requirements for the award of the degree of **Masters in Animal Nutrition**, to the **Faculty of Veterinary Sciences & Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir** is a record of bonafide research work carried out by **Naveed Ahmed Malik(MSV-2019-442)**under my supervision and guidance. No part of the thesis has been submitted for any degree or diploma.

It is further certified that information received during the course of investigation has duly been acknowledged.

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ABSTRACT

The present study is aimed to evaluate the effect of dietary supplementation of saffron petals as feed additive on the performanceof broilers. In the experiment, 140 day-old chicks were randomly distributed in five treatment groups having four replicates of seven chicks each.Birds of treatment group T_0 (control)were offered basal diet without feed additives. Birds of treatment group T_1, T_2 T_3 and T_4 were offered basal diet supplemented with grinded saffron petals as feed additive @ 0.5,1, 1.5 and 2g/kg feed on dry matter basis. The experimental diets were formulated to contain 3000 Kcal ME/kg and 23.20% CP for pre-starters, 3100 Kcal ME/kg and 20.16% CP for starters and 3191 Kcal ME/kg with 18.00% CP for finishers. The overall temperature and humidity of experimental groups was recorded 23.43°C and 55.19% during day and 23.17°C and 56.01% during night throughout the experiment. The Saffron petals (*Crocus sativus* L.) used in the study as feed additive contained 85% DM, 11.94% CP, 5.03% EE, 7.85% CF, 52.81% NFE, 5.37% total ash, 1.33% acid insoluble ash, 36.10% NDF, 30.00% ADF, 6.10% hemicelluloses and 5.90% cellulose.

The average weekly live body weights (g) of experimental birds subjected to different levels of saffron petals as feed additive in dietary treatment groups (T_0, T_1, T_2, T_3 and T_4) had non-significant differences at the first and second weeks of age. However, the average body weight of birds at the end of 3^{rd}, 4^{th} and 5^{th} week was significantly lower ($P \leq 0.05$) in the T_0 (control) group as compared to

T_1, T_2, T_3 and T_4, with T_4 treatment group having significantly highest ($P \leq 0.05$) body weight followed by T_3, T_2 and T_1 groups.

During entire experimental period among groups supplemented with the feed additive feed intake were numerically higher as compared to control but could not reach to statistical significant difference. Initially, during the first week of the experiment there was no significant difference among the feed conversion ratio (FCR)and feed efficiency ratio(FER) values in the treatments T_0, T_1, T_2, T_3 and T_4; however, as the age progressed, a significantly ($P \leq 0.05$) better feed efficiency (lower FCR and higher FER) was recorded in the birds of T_3 and T_4 groups as compared to control (T_0). The overall FCR and FER of the experimental birds during entire experimental period were significantly better in supplemented (T_1, T_2, T_3 and T_4) groups as compared to control group (T_0). The results of weekly performance index depicted that treatments T_3 andT_4 had significantly ($P \leq 0.05$) higher performance index as compared to control (T_0), T_1 and T_2 groups. The overall performance index of broiler chicken was significantly ($P \leq 0.05$) higher in T_4 followed by T_3,T_2 and T_1treatments with statistically ($P \leq 0.05$) lowest performance index values in control (T_0). The results of average nutrient digestibility in experimental birds show significantly ($P \leq 0.05$) higher dry matter digestibility in treatment T_2, T_3 and T_4 as compared to control (T_0) and T_1 treatment groups. Birds of treatment group T_4 also had significantly($P \leq 0.05$) higher digestibility of crude protein (CP) as compared to control group with no significant difference among T_0, T_1, $T_2$and T_3 treatments.There was nosignificant effect of feed additive supplementation on digestibility of ether extract (EE), crude fibre (CF) and nitrogen-free extract (NFE). There has been no mortality reported of experimental birds during experiment. The production cost per kg live weight was least in treatment T_4 and highest in control (T_0).

All the haemato-biochemical parameters were within normal range indicating no deleterious effect of saffron petal as feed additive in the diet of broiler birds. The blood haemoglobin (Hb) concentration of birds of T_3 and T_4 group were significantly ($P \leq 0.01$) higher as compared to control (T_0) and T_1 group. However, there was no significant difference in Hb concentration among birds of T_1, T_2 and T_3 treatment groups. Red blood cell (RBC) and white blood cell (WBC) count, packed cell volume (PCV), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) values of birds of control (T_0) and treatment groups could not reach to statistical significant difference.The blood glucose values of the experimental birds of group T_0 and T_1 showed significantly lower ($P \leq 0.01$) levels than the birds in T_2, T_3 and T_4, with no significant difference between T_2, T_3 and T_4 treatments. Significantly ($P \leq 0.05$) lower cholesterol and serum triglycerides levels were found in birds of T_4 treatment as compared to control (T_0) with non-significant difference between T_1, T_2 and T_3 treatments. There was no significant difference in serum creatinine, high density lipoprotein (HDL), low-density lipoprotein (LDL) values, serum aspartate transaminase (AST) and serum alanine

transaminotransferase (ALT) activity among birds of control and treatment groups. The birds of group T_4 produced a significantly ($P \leq 0.05$) higher antibody response than the birds of control (T_0), T_1 and T_2 groups with no significant difference with birds of T_3 treatment. Significantly ($P \leq 0.05$) lower dinitrochlorobenzene(DNCB) values were reported in birds of control (T_0)group as compared to birds of T_2, T_3 and T_4 treatments with non-significant difference with birds of T_1 group. The mean weight (g) of different immune organs of the birds of group T_0, T_1, T_2, T_3 and T_4 have no significant difference in average weights of bursa, caecal tonsils and ileum. However, a significantly ($P \leq 0.05$) higher mean spleen weight was recorded in birds of T_4 treatment as compared to control (T_o). Significantly ($P \leq 0.05$) lower mean total oxidant status (TOS) values were recorded in birds of T_2, T_3 and T_4 treatments as compared to control (T_0), with non-significant difference between T_0 and T_1 group. The total antioxidant status (TAS) values in the treatment groups (T_1, T_2, T_3 and T_4) have significantly ($P \leq 0.01$) higher values as compared to control (T_0). The thiobarbuturic acid reactive substances (T-BARS)-Breast values were significantly ($P \leq 0.01$) lower in T_4 treatment as compared to T_0, T_1, T_2 and T_3. The T-BARS (Thigh) values were significantly ($P \leq 0.01$) higher values in control (T_0) followed by T_1, T_2, T_3 and T_4 treatments respectively.

The results of carcass parameters (%) of the birds of group T_0, T_1, T_2, T_3 and T_4 depicted that dressing percentage was significantly ($P \leq 0.05$) higher in birds of T_3 and T_4 groups compared to that of T_0 group. Dressing parameters like feathering loss and bleeding loss of the experimental birds were without any statistical significant difference. The breast, back, drumstick, thigh, wing, neck and total giblet percentage of experimental birds of all treatment groups also could not reach to significant difference statistically.

Key words: Broiler, Carcass parameters, Immune status, Oxidative status, Performance, Saffron petal.

Signature of Student Signature of Major Advisor

Date_____ Date _____

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Dr. Naveed Ahmad Malik

Place:Shuhama, Srinagar

Dated:

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Abbreviations

%	:	Per cent
% kg BW	:	Per cent body weight
°C	:	Degree Celsius
ADF	:	Acid detergent fibre
ad lib	:	Ad libitum
ADG	:	Average daily gain
A: G	:	Albumin: globulin ratio
ALT	:	Alanine aminotransferase
ALP	:	Alkaline phosphatase
AM	:	Ante meridian
ANOVA	:	Analysis of variance
AST	:	Aspartate aminotransferase
AOAC	:	Association of Official Analytical Chemists
BW	:	Body weight
CF	:	Crude fibre
CP	:	Crude protein
d	:	Day
DCP	:	Digestible crude protein
DCPI	:	Digestible crude protein intake
DDM	:	Digestible dry matter
DDMI	:	Digestible dry matter intake
DE	:	Digestible energy
dL	:	Decilitre
DLC	:	Differential leucocytes count
DM	:	Dry matter
DMI	:	Dry matter intake
DNCB	:	Dinitrochlorobenzene
DOM	:	Digestible organic matter
DOMI	:	Digestible organic matter intake
EDTA	:	Ethylene di amine tetra acetic acid
EE	:	Ether extract
EFEs	:	Exogenous Fibrolytic Enzymes
EPG	:	Eggs per gram
FCR	:	Feed conversion ratio
FER	:	Feed efficiency ratio
FECR	:	Faecal egg count reduction
FI	:	Feed intake
g	:	Gram
h	:	Hour (s)

Hb	:	Haemoglobin
HDL	:	How-density lipoprotein
HI	:	Humoral Immune
IVDMD	:	*In-vitro* dry matter digestibility
IVOMD	:	*In-vitro* organic matter digestibility
IVNDFD	:	*In-vitro* neutral detergent fibre digestibility
Kcal	:	Kilocalorie
kg	:	Kilogram
L	:	Litre
LDL	:	Low-density lipoprotein
MCH	:	Mean corpuscular haemoglobin
MCHC	:	Mean corpuscular haemoglobin concentration
MCV	:	Mean corpuscular volume
ME	:	Metabolizable energy
mg	:	Milligram (s)
mL	:	Mililitre
mm	:	Milimeter
MPV	:	Mean platelet volume
N	:	Nitrogen
NDF	:	Neutral detergent fibre
NFE	:	Nitrogen free extract
NH_3	:	Ammonia
NH_3-N	:	Ammonia nitrogen
NPN	:	Non protein nitrogen
NR	:	Nutritive ratio
OM	:	Organic matter
OMI	:	Organic matter intake
Pct	:	Platelet percent
PCV/Hct	:	Packed cell volume/Haematocrit
PDW	:	Platelet distribution width
RDW	:	Red cell distribution width
RBC	:	Red blood cell
ROS	:	Reactive oxygen species
PI	:	Performance index
rpm	:	Revolutions per minute
SE	:	Standard error
SRL	:	Strained rumen liquor
TA	:	Total ash
TAS	:	Total antioxidant status
T-BRAS	:	Thiobarbuturic acid reactive substances
TCA-ppt- N	:	Tri-carboxylic acid precipitable nitrogen

TDN	:	Total digestible nutrients
TEC	:	Total erythrocyte count
TOS	:	Total oxidant status
TLC	:	Total leucocytes count
TTC	:	Total thrombocyte count
TVFA	:	Total volatile fatty acids
$W^{0.75}$	:	Metabolic body weight
WBC	:	White blood cell
wt.	:	Weight

Chapter – 1

INTRODUCTION

In India, poultry industry has emerged as the most dynamic and fastest expanding segment in animal husbandry sector with around eight percent growth rate per annum (Anonymous, 2019). Among different sectors in India, poultry industry represents a major success story, what was largely a backyard venture few decades earlier has been transformed into a vibrant agribusiness (Mehta and Nambiar, 2008). The various favorable factors that are responsible for the growth of poultry industry and business in India are good climate, low cost of labor and inland feed ingredients. The recent growth of Indian poultry sector has been derived from some other key factors such as increase in daily income, young population and gradual shift from vegetarianism to non-vegetarianism preferring chicken meat mostly.

As per 2019 livestock census, annual growth rate of broiler production is about 11% while egg production is about 8.51% with poultry meat contributing about 50.06% of the total meat produced in the country. The annual per capita availability also increased to 60 eggs and 2.5 Kg of meat, consistently with increase in productivity. People of Jammu and Kashmir predominately being non-vegetarian consumed 121 million eggs and 7.4 million Kg poultry meat in year 2016-17 according to official reports of J&K's Animal and Sheep Husbandry Department. The region is managing almost three-fourth of its requirements locally with large quantity of poultry meat being consumed in Kashmir (Anonymous, 2017). However, in Kashmir valley, the poultry production is different from the rest of the country due to its temperate climate and higher altitude. Because of extreme cold environmental temperature particularly from November to March, poultry production becomes a challenging task in Kashmir during winters. Despite of these difficulties, the last decade has witnessed tremendous growth in the poultry sector in J&K with large

number of educated unemployed youth taking poultry farming as a sustainable means of earning their livelihood. Still there is significant gap between requirement and production of poultry and poultry products in the state. Moreover, the increasing human population also increases the demand and in order to meet the objective, the efficient poultry production is need of an hour. There is an extreme selection pressure on broilers for both high growth rate and feed efficiency which puts high demand on the metabolic activities and hence causes extreme physiological oxidative stress resulting due to excessive production of free radicle (Mishra and Jha, 2019). Another important factor for economical and profitable broiler production is maintenance of thermo neutral environment (i.e., 18 to 25 ºC) thereby increasing the extra expenditure on maintenance (Pawar *et al.,* 2016).

Efficient poultry production necessitates the usage of antibiotics as curative and prophylactics along with use of antioxidant supplementation to maintain effective antioxidant defenses to ensure maximum gain in minimum time with better health. However, use of antibiotics as a growth promoter in livestock feed has been fully banned in the European Union since January 2006 (Regulation 1831/2003/EC) owing to antibiotic resistance hazard (Mund *et al.,* 2017). These concerns about antibiotics initiated the surge of exploring alternatives feed additives with similar antimicrobial and growth-promoting effects. In the recent years, some feed additives such as probiotics (Musa *et al.,* 2009), prebiotics (Gibson *et al.,* 2004; Tavaneillo *et al.,* 2018; Kridtayopas *et al.,* 2019), organic acids (de Lange *et al.,* 2010; Upadhaya *et al.,* 2016), enzymes (Khattak *et al.,* 2006, Hashemi and Davoodi 2010; Bedford and Cowieson, 2012) and phytogenics (Randrianarivelo *et al.,* 2010; Gong *et al.,* 2014) are used as a replacement for antibiotics as animal growth promoters (AGP). Due to the increasing awareness, health consciousness and preference for natural foods among consumers scientists are compelled to use natural herbs as feed additive in poultry production. Phytogenic feed additive has been reported to enhance

performance, feed conversion ratio, carcass meat safety and quality in animals (Stanacev *et al.,* 2011). Besides enhancing performance, phytogenic also has anti-oxidant (Nakatani, 2000; Wei and Shibamoto, 2007; Alagawamy *et al.,* 2016), antimicrobial (Jamroz *et al.,* 2006; Mitsch *et al.,* 2004), anticoccidial and immunogenic (Cao and Lin, 2003; Hashemi and Davoodi 2010) properties. They also improve the palatability of the feed and have beneficial effects on nutrient utilization (Platel and Srinivasan, 2004) by improving gastrointestinal morphology (Jamroz *et al.,* 2006; Upadhaya *et al.,* 2016_{ab}). The supplementation of blends of essential oils demonstrated stable egg yield and reproductive performance in birds compared with that of an antibiotic (Bozkurt *et al.,* 2009_{ab}).

Saffron (Crocus sativus L.) is a perennial spicy herb (Iridaceae family) and well known as Red Gold as this plant is the most expensive cultivated herb in the world (Saeidnia *et al.,* 2015). *C. sativus* is cultivated in west of Asia and Mediterranean countries which have cold winter and warm summer especially with less humidity like Iran, Spain, Italy, Greek, Morocco, Azerbaijan and Jammu and Kashmir. In India this high value aromatic spice is called Kesar and in Kashmiri it is called Koung. The world's total production of dried saffron is estimated to be around 325tones per year with more than 90% originating from Iran (Jha, 2019). India produces approximately 7% of the total world saffron exclusively from Jammu and Kashmir (Ganaie and Singh, 2019). In Jammu and Kashmir saffron is cultivated in the district of Pulwama (74.6%) especially in Pampore, Budgam (16.13%) and Srinagar (6.68%). Pampore has the rich heritage of cultivating this golden spice which is also known as the 'saffron town of Kashmir', which alone contributes about 2128 kg saffron annually. It has been recognized as Globally Important Agricultural Heritage Site (GIAHS) by F.A.O. In Jammu and Kashmir, the area under saffron crop is 3674 hectares and production are 9.6 tones with the yield rate of 2.61 kg/h(Ganaie and Singh, 2019).

The plant of *C. sativa* has mainly three parts viz; bulb, stem and flower. The underground parts of the plant, corm or bulb is used to produce new plant as this plant has no seed propagation. The flower of saffron is light purple color having three main parts; namely, stigma, petal and anther, with three stigmas (25-30 mm long) drooping over the petals being outstanding feature. In each kg of harvested fresh flower, there are 2,170 flowers, and therefore 13,020 petals and sepals. It takes about 36,000 flowers to yield just one pound of stigmas (Gohari *et al.,* 2013). From ancient times, the flower of the plant *Crocus sativus* is widely used to promote health and fight diseases in view of its wide range of medicinal uses. Saffron is the rich in carotenoids and contains about 150 volatile aromatic substances. It contains crocin, which is having strong coloring property, distinctive aroma with taste and essential oils which are responsible for its therapeutic properties (Rios *et al.,*1996).

In the literature, little information on the use of dietary saffron as animal feed additive is available and is limited to layers and broiler chickens (Botsoglou*et al*., 2005a; Florou-Paneri *et al*., 2019). One doubt to the use of saffron as a feed additive in animals could be the fact that saffron is the most expensive cultivated spice. However, it may be noted that at least 30% of saffron samples do not fulfil quality specifications and are considered as waste products (Hensel *et al.,* 2006). These saffron by products that have the same composition as the rest of the spice, are discarded for aesthetic reasons and marketed as a relatively cheap byproduct of this industrial production. The low cost of this material encouraged its investigation to be used as a promising and sustainable feed additive for its antioxidant and coloring properties, as well as the health promoting effects. Petals, which form major part of saffron flowers by weight, are currently considered as waste material though their chemical profile is similar to that of the stamens. Phytochemical analysis has shown that saffron flowers are rich in antioxidant compounds like flavanols, flavanones, crocins and crocetin responsible for variety of health benefits traditionally attributed to

saffron (Montoro *et al.,*2012; Goli *et al.,* 2012). Crocins form the main pigment of *C. sativus*stigmas, have also been identified in petals (Moraga *et al.,* 2013). In traditional medicine, saffron petal is consumed as antispasmodic, stomachic, curative of anxiety, antitumor and antidepressant (Mortazavi *et al.,* 2001). Fahim *et al.* (2012) reported that saffron petals contain 10.2% protein, 5.3% fat, 7.0% ash, 8.8% fiber with rich source of minerals like Calcium (486.25mg/100g), Phosphorus (209.90mg/100g), Sodium (25.75mg/100g), Potassium (542.13mg/100g), Copper (0.87mg/100g), Iron (17.99mg/100g), Magnesium (2.93mg/100g) and Zinc (1.8mg/100g). Keeping the above adduced facts in view the present study will be conducted to investigate the effect of saffron petals as feed additive in broiler diet with the following objectives: -

- To study the effect of saffron petals as feed additive on performance parameters and nutrient utilization in broiler chicken
- To study the effect of saffron petals as feed additive on blood biochemical, immune, oxidative and carcass parameters of broiler chicken

Chapter-2

REVIEW OF LITERATURE

The escalating human population is increasing the demand for animal protein, especially broiler meat. To achieve the goal, there is requirement of advanced genetics, nutrition and management in birds which results in improved performance and rapid growth with less feed consumption (Owens *et al*., 2008; Petek *et al*., 2010). Such scientific evolution makes these birds prone to factors that can alter their health, welfare and productivity (Nain *et al*., 2008). Since ages the antibiotics are being used as a prophylactic measure as well as growth promoter in poultry diets. The use of antibiotics as growth promoter in animals has been questioned due to the development of potential antibiotic resistance, transmission of this resistance to humans through the food chain and risk of accumulation of residues in animal products (Wegener *et al.,* 2012). Increased consumer's concern about safety, quality of animal products and also environmental issues, forced animal nutritionist to use "natural additives" in animal diets. These natural feed additives apart from increasing productivity, decrease the antibiotic load with no risk of antibiotic resistance. So, feed additives of plant origin such as herbs as such, essential oils or extracts of aromatic plants have received considerable attention as an alternative to traditional antibiotics.

Apart from antibiotic growth promoters, use of antioxidants in poultry diets are in rise owing to development of new broiler strains with faster growth rate at lower feed consumption. Use of synthetic antioxidants (butylated hydroxyanisole, butylated hydroxytoluene), despite the effectiveness of their use, provoke occurrence of various chronic diseases among both birds and consumers, which significantly limits their use. Natural antioxidants which are safer, cheaper, do not cause metabolic diseases in birds are also able to prevent oxidative reactions in animal products during storage. Among natural antioxidants herbal feed additives has been found to be a simple and convenient strategy to introduce

natural antioxidants in poultry diets (Botsoglou *et al.,* 2002). Supplementation of poultry diets with rosemary and sage (Lopez-Bote *et al.,* 1998), tea extracts (Tang *et al.,* 2000; 2001) and essential oil of oregano (Botsoglou *et al.,* 2002; 2003a,b,c; Papageorgiou *et al.,* 2003) could increase the oxidative stability of chicken and turkey tissues. These natural antioxidants affect lipid oxidation in meat after slaughter during storage and prevent meat from oxidative rancidity (Caleja *et al.,* 2017).

2.1 Use of Mediterranean diet spices as feed additives

In a study assessing the antioxidant properties of some mediterranean diet spices, rosemary, oregano and saffron were reported to be effective scavengers of peroxyl radicals in an ox brain phospholipid system compared with other common food additives (Martinez-Tomme *et al.,*2001). This antioxidant properties of natural phytobiotics are mainly related to their phenolic content, thus, their action is similar to synthetic phenolic antioxidants (Duong *et al.,* 2016). Green tea (*Camellia sinensis*) is well-known antioxidant contains epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG), epicatechin (EC), and flavonoids that helps to reduce the risk of a wide range of chronic diseases such as cancer, diabetes and cardiovascular diseases (Charradi *et al.,* 2017). Grape seed extract contains biologically active phenolic compounds such as flavonols, plant polyphenols (flavan-3-ol), anthocyanins, tannins and derivatives of phenolic acid (Sano., 2017). Grape seeds are a particularly rich source of proanthocyanidins (PAs), that consist of procyanidin and esterified gallic acid which inhibits the lipid oxidation of poultry during gastric digestion (Bonilla and Sobral, 2016). Clove oil a natural preservative inhibits the growth of bacteria and mould results in improving live weight of broilers when incorporated in poultry diets at a rate from 300 to 500 mg perkg of feed (Valenzuela-Grijalva *et al.,* 2017). Cinnamon is a spice with strong antimicrobial and antioxidant activity (Su *et al.,* 2016). Coloured berries (*Vaccinium uliginosum L.*,) also known as marsh blueberry, is rich in anthocyanins and flavonols. Sunflower seeds and

pomegranate peel are good sources of tannins, anthocyanins and flavonoids (Bazargani-Gilani *et al.,* 2015).

Positive effect on growth and development of broiler chickens and laying hens with improved feed conversion was noted, when instead antibiotics, they obtained mandarin peel extract and crushed leaves of *Moringa oleifera*(Kutlu *et al.,* 2018). An increase in the intestinal population of the *Lactobacillus bacteria* and the suppression of the *Escherichia coli* population in the cecum compared to control group of broilers after the introduction of eugenol (4-allyl-2-methoxyphenol), obtained from a tropical plant *Eugenia uniflora L.,* has been reported (Peek *et al.,* 2013). *Echinacea purpurea* extract, containing β-glucan, shiitake mushroom extract (*Lentinula edodes*) that contains betaine and β-curcumin obtained from turmeric (*Curcuma longa L.*) are effective antioxidants for broilers that suppress the activity of *Escherichia coli* (Alp *et al.,* 2012). Anticoccidial effect was observed when thymol and carvacrol (obtained from oregano leaves) were added to broiler feed with improved feed conversion (Surai, 2013). A number of researchers have noted the positive effect of using essential oils of oregano (*Origanum sp.*), leaves of laurel (*Laurus nobilis L.*), sage (*Salvia triloba L.*), myrtle (*Myrtus communis L.*), fennel (*Foeniculum vulgare Mill*) and citrus peel (*Citrus sp.*) in an amount of up to 24 mg per kg of feed as a feed additive for the production of quail eggs (Adaszyńska-Skwirzyńska and Szczerbińska, 2017). The use of lavender oil (*Lavandula stoechas L.*) for 21 days during the growth period of broiler chicken in an amount of 24mg per kg of feed led to an increase in live weight and a decrease in mortality. A mixture of essential oils containing capsaicin, carvacrol and cinnamon aldehyde on broilers in an amount of 400 mg per kg of feed during the period of intensive growth and 150 mg per kg of feed during the final stage of fattening (31 - 42 days) increased body weight and feed efficiency. Oskoueian *et al.* (2013) confirms the suppression of the vital activity of *Campylobacter jenuni*, which cause campylobacteriosis with bioactive phenols extracted from blackberry (*Rubus*

fruticosus L.) and blueberry (*Vaccinium corymbosum L.*). Patra and Yu, (2012) noted a positive effect on the overall health of a bird as a result of the consumption of extracts of cumin, anise, coriander and fennel. Buo *et al.,*(2009)reported that antioxidants such as vitamin E, ascorbic acid, selenium, polyphenols of oats, rosemary, sage, oregano and milk thistle extract (Embuscado, 2015) improve the antioxidant protection and preservation of broiler chickens and further improve the quality of poultry meat and eggs.

Mir *et al.* (2013) investigated the presence of bioactive constituents (Saponins, flavonoids, alkaloids, phenols) of Dandelion in its aqueous and methanol extract. Dandelion reduces serum cholesterol and triglycerides because it intensifies bile secretion (Jassim *et al*., 2012). Galib *et al.* (2010) reported that the supplementation of Dandelion in broiler chicken @ 0.5% of diet improved body weight gain with better feed conversion ratio and reduced mortality. Meghwal and Goswami (2012) and Mullaicharam *et al.*, (2013)reported that Fenugreek seeds contain rare chemical constituents (saponins, coumarin, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin and trigonelline), exhibit pharmacological properties such as antitumor, antiviral, arteriosclerosis, antimicrobial, anti-inflammatory, hypotensive and antioxidant activity.The use of fenugreek seeds as feed additive in broiler diets @ 5.33 kg per ton (Abdel-Rahman *et al.,* 2014), @ 0.3% level (Aksa *et al*., 2012; Rabia, 2010), @ 0.5% level (Abdel-Azeem 2006), @ 1% level (Mamoun *et al*., 2014; Weerasingha and Atapattu, 2013; Yatoo *et al*., 2012), @ 1.5% level (Magda, 2012), 2% level (Hind *et al*., 2013), 4% level resulted improvement in the live body weight, feed intake and feed efficiency with reduced mortality. Raziq (2012), Abaza (2001), Guo *et al*., (2004)and Farman Ullah *et al.*, (2009) also reported improved body weight and feed efficiency with reduction in feed cost when the diets of broiler chicken were supplemented with Fenugreek seeds as natural feed additives. Qureshi *et al.,* (2016) reported that diets supplemented with either Dandelion leaves @ 5g/kg feed or Fenugreek seeds@ 10g/kg feed alone or in combination with or without

enzyme addition resulted into an improvement in the body weight, feed conversion ratio and economics with reduced caecal viable and coliform counts with beneficial effect on liver and small intestines histomorphology of broiler chicken. There was no effect on other carcass parameters with only higher dressing percentage in supplemented groups as compared to control. Suntres, (2011) have made attempted to systematize plant antioxidants and their effects. The results are summarized in table 2.1.

Table 2.1: Mediterranean diet spices as feed additives

Plant	Antioxidant compound	Antioxidant action
Rosemary (*Rosmarinus officinalis L.*)	L-carnosine, carnosic acid, rosmadial, diterpenes (epirosmanol, isosmanol, rosmaridiphenol, rosmarichinon, rosmarinic acid)	Free radical acceptor
Sage (*Salvia officinalis L.*)	Carnaic acid, rosmanol, rosmadial, methyl and ethyl, rosmarinic acid	
Oregano (*Origanum vulgare L.*)	Rosmarinic acid, 3,4-dihydroxycinnamic acid, phenylpropionic acid; flavonoids - apigen, eridictil, dihydroquercetin; carvacrol, thymol	
Thyme (*Thymus vulgaris L.*)	Thymol, carvacrol, phenolic acids, phenolic diterpenes, flavonoids	
Ginger (*Zīngiber officināle*)	Gingerols	
Turmeric (*Curcuma L.*)	Turmeric, 4-Hydroxycinnamoylmethane	
Black pepper (*Piper nigrum L.*)	Kempferol, ramnetin, quercetin	
Hot red pepper (*Capsicum frutescens*)	Capsaicin, capsaicinol	
Carnation (*Dianthus caryophyllus L.*)	Phenolic acids (gallic acid), flavonol glucosides, phenolic volatile oils (eugenol, acetylevenol, isoegenol), tannins	
Marjoram (*Majorana majorana L.*)	β-carotene, β-sitosterol, caffeic acid, carvacrol, eugenol, hydroquinone, rosmarinic acid, terpinen4-ol	
Cumin (Carum carvi L.)	Kumin, γ-terpinene, pinocarveol, linalool, 1-methyl-2- (1-methylethyl) benzene, carotene	

2.2 Saffron

Crocus sativus L.,commonly known as saffron, a perennial stem less bulbous plant of the Iridaceae family, is well adapted to arid and semi-arid lands adaptable to temperate and sub-tropical climates. It has been originated from Eastern Greece or Crete named as 'red gold' as saffron is most expensive spice in the whole world.Saffron is a global spice and is named as Azafran (Spanish), Safran (French), Zafferano (Italian), Agafrao (Portuguese), Saffran (Swedish and German), Saframi (Finnish), Safrany (Hungarian), Shafran (Russian), Zafraran (Arabic), Fan nung hua (Chinese) and Safuran (Japanese). Zaafarn is the original Persian name of saffron, meaning yellow flowers. In India this high value aromatic spice is called Kum Kumin or Kesar and in Kashmiri it is called Koung.

Cultivation history of saffron returns to 2500-1500 BCE as it has been used in folk medicine for more than 3000 years. Iran, saffron's origin, owns more than 80% of its world's production, although it is cultivated around the world e.g., in China, Spain, Italy, India, Pakistan, Turkey, France, Switzerland and Greece (Cavuolu *et al.,* 2009; Hosseinzadeh *et al.,* 2008b; Bolandi & Ghoddusi, 2006). Total world production of saffron is around 300 tons per year with Iran, India, Spain and Greece major saffron producing countries. India produces approximately 7% of the total world saffron production with Jammu and Kashmir only state where saffron is produced (Anonymous, 2015). In Jammu and Kashmir saffron is cultivated in the districts of Pulwama (74.6%) especially in Pampore, Budgam (16.13%), Srinagar (6.68%). Pampore has the rich heritage of cultivating this golden spice which is also known as the 'saffron town of Kashmir', which alone contributes about 2128 kg saffron annually. It has been recognized as Globally Important Agricultural Heritage Site (GIAHS) by F.A.O. In Jammu and Kashmir, the area under saffron crop is 3674 hectares and production are 9.6 tones with the yield rate of 2.61 kg/ha (Anonymous, 2015).

2.2.1 Structure of saffron plant:

The saffron plant is made up basically from corn (bulb), stem, 2 to 3 cm triple stigma, three anthers and six light purple tepals (petals), stigma being the main component of saffron plant (Fig. 1). The female sexual part of saffron flower consists of stigma, style, ovary, peduncle and its male part, stamens are yellow in color (Aytekin & Acikgoz, 2008).

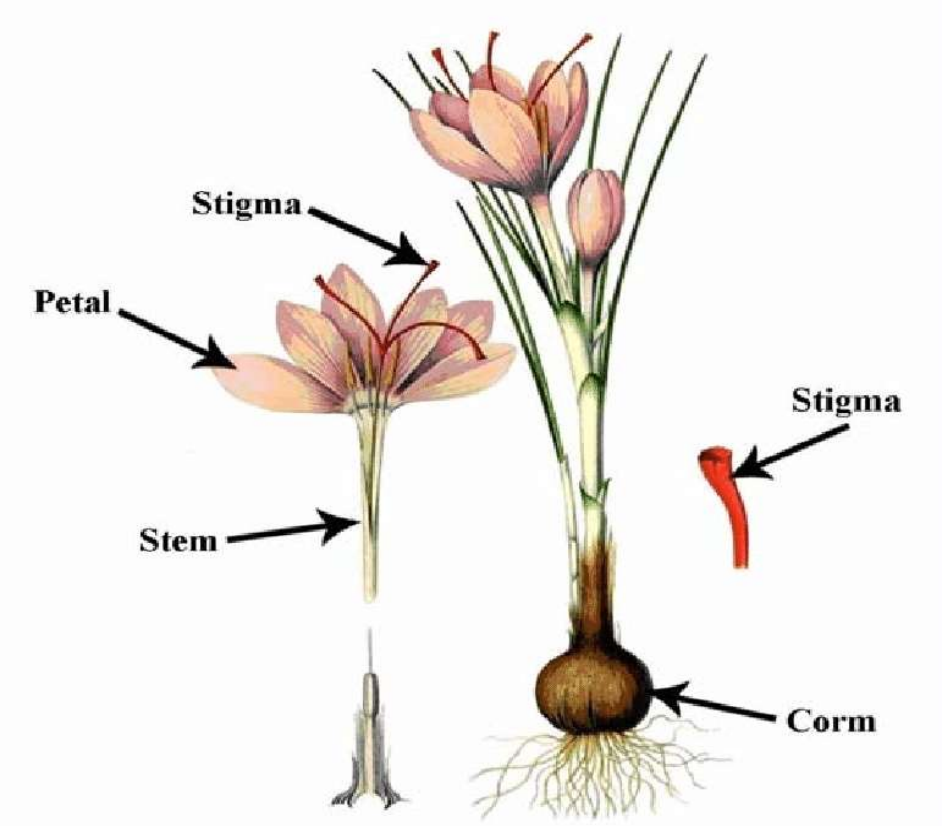


Fig. 2.1.Structure of saffron plant (Rubio-Moraga *et al.,* 2010; Ozdemir & Kilinc,2008).

2.2.2 Chemical composition of saffron (stigma)

Analysis has shown the presence of more than 150 chemical components in saffron stigmas, which contain carbohydrates, proteins, amino acids, minerals, mucilage, gums, vitamins (especially riboflavin and thiamine), alkaloids, saponins, pigments (crocins, carotenes, carotenoids, anthocyanin, lycopene, flavonoids and zeaxanthin), terpenes (saffranal and picrocrocin) together with other chemical compounds (Carmona et al., 2006; Samarghandian and Borji, 2014; Singla & Bhat, 2011; Zarinkamar *et al.,* 2011). The main components of stigma are crocetin, its glucosidic derivatives, crocins, picrocrocin, safranal (Rubio-Moraga *et al.,* 2010) and flavonoids including quercetin and kaempferol (Nikolaos*et al.,* 2008). Quality of saffron is highly influenced by the presence of its three major compounds (crocin, picrocrocin, and saffranal). Proximate composition of saffron stigma is 90% dry matter, 12 % protein, 5% fat, 5% crude fibre, 5% minerals (mainly calcium, phosphorous, potassium, sodium, zinc and manganese), crude fibre (5%w/w) and 63% nitrogen free extract (starch, reducing sugars (glucose, fructose, gentiobiose and a small quantity of xylose and ramnose), pentosans, gums, pectin, and dextrins (Habibi & Bagheri, 1989; Wang *et al.,* 2014).

2.2.3 Applications of saffron in the food and pharmaceutical industry

Saffron is widely used in food flavouring and colouring, cosmetics, textile dye, perfume and pharmaceutical industry (Tarantilis *et al.,* 1998). In the food industry, saffron is used as an odoring, tasting and coloring agent in cream, cheese, butter, ice cream, bouillabaisse, chicken, bread, soups, sauces, puddings, rice and paella in industrial scales (Tarantilis *et al.,* 1994; Nilkashi *et al.,* 2011). Recently, there have been some efforts for the extraction of bioactive components from stigma (Sarfarazi *et al.,* 2015) and tepals (Khazaei *et al.,* 2016) of saffron along with micro/nano encapsulation of these nutraceuticals (Khazaei *et al.,* 2016; Jafari *et al.,* 2016).

Studies related to the influence of saffron on human health are due to multitude of bioactive agents present. The content of these bioactive agents gets influenced by different environmental conditions like temperature, soil type, humidity, drought, altitude, UV etc. (Zarinkamar *et al.,* 2011). Saffron has been traditionally used as an aphrodisiac agent, anticatarrhal, laxative, eupeptic, timulant, antispasmodic, antidepressant, a respiratory decongestant, nerve sedative, stomachic, expectorant, emmenagogue, carminative, diaphoretic, anodyne, gingival sedative, galactogogue, amenor-rhea, dysmenorrhea and in treatment of bladder, liver, kidney, eye, acne, and several skin diseases (Hosseinzadeh *et al.,* 2008b; Nikolaos*et al.,* 2008). Other pharmaceutical use of saffron are treatment of ascites, naevus, freckles, ulcer, headache, cold, smallpox, scarlet fever, bronchitis, pharyngopathy, vomiting, asthma, myopia, discomfort of teething infants, cholera, inflammation, melancholia, epilepsy, menstruation disorder, painful labor, strengthening the heart and memory enhancer (Nilkashi *et al.,* 2011).

2.2.4 Safety of saffron

Reports suggest that saffron is fully a nontoxic plant (Abdullaeva *et al.,* 2004; Abdullaev & Espinosa-Aguirre, 2004). Ethanolic extracts of saffron, at doses rate of 5 g/kg b.wt, did not cause any toxic changes in liver and kidney of mice (Abdullaeva *et al.,* 2004). In another study, saffron tablets at doses rate of 200 and 400 mg (4-6 times) for 7 days decreased blood pressure of volunteers and changed their hematological and biochemical factors (triglyceride, cholesterol, HDL and LDL), which were not clinically important (Modaghegh *et al.,* 2008). The results of double-blind, placebo-controlled study performed in healthy volunteers (male and females) also showed that administration for one month of crocin tablets (20 mg/day) did not elicit significant alterations of different hematological, biochemical, hormonal and urinary parameters recorded (Mohamadpour *et al.,* 2013). Another double-blind, placebo-controlled study performed on patients (all male) with schizophrenia during 12 weeks of capsules

of saffron aqueous extract (15 mg twice daily), crocin (15 mg twice daily) showed that markers of thyroid, liver and kidney or inflammation markers had no statistically significant differences among the groups (Mousavi *et al.,* 2015). However, some studies reported that saffron at dose rate of 1.2-2 g caused nausea, vomiting, diarrhea and bleeding (Schmidt *et al.,* 2007).

2.3 Saffron petals

The harvested stigma from saffron flower is used as a food spice or as an herb with medicinal properties, whereas Saffron petal is a by-product and is usually discarded as a waste. Petals are the major part of saffron flower as (96.36% on dry matter basis) are a good, low cost source of bioactive components. (Khazaei *et al.,* 2014). Tepals of *Crocus sativus L.*flower, are a rich source of phenolic and biologically active compounds; such as, flavonoids (kaempferol, rutin, quercetin, luteolin, hesperidin, and bioflavonoids), tannins and anthocyanins (Kanakis *et al.,* 2006; Srivastava *et al.,* 2010). Traditionally saffron petal is consumed as antispasmodic, stomachic, curative of anxiety, antitumor and antidepressant (Mortazavi *et al.,* 2001). In addition, saffron tepal is a source of protein, fiber, fats and essential minerals (K, Ca and P), necessary for the growth of animals, hence can be used as an animal feeding source (Fahim *et al.,* 2012).

2.3.1 Chemical composition and health-promoting effects of tepal

Proximate analysis revealed that saffron petal contains 10.20% crude protein, 5.3% ether extract, 8.80%crude fiber and 7.00% ash (Fahim *et al.,* 2012). Being rich source of minerals, saffron petals contain sodium (25.75 mg/100 g), potassium (542.13 mg/100 g), calcium (486.25 mg/100 g), copper (0.87 mg/100 g), iron (17.99 mg/100 g), magnesium (2.93 mg/100 g), zinc (1.80 mg/100 g) and phosphorus (209.90 mg/100 g) (Fahim *et al.,* 2012). Also, it is composed of flavonoles (kaempferol, 12.6%w/w) (Hadizadeh *et al.,* 2003; Eka *et al.,* 2015), carotenoids (crocin, 0.6%w/w and crocetin) (Eka *et al.,* 2015) anthocyanins (Khazaei *et al.,* 2016) phenolic compounds (Goli *et al.,* 2012) terpenoids and

alkaloids(Termentzi and Kokkalou, 2008). The analysis of ethanolic extract of saffron petal by HR-NMR spectroscopy confirmed the presence of kinsenoside, goodyeroside A, kaempferol 3-O-3 sophoroside and 3-hydroxy-γ- butyrolactone (Righi*et al.,* 2015). The stigma and petal are containing terpenoids such as crocusatins and anthocyanin which causes the purple color of saffron (Li*et al.,* 2004).

Table 2.2: Proximate, mineral and bioactive compounds in saffron petal

Compound	Amount	Reference
Protein	10.20%	(Fahim *et al.,* 2012)
Fat	5.3%	(Fahim *et al.,* 2012)
Ash	7%	(Fahim *et al.,* 2012)
Fiber	8.80%	(Fahim *et al.,* 2012)
Sodium	25.75 mg/100 g	(Fahim *et al.,* 2012)
Potassium	542.13 mg/100g	(Fahim *et al.,* 2012)
Calcium	486.25 -542.13 mg/100 g	(Fahim *et al.,* 2012)
Copper	0.87 mg/100g	(Fahim *et al.,* 2012)
Iron	17.99 mg/100g	(Fahim *et al.,* 2012)
Mg	2.93 mg/100g	(Fahim *et al.,* 2012)
Zinc	1.80 mg/100g	(Fahim *et al.,* 2012)
Phosphorus zinc	209.90 mg/100g	(Fahim *et al.,* 2012)
Kaempferol	12.6%w/w	(Hadizadeh *et al.,* 2003)
Crocin	0.6%w/w	(Eka *et al.,* 2015)
Anthocyanins	1712 mg/l extract	(Khazaei*et al.,* 2016)
Phenolic compounds	3.42 mg	(Goli *et al.,* 2012)
Terpenoids		(Goli *et al.,* 2012)
Alkaloids		(Fahim *et al.,* 2012)

2.3.2 Pharmacological properties of saffron petals

2.3.2.1 Antibacterial

There has been reports that methanolic, ethyl acetate and aqueous extracts of saffron petals exhibit antimicrobial activity. The methanolic extract of saffron petal showed antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus, Salmonella typhi, Escherichia coli* and *Shingella dysenteriae* at concentration of 1000 mg/ml, whereas ethyl acetate extract prevented the growth of *B. cereus.* The water and chloroform extracts had lesser activity against mentioned strains (Asgarpanah *et al.,* 2013).

2.3.2.2Antispasmodic effects

To investigate the effect of saffron petal on tonicity of smooth muscles, rat isolated vas deferens and guinea pig isolated ileum were used. The study revealed that the petal extracts reduced electrical field stimulation (EFS)-induced contraction in rat isolated vas deferens by reduced responses to epinephrine and EFS induced contraction in guinea-pig isolated ileum via inhibition of muscarinic receptors (Fatehi *et al.,* 2003).

2.3.2.3Antidepressant effect:

According to medical studies, saffron tepal can be used to treat depression (Melnyk *et al.,* 2010; Moshiri *et al.,* 2007; Karimi *et al.,* 2001). This anti-depressant property of saffron petal is related to safranal and crocin bioactive agents (Hausenblas *et al.,* 2013). Comparing antidepressant effect of tepal form *C. sativus* (15 mg bid) with fluoxetine (10 mg bid), Basti *et al.,*(2007) reported no considerable difference in Hamilton Rating Scale for Depression score. In another study, stigma and tepal extracts of *Crocus sativus* and its constituents, safranal and crocin, have shown antidepressant activity in the forced swimming test in mice and rats (Hausenblas *et al.,* 2013).

2.3.2.4Antinociceptive and anti-inflammatory activity

Ethanolic and aqueous extracts of saffron tepal exhibited significant anti-inflammatory activity in in mice as evident from the chronic inflammatory test. Hosseinzadeh and Younesi (2002). Anti-inflammatory potentials of tepal extracts of *Crocus sativus* have been evaluated via in vitro (human red blood cell) and in vivo (rat) experiments by Bhat *et al.,* 2012. The results of tepal extract (400 mg/ml) were compared to those of Diclofenac (10 mg/ml) for in-vitro and in-vivo models and both showed similar anti-inflammatory activities of 63.16% and 71.05%, respectively. Antinociceptive and anti-inflammatory effects of the extracts are attributed to their content of flavonoids, tannins, anthocyanins, alkaloids, and saponins (Hosseinzadeh *et al.,* 2000).

2.3.2.5Antioxidant and antiproliferativeeffect of saffron tepal

Antioxidant and antiproliferative effect of saffron tepal were investigated by Sanchez-Vioque *et al.*(2012) on human colon adenocarcinoma cells and reported best antioxidant properties for saffron petal extract, which totally inhibited the oxidation of b-carotene at 10mg/ml and showed a DPPH scavenger activity up to 32 times higher than those reported for traditional sources of antioxidants like grapes and berries (Sanchez-Vioque *et al.,* 2012).

2.3.2.6Cardiovascular effect

In an in vivo study, it was reported that saffron extracts, particularly the mixture of extracts from stigma and tepal, ameliorated dyslipidemia in obese rats, leading to decreased atherosclerosis and insulin resistance (Hoshyar *et al.,* 2016). Animal studies have also shown the effect of saffron tepals in hypertension as aqueous and ethanol extracts of *C. sativus* tepals reduced blood pressure in a dose-dependent manner in rats in an experiment carried out by Fatehi *et al.* (2003). It was reported that 50 mg/100 g of aqueous extract reduced blood pressure from 133.5 to 117 (mmHg), possibly due to antioxidant and anti-inflammatory properties of saffron tepals (Milajerdi *et al.,* 2016).

2.3.2.7Hepatoprotective and Renoprotective

The levels of liverfunction enzymes (ALT and AST) decreased following treatment by ethanolic and aqueous extracts of saffron petal reducing carbon tetrachloride, acetaminophen and Cisplatin hepato toxicity. Histopathological studies showed the petal extracts reduced liver lesions induced by carbon tetrachloride, acetaminophen and Cisplatin as antioxidant properties of petal reducing production of free radicals (Iranshah *et al.,* 2011; Omidi *et al.,* 2014a). A study investigated the protective effect of saffron petal against gentamicin-induced peliosis hepatis in rats (Nakanuma, 1995). Saffron petal extract reduces renal toxicity induced by Acetaminophen drug via reduction of uric acid and creatinine levels (Omidi *et al.,* 2015).

2.3.2.8Antiobesity and Antidyslipidemia

In a study saffron stigma (40 mg/ kg), petal (80 mg/kg) and combination of them (80 mg/kg) were gavaged to rats for 3 weeks received high-fat diet for 10 weeks. The results showed the extracts decreased total cholesterol, triglyceride and LDL, while, increased HDL levels. Also, the extracts reduced atherosclerosis-index (LDL/HDL), atherogenic index (TC/HDL), liver enzymes (ALT, AST and ALP), leptin and insulin. Results indicated that saffron extracts ameliorated dyslipidemia in obese rats via reduction of atherosclerosis and insulin resistant (Hoshyar *et al.,* 2016).

2.3.2.9Antidiabetic and Antioxidant activity

In Streptozotocin -diabetic rats, saffron petal extract was given orally at doses of 100 or 200 mg/kg for 28 days. The extract at dose of 200 mg/kg reduced fasting blood sugar, while, urine volume and BUN level decreased by both of doses with no effect on creatinine level. Also, the extract improved the histological damages induced by Streptozotocin (Zarezadeh*et al.,* 2017). In another study Amraei *et al.* (2018) reported that 100 and 200 mg/kg dosages of saffron petal extract caused significant reductions in serum glucose levels in

contrast to the diabetic groups with significant increase in serum insulin levels ($P<0.001$) in Streptozotocin induced diabetic Rats.

Antioxidant activity of saffron petal in lambs has been evaluated by (Ardalan *et al.,* 2012) using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free-radical method. The extracts of saffron petal were gavaged at doses of 500, 1000 and 1500 mg/kg for 15 days, reported increased anti-oxidant status at all doses. The extract did not change the levels of glucose, uric acid, creatinine, AST, ALT, ALP, MDA, total thiol, BUN and other indexes.

2.3.2.10Immune system

In a study conducted on rats received graded doses saffron petal extract @ 0, 75, 150, 225, and 450 mg/kg for 14 days increased IgG at dose of 75 mg/ kg in comparison with other groups, with no difference in hematological parameters such as red blood cells, hemoglobin, hematocrit, and platelet has been observed between treated groups with control (Babaei*et al.,* 2014).

2.3.2.11Antitumor activity

Anti-tumor activity of saffron stigma and petal was evaluated using brine shrimp and potato disk. Results showed that the IC50 values of saffron extracts were 5.3mg/ml and 10.8 mg/ml for petal and stigma extracts against tumor, respectively (Hosseinzadeh *et al.,* 2005).

2.3.3 Toxicity of saffron petal

According to toxicological studies, toxicity of stigma is more than petal. Study reported that consumption of 1.2 g saffron led to diarrhea, bleeding, nausea and vomiting (3). For determination of LD50, different doses of saffron stigma and petal were injected to rats intraperitoneally (IP) and the mortality was evaluated after 24 hr. The LD50 values of saffron stigma and petal in mice were 1.6 and 6 g/kg, respectively (Karimi *et al.,* 2004). In a sub-acute toxicity study, saffron stigma was injected IP at doses of 0.16, 0.32 and 0.48 g/kg, while, petal was administrated at doses of 1.2, 2.4 and 3.6g/kg for two weeks. This study

reported that saffron petal and stigma extracts reduced body weight, hematocrit, hemoglobin and erythrocytes. Pathological examination showed stigma did not cause damage in different organs significantly, while, liver and lung injuries were observed in animals received saffron petal (Karimi*et al.,* 2004).

2.3.4 Use of saffron/saffron petal as a feed additive

Poultry industry appreciate natural antioxidants that could replace synthetic ones (hydroxyanisole, butylated hydroxytoluene, or *tert*-butylated hydroxyl- quinone) for increasing the oxidative stability of foods and satisfy consumer demands for production of eggs and meat without residues from substances that have the potential to harm humanhealth. In a study to assess the antioxidant properties of some spices, saffron was found to be an effective scavenger of peroxyl radicals in an ox brain phospholipid system compared with rosemary, oregano, annatto, paprika, cumin and common food additives including butylated hydroxyanisole, buty- lated hydroxytoluene and propyl gallate (Martinez-Tomé *et al.,*2001). Both crocins and crocetins have exhibited substantial antioxidative activity; however, their antioxidative mechanism has not been yet adequately elucidated (Matheson and Rodgers 1982; Ríos *et al.,*1996; Pham *et al.,*2000). Incorporation of the antioxidant constituents of saffron into egg yolk and meat through addition of saffron to poultry feeds might help in reducing or eliminating the need for additional oxidative stabilization.

In the literature, little information on the use of dietary saffron as a feed additive is available and is limited to layers and broiler chickens (Botsoglou *et al*., 2005a; Florou- Paneri *et al*., 2006; Naghous *et al.,* (2015); Zeweil *et al*. (2015). Botsoglou *et al*. (2005a) investigated the effect of feed supplementation with red stigmas of saffron on the oxidative stability of shell eggs and liquid yolks during refrigerated storage. In this study, laying hens were given feeds supplemented with 10 or 20 mg saffron or 200 mg *a*-tocopheryl acetate/kg feed. Following 6

weeks feeding, the rate of lipid oxidation was determined in shell eggs refrigerated stored for two months. Results showed that the extent of lipid oxidation in shell eggs, as measured by malondialdehyde (MDA) formation (Botsoglou *et al.,* 1994), did not change with the storage time but differed significantly between the dietary treatments with better results in saffron supplemented group. Pham *et al.* (2000) have shown that crocins, the main biologically active constituents of saffron, could prevent the oxidation of linoleic acid *in vitro* as their anti- oxidative activity was found to be comparable to that of butylated hydroxyanisole.

Since shell eggs were inherently resistant to oxidative deterioration upon refrigerated storage, the same authors (Botsoglou *et al*., 2005a) performed additional experiments in order to evaluate the oxidative stability of the yolk lipids under conditions that could promote lipid oxidation. Thus, the effect of dietary treatments on the oxidative stability of yolks adjusted at pH of 6.2 or 4.2 and agitated for 20 days in presence of light was investigated. Results showed that the extent of lipid oxidation, as measured by MDA formation, differed between the dietary treatments at all time points. Dietary treatments exhibited progressively higher MDA values compared to day 0, however the rate of lipid oxidation was rather low by day 12 of agitation and it was more intense thereafter. Control yolks at pH 6.2 presented mean MDA values that were higher than those of the group fed 10 mg saffron/kg feed, which in turn were higher than those of the group fed 20 mg saffron/kg feed, a finding suggesting that dietary saffron exerted a dose dependent anti-oxidative activity. The *a*-tocopherol supplemented group presented MDA values that were lower compared to all other treatments at all time points. The oxidation profile of yolks adjusted at pH 4.2 showed a similar pattern, however, the rate of oxidation was more intense. Considering that egg yolks from the saffron groups exhibited increased resistance to lipid oxidation compared to the control, it could be assumed that components from saffron passed throughfeeding into egg yolks providing antioxidant properties. In another study,

Botsoglou *et al*. (2005b) investigated the effect of feeding rosemary, oregano, saffron or *a*-tocopheryl acetate on hen performance and egg quality. In this study, the experimental feeds contained 200 mg *a*-tocopheryl acetate, or 5 g rosemary, or 5 g oregano, or 20 mg saffron/kg. Results showed no significant differences in egg production, feed intake, feed conversion ratio, egg weight and shape, yolk shape, haugh units and shell thickness among treatments. However, yolk colour was significantly improved in the saffron group compared to other groups. The extent of lipid oxidation in shell eggs differed among the dietary treatments, but did not change with storage time. In liquid yolk at pH 6.2, lipid oxidation was higher in the control group compared to other groups. The oregano group presented lower oxidation rate than the rosemary group, but higher than the saffron group, which in turn exhibited higher oxidation rate than the tocopherol group. When liquid yolk was acidified at pH 4.2, the lipid oxidation profile remained unchanged but the rate of lipid oxidation was much more intense.

Florou-Paneri *et al.* (2006) investigated the effect of feed supplementation with red stigmas of Greek saffron at dose rate of 10 or 20 mg saffron or 200 mg *a*-tocopheryl acetate/kg feed on the oxidative stability of breast and thigh muscle tissues. Following 42 days feeding, birds were slaughtered, and the oxidative stability of the collected breast and thigh muscle tissue samples were assessed by monitoring malondialdehyde (MDA) formation during refrigerated storage at 4°C for 9 days. The results showed that refrigeration of both raw and cooked tissue samples led to spontaneous increase in the malondialdehyde content in all groups. Tissue samples from birds fed the feed supplemented with 200 mg/kg *a*-tocopheryl acetate showed lowest mean levels of malondialdehyde during the 9-day period of refrigerated storage compared to the other groups. The incorporation of saffron in feeds at the level of 20 mg/kg feed was more effective in retarding lipid oxidation in both raw and cooked samples compared to the level of 10 mg saffron /kg feed. Thigh muscle was more susceptible to oxidation than breast muscle in allgroups.Naghous *et al.* (2015) reported that saffron petals at rate of 2.5g/ kilogram increases Malon Di

Aldehyde in poultry meat depicting better keeping quality with decreased tenderness and juiciness. Zeweil *et al*. (2015) conducted a study on effect of vitamin E and phytogenic feed additives on performance, blood constituents and antioxidative properties of broiler chicks. Two hundred and sixteen, 7 day old unsexed Hubbard broiler chicks were divided randomly into nine treatments divided into 3 replicates with 8 chicks each. All treatments received basal diets as per requirement, with chicks in treatment (T_1) were fed basal diets without any addition (control), whereas chicks in group (T_2) were fed basal diets supplemented with vitamin E @ 200 mg/kg feed. In groups (T_3), (T_4) and (T_5) chicks were fedbasal diets supplemented with Rosemary @1.5 g/kg feed, Rosemary @ 3 g/kg feed and Rosemary @ 9 g/kg feed respectively. Chicks in group (T_6), (T_7), (T_8) and (T_9) were fed basal diets supplemented Saffron @15 ppm/kg feed, Saffron @ 30 ppm/kg feed, Rosemary+ Saffron @ (0.75g +7.5 ppm) / kg feed and Rosemary+Saffron @ (1.5g +15ppm)/kg feed, respectively. There was significantly ($P\leq0.05$) lower body weight, body weight gain and feed intake of broilers fed Rosemary 9.0 g/kg than other treatments with worst feed conversion ratio. No significant effects were observed on carcass characteristics and meat chemical analyses except abdominal fat wassignificantly ($P\leq0.05$) higher in birds of control (T_1) group as compared to other treatments. Plasma total proteinwere found significantly ($P\leq0.05$) lower, whereas γGT, glucose, LDL cholesterol and triglyceride were found significantly ($P\leq0.05$) higher in control (T_1) compared to treatments fed phytogenic feed additives and Vitamin E. There was no effect of saffron alone or in combination on plasma albumin or gulbulin, however birds fed vitamin E and Rosemary show significantly higher plasma globulin as compared to control group. Birds fed diet supplemented with Vitamin E (T_2) @ 200 mg/kg feed and Rosemery @ 3 g/kg feed(T_4) reported significantly ($P\leq0.05$) lower total cholesterol and HDL as compared to birds of control. Total antioxidant capacity was found significantly ($P\leq0.05$) higher in birds of supplemented groups as compared to control, whereas MDA was found significantly ($P\leq0.05$) lower in birds of supplemented groups as compared to

control. So, these data indicate that, vitamin E and phytogenic feed additives exert a beneficial effect on performance, carcass meat quality and plasma blood constituents in broiler chicks.

Hosseini-Vashan *et al.* (2018)investigated the effect of hydroethanolic saffron petals' extract (HSPE) on yield, carcass characteristics and blood biochemical parameters of Japanese quail. A total of 120 quails were arranged into 12 units with 3 treatments based on a completely randomized designreceiving isocaloric and isonitrigenous diets. Each dietary treatment had 3 replications with 10 birds. Treatments were included 350 and 700 ppm of hydroethanolic saffron petals' extract and control. Two quails from each pen were selected and slaughtered. The results revealed that inclusion of hydroethanolic saffron petals' extract to quail diets were improved the body weight and FCR. The relative weight of breast, thigh, pancreases, and heart did not affected by dietary treatments. The relative weight of fabricus bursa was increased when birds received the HSPE. Addition of hydroethanolic saffron petals' extract decreased serum cholesterol and triglyceride of quail. The HDL, LDL and total protein concentration were not affected by the treatments. Therefore, supplementation of hydroethanolic saffron petals' extract to diet may improve the yield and cholesterol of Japanese quail.

One doubt to the use of saffron as a feed additive could be the fact that saffron is the most expensive cultivated spice, however, saffron by products that have the same composition as the rest of the spice, are discarded for aesthetic reasons and marketed as a relatively cheap byproduct. The low cost of saffron byproducts can be used as a promising and sustainable feed additive for his antioxidant and colouring properties, as well as the health promotingones. Hensel *et al.* (2006) stated that at least 30% saffron samples do not fulfill quality specifications or are considered as waste products so, these saffron byproducts that have the same chemical composition as the rest of the spice, are discarded for aesthetic reasons and marketed as a relatively cheap by product of this industrial

production .The low cost of this material encouraged its investigation to be used as a promising and sustainable feed additive for antioxidant and coloring properties. Omidi *et al.* (2014b) showed antioxidant activity of saffron petal in lambs by using 2, 2-diphenyl -1-picrylhydrazyl (DPPH) free radical method. The extracts of saffron petal were gavaged at doses of 500, 1000, 1500 mg/kg for 15 days. Results showed that saffron petal increased antioxidant content at all doses with no effect on hematobiochemical parameters.Alipour*et al.* (2019) conducted a study to investigated the effects of the administration of ethanolic saffron petal extract (SPE) and vitamin E on growth performance, blood metabolites and antioxidant status in Baluchi lambs. Thirty-two Baluchi male lambs (35.22±5.75 kg) were randomly divided into 4 groups. The 1st (control), 2nd (injectable saffron petal extract [ISPE]), and 3rd (Vit E) groups were respectively injected subcutaneously with either physiological saline (5 mL), SPE (25 mg/kg body weight) or DL-α-tocopheryl acetate (225 IU) once a week. An oral dose of SPE (500 mg/kg BW) was also administered to the 4th group (oral saffron petal extract [OSPE]). Feed intake and bodyweight were measured for 42 days and blood samples were taken on days 1, 14, 28, and 42. The lambs were slaughtered, and tissue samples were taken. There was no significant effect of supplementation on feed intake, body weight gain, feed conversion ratio and carcass parameters. Also no significant effect of supplementation on haemato-biochemical parameters,except in supplemented groups plasma cholesterol was significantly ($p< 0.01$) lower, higher ($p<0.01$)plasmaGPx activity, higher ($p<0.01$) plasma SOD activity than control. The ISPE group showed significantly lower ($p<0.05$) antioxidant status of both longissimus dorsi muscle and liver among the groups. Moghaddam *et al*., (2015) evaluated nutritive value of saffron residues through determination of chemical compositions, in situ degradability and in vitro gas production techniques using two permanently fistulated Holstein heifers. The gas production and degradability characteristics were measured at 0, 2, 4, 8, 16, 24, 48, 72 and 96 hours. Author reported the saffron forage contained 96.8, 6.7, 45.9, 38.0, 5.2, 4.7 and 42.5 % of organic matter, crude protein, neutral detergent fiber,

acid detergent fiber, ash, ether extract and nonfiber carbohydrates, respectively. The study showed that Na, Mg, Zn and Fe were insufficient for ruminant requirement. The results obtained from degradability of dry matter showed that fraction “a” (rapidly degradable), “b” (slowly degradable) and “c” (constant degradable rate) were calculated 32.0%, 39.2% and 0.043/h, respectively. Also, in vitro gas production parameters (b and c), organic matter digestibility, metabolisable energy and short-chain fatty acids values of saffron forage were 49.8 ml/200 mg of DM, 0.091 ml/h, 53.9 %, 8.0MJ/kg DM and 0.89 mmol, respectively. Phenolic compounds, total tannin and condensed tannin contents were 2.93%, 0.97% and 0.31%, respectively.

Chapter – 3

MATERIAL AND METHODS

The present study has been carried out to evaluate the response of broiler chickens to dietary supplementation of graded level of saffron petal as feed additive on feed intake, growth performance, nutrient digestibility, health status, immune response, carcass quality, and production economics.

3.1. Place of study

The study was conducted in the Division of Animal Nutrition, FVSc & AH, and Instructional Poultry Farm of the Division of Livestock Production and Management, FVSc & AH, Sher-e-Kashmir University of Agricultural Sciences and Technology- Kashmir, Shuhama, Alusteng (J&K).

3.2. Procurement of Saffron Petals

Petals are procured from saffron growing farmers of District Pulwama. These petals were freed from impurity and other plant parts like leaves or stem. These petals were then dried under shade, grinded to fine powder and stored till further use.

3.3 Biological trial

One hundred forty (140), day old commercial broiler chicks were procured from a reputed hatchery and brooded for one week under common brooding conditions. On 8^{th} day, the chicks were distributed randomly into five treatment groups having four replicates of seven chicks each. Birds of treatment group T_0 were offered basal diet without feed additives. Birds of treatment group T_1, T_2 T_3 and T_4 were offered basal diet supplemented with grinded saffron petals as feed additive @ 0.5,1, 1.5 and 2g/kg feed on dry matter basis. Diets were formulated as per ICAR (2013) requirements. The experimental chicks were reared in a battery brooder. The battery brooder was cleaned, washed and disinfected by blow

lamping and complete house was fumigated using formaldehyde and potassium permanganate four days before the commencement of the experiment. All the treatment groups in the trial were reared under similar managemental conditions like space, light and ventilation, under thremoneutral environment (23.28 - 23.50°C) with the help of room heating equipment.

3.4. Treatment Description and Experimental Design

Completely Randomized Design

Groups	Replicates	No. of Broilers	Treatments
T_0 (n=28)	T_0R_1 T_0R_2 T_0R_3 T_0R_4	7 7 7 7	Basal diet as per ICAR (2013) requirements without saffron petal as feed additive.
T_1 (n=28)	T_1R_1 T_1R_2 T_1R_3 T_1R_4	7 7 7 7	Basal diet + saffron petal @ 0.5 g/kg of feed on Dry Matter basis.
T_2 (n=28)	T_2R_1 T_2R_2 T_2R_3 T_2R_4	7 7 7 7	Basal diet + saffron petal @ 1.0 g/kg of feed on Dry Matter basis.
T_3 (n=28)	T_3R_1 T_3R_2 T_3R_3 T_3R_4	7 7 7 7	Basal diet + saffron petal @ 1.5 g/kg of feed on Dry Matter basis.
T_4 (n=28)	T_4R_1 T_4R_2 T_4R_3 T_4R_4	7 7 7 7	Basal diet + saffron petal @ 2.0 g/kg of feed on Dry Matter basis.

3.5. Duration of experiment

The experiment was conducted for 5 weeks from 17th of April 2021 and to 25th of May 2021.

T_0R_1

T_0R_2

T_0R_3

T_0R_4

Plate 1A: Experimental Birds

T_1R_1

T_1R_2

T_1R_3

T_1R_4

Plate 1B: Experimental Birds

T_3R_1

T_3R_2

T_3R_3

T_3R_4

Plate 1C: Experimental Birds

T_4R_1

T_4R_2

T_4R_3

T_4R_4

Plate 1D: Experimental Birds

3.6. Determination of chemical composition of experimental feeds

Feed ingredients used in the study were analyzed in triplicates for the proximate constituents, calcium and phosphorus contents. The moisture, crude protein, crude fibre, ether extract, nitrogen-free extract and ash were estimated following the procedures laid down by AOAC (2005), while the calcium and phosphorous contents of the samples collected in the study were estimated using the methods described by Talpatra *et al.* (1940). The method of Van Soest *et al.* (1991) was adopted to evaluate the cell wall constituents. Saffron components (Crocin) concentrations from petals were estimated by High performance liquid chromatography (Thermo Scientific™ Ulti Mate™ 3000 Standard Dual System). The extract was prepared by suspending Saffron petals (1g) in 50 ml of 70% ethanol overnight. After extraction the samples were filtered to eliminate plant residues and filtrate dried at room temperature by evaporation (Pamela *et al.,* 2008). The residues were resolved in EtOH/H_2O (70:30) to final volume of 3 ml. the extracts were analysed by HPLC trough calibration curve.

3.7. Parameters studied

The following parameters were studied during the experimental period

3.7.1 Physiological Responses (Temperature and humidity)

Inhouse environmental temperature and relative humidity were recorded daily in the morning and night hours during the experimental period using a thermo-hygrometer clock.

3.7.2 Production performance for broiler

3.7.2.1 Body weight

The body weight of all experimental birds was recorded individually at the weekly interval by using an electronic weighing balance in the morning hours before feeding. Weight gains in different broiler were calculated separately on a

Cleaning of saffron petals from residues

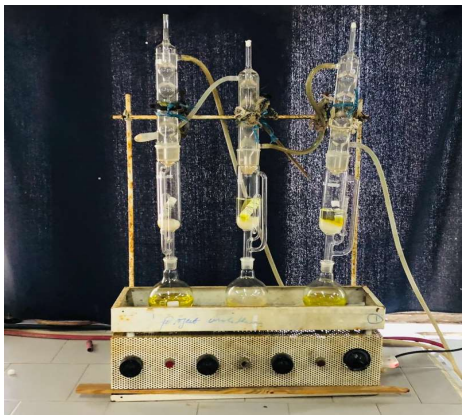
Saffron petals

Grinded Saffron petals

Plate 2: Saffron petal as feed additive

Crude fibre Estimation

Dry matter estimation



Ether Extract Estimation

Mineral estimation

Protein Estimation

Estimation of Ash

Plate 3: Proximate analysis

Preparation of premix

Weighing of feed and feed residue

Weighing of body weight

Plate 4: Feeding and weighing of experimental birds

Table-3.1: Proximate composition of experimental diets (DM basis).

Ingredients	Diet		
	Pre-starter	**Starter**	**Finisher**
Maize (yellow)	45.00	45.00	48.12
Soybean meal	38.00	29.00	23.73
Wheat bran	7.50	16.68	20.00
Vegetable oil	5.00	5.50	4.50
Lime stone powder (LSP)	1.97	1.70	1.60
Dicalcium phosphate (DCP)	1.33	1.00	0.95
Salt	0.29	0.29	0.29
Mineral premix +Vitamins*	0.75	0.75	0.75
Methionine	0.16	0.08	0.06
Total	**100.00**	**100.00**	**100.00**
Proximate composition (%)			
Dry matter (DM)	91.00	92.30	90.32
Crude protein (CP)	23.20	20.16	18.00
Crude Fibre (CF)	2.33	3.22	4.28
Ether extract (EE)	11.99	10.80	9.06
Ash	3.87	4.04	4.16
Calcium (Ca)	1.10	1.00	0.89
Phosphorus (P)	0.49	0.44	0.40
ME (Kcal/kg) Calculated	3,000	3,100	3,191

*Supplied/kg of diet: Vitamin D_3-3300IU; Vitamin A-8800IU; Vitamin E-1100IU; Riboflavin-9mg; Biotin-0.30mg; Thiamine-4mg; Pantothenic acid-11mg; vitamin B_{12}-13µg; Niacin-30mg; Choline-950mg; Vitamin K-1.5mg; Folic acid-1.5mg; Ethoxyquin-

Crocin extraction

Filtration

Crocin extract

HPLC

Plate 5: HPLC estimation of Crocin

130mg; Manganese-50mg; Copper 5.5mg Iron30mg and Selenium0.1mg.
weekly basis considering the body weights of broilers, recorded during different intervals.

3.7.2.2 Average body weight gain

Body weight gain was calculated as

Body weight gain = Final body weight - Initial body weight

3.7.2.3 Feed Intake

The feed intake in different treatments was calculated by subtracting the weight of left-over feed from the weight of total feed offered weekly. During the metabolic trial, a separate record of feed consumption and left-over feed was maintained.

3.7.2.4 Feed conversion ratio (FCR)

FCR was calculated using the following formula.

FCR = Feed consumption (g) / Body weight gain (g)

3.7.2.5 Feed efficiency ratio

The feed efficiency ratio was calculated based on weekly body weight gain and weekly feed intake for each week during the experiment. FER was calculated using the following formula.

FER = Body weight gain (g) / Feed consumption (g)

3.7.2.6 Performance index

The performance index was calculated as per the formula proposed by Bird (1995).

$$PI = \frac{\text{Live weight (g)}}{\text{Feed Conversion Ratio}}$$

3.7.2.7 Balance studies

A metabolism trial of four days was conducted in the last week of the experiment. During the trial, a daily record of feed offered, the residue left and the

faeces voided out was maintained on a 24-hourly basis. During this period, representative samples of about 200 g each from feed offered, the residue left and the faeces voided were taken daily and kept in the hot air oven for drying. After drying, samples were kept in airtight polythene bags daily so that at the end of the collection period, all the samples were mixed thoroughly and pooled samples were analyzed for ether extract and crude fibre. For ash, the digestibility coefficient was not calculated since most of the minerals were re-excreted in the gut. For determination of digestibility of crude protein of faeces voided out, representative sample about $1/50^{th}$ of total faeces voided out was preserved separately 5 to 10 ml of 1:4 sulphuric acid in airtight containers to fix the nitrogen content of faeces. The dry matter of the faeces was calculated by subtracting the weight of sulphuric acid and water present in the sample.

3.7.2.8 Mortality

Mortality, if any, was recorded on a daily basis and the mortality rate was calculated for the experimental period.

3.7.2.9 Economics

At the end of the study, the economics were worked out considering the purchase rate of chicks, average feed consumption of birds, feed cost, cost of feed additive (saffron petals), mortality, total weight gain of birds and management expenditure (cost of medication, vaccination, litter and other overheads) during the experimental period.

3.7.3 Heamato-biochemical parameters

The blood samples of 3 birds from each replicate were collected on 35^{th} day of the experiment from the jugular vein for biochemical and physiological stress enzyme activities in the clot activator vials (for serum extraction). The serum samples were maintained at -20 ℃ for further analysis.

3.7.3. 1. Hematological parameters

Hb, PCV, RBC's, WBC's, lymphocytes, granulocytes etc. were estimated by automatic hematology analyzer (SB-21 Vet).

3.7.3.2. Biochemical parameters

Glucose was estimated by glucometer instantly after blood collection. Creatinine and serum enzymes (AST and ALT) were estimated by using commercial diagnostic kits (Span diagnostics limited, Surat, Gujarat, India). Serum cholesterol, lipoproteins and triglycerideswere estimated by using commercial diagnostic kits (DiaSys diaganostic systems).

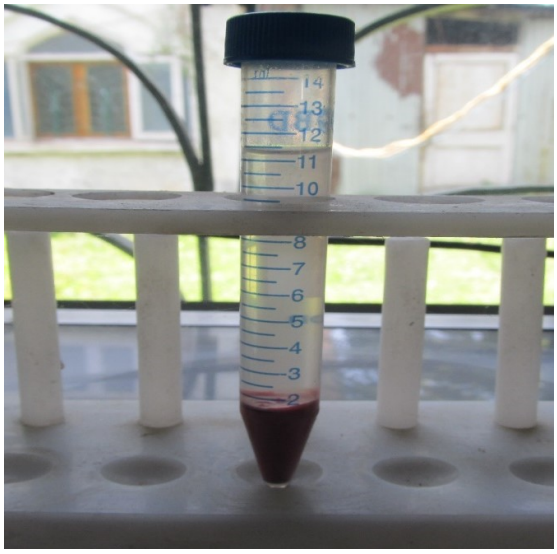
3.7.4 Immunological parameters

3.7.4.1 Humoral Immune Responses

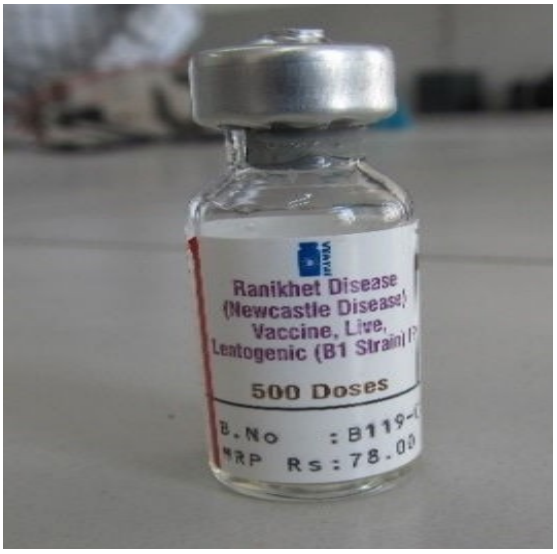
The HI-test against Newcastle disease vaccine was done on the 21st and 28th day after vaccination by β-method (diluted serum and constant virus) to monitor the immune response of birds. There are two different methods of HI-test, α-method (decreasing virus constant serum) and β-method (diluted serum and constant virus).

The following procedure was followed:

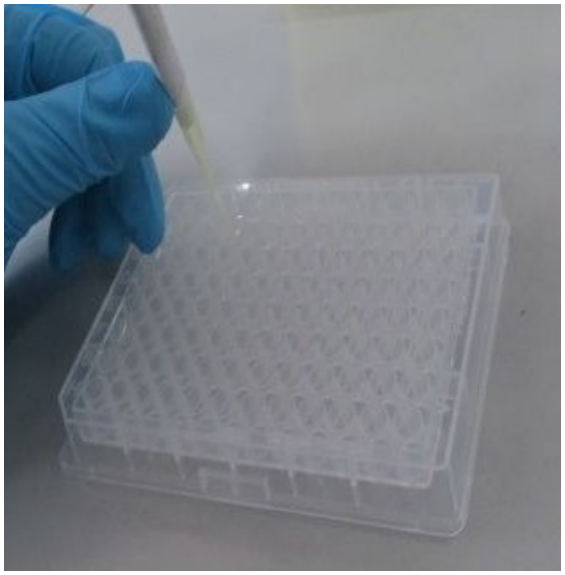
1. Collection of blood and preparation of 0.5% chicken RBC suspension.
2. Initial dilution of Viral antigen (Newcastle disease vaccine).
3. Preparation of working haemagglutinin: For this, first perform HA-test with ND virus. For HI-test, 4HA units of virus suspension are required.
4. Add 50µl of NSS in all the 12 wells of one row of a microtiter plate.
5. Add 50µl of test serum to the first well and make a serial two-fold dilution with a micropipette. Finally, discard 50µl mixture from the last well.



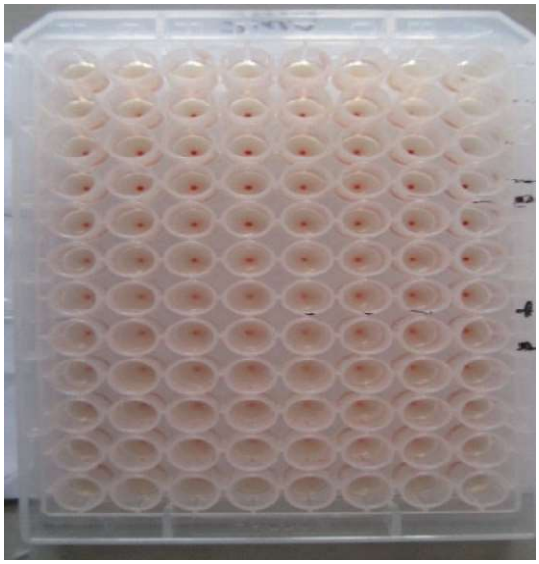
Chicken Cell RBC



Viral antigen



HAtestforViral Antigen



MicrotitreplateforHI test

Plate 6: Estimation of Humoral immunity

6. Add 50µl viral suspensions (which contain 4HA units) in all wells. Mix the contents by slight agitation of the plate. Keep the plate at room temperature for 15-20 minutes.
7. Add 50µl of RBC suspension in all wells and mix the contents by slight agitation of the plate.
8. Keep the plate at room temperature for 30 minutes and observe the results {RBC control: 100µl NSS+50µl RBC suspension and there should not be any agglutination; Serum control: 50µl NSS+ 50µl serum+50µlRBC suspension and there should not be any agglutination; Virus control: 50µl NSS+50µl virus+ 50µl RBC suspension. There should be complete agglutination}.
9. The HI-titre is expressed as reciprocal of the highest dilution of serum which causes at least 50% inhibition of haemagglutination.

HI titre = Serum endpoint (reciprocal) × number of HA Units.

3.7.4.2 Cell Mediated Immunity

Cell mediated immunity was determined by using DNCB (1-Choloro Di nitro Benzene).

Preparation of DNCB

- 2% solution of DNCB was prepared using acetone; olive oil in the ratio of 4:1 as vehicle
- 16 ml of acetone and 4ml of olive oil were taken and mixed properly to make a final volume of 20 ml

Injection of DNCB.

1. 0.25 ml of DNCB solution was injected to 3 birds in each replicate with the help of an insulin syringe intra dermally at inter digital space between 3rd and 4th digit of right leg.
2. 0.25 ml of NSS was injected in the same 3 birds of each replicate at the same site of left leg which was taken as control.

3. The thickness of the skin at the site of injection in both the legs was measured before and after injection of DNCB, using verniercalliper in all the replicates.

   The cell mediated immune response (CMIR) or Foot Web Index was determined by using the following formulae

   $$FW1- (R_2-R_1) - (L_2-L_1)$$

   Where

   R_2 thickness after 24hrs of DNCB injection

   R_1thickness before injection of DNCB injection

   L_2 thickness after 24 hrs of NSS injection

   L_1 thickness before injection of NSS injection.

3.7.4.3 Immune organ weight:

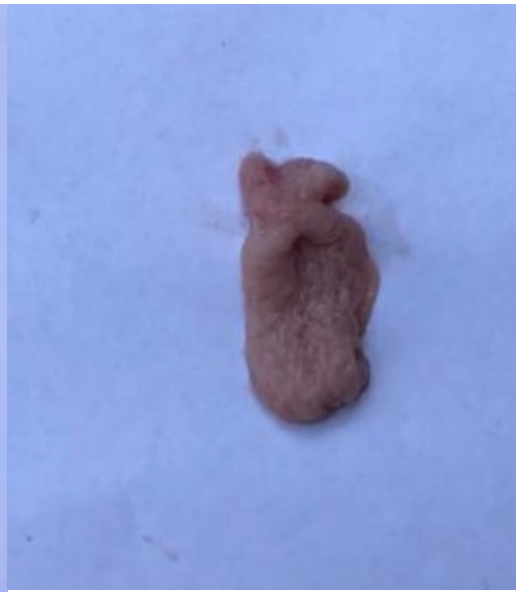
Three birds of average body weight per replicate were sacrificed by the *halal* method. Immediately after bleeding, immune organs like the ileum, cecal tonsils, bursa of Fabricius and spleen were harvested and weighed individually.

3.7.5 Oxidative stress Parameters:

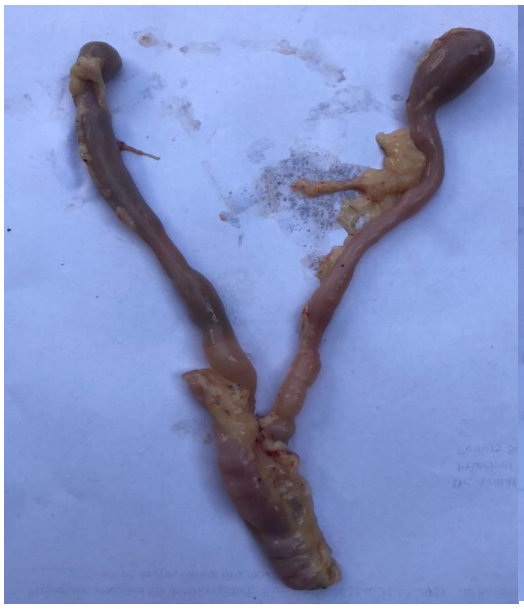
3.7.5.1. Thiobarbituric Acid Reactive Substance (TBARS) value

The estimation of TBARS value was done by following the method of Witte *et al*., (1970) with slight modifications. 10 g of sample was triturated with 25 ml of pre cooled 20% tricholoroacetic acid (TCA) in 2 molar orthophospheric acid solution for 2 minutes. The content was then quantitatively transferred into a beaker by rinsing with 25ml of chilled distilled water. After proper mixing, the contents were filtered through ash less Whatman filter Paper No 1. 3ml of TCA extract (filtrate) was mixed with 3ml of TBA reagent (0.005M) in test tubes and placed in a dark room for 16 hrs. A blank sample was made by mixing 3ml of 10%TCA and 3ml of 0.005 molar TBA reagent. Absorbance (O.D) was measured at fixed wavelength of 532 nm using UV-VIS spectrophotometer (HITACHI, UV-Spectrophotometer U-1800). TBARS value

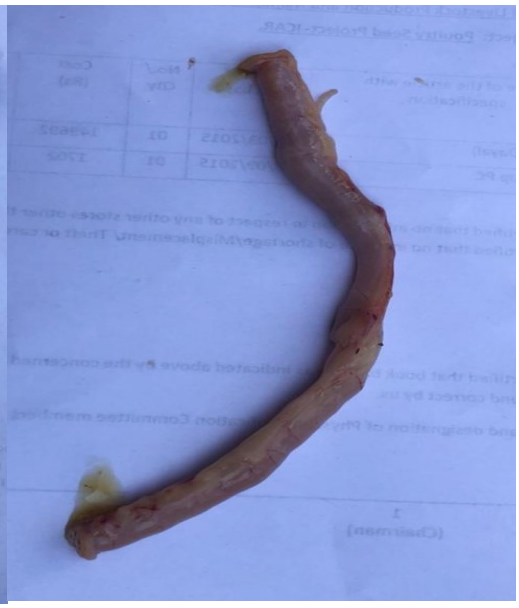
Spleen



Bursa



Ceacal Tonsils



Ilium

Plate 7: Immune organ

was calculated as mg Malonaldehyde per kg of sample by multiplying O.D value with K factor 5.2.

3.7.5.2. Estimation of Total Antioxidant Status (TAS)

In order to calculate the total antioxidant status following method of Erel (2004) was used. A 200µl of reagent 1 (Reagent 1: acetate buffer 0.4 mol/l pH 5.8) was taken in an uncoated plate to which 5µl of sera and 20µl of reagent 2 (Reagent 2: the $ABTS^{\bullet+}$ in acetate buffer 30 mmol/l pH 3.6) was added and absorbance was calculated at 600 nm wave length. Where ABTS is 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonate).

3.7.5.3. Estimation of Total Oxidant Status (TOS)

Total oxidant status was calculated by using Erel (2004) method. A 225µl of reagent 1 (Reagent 1: acetate buffer 0.4 mol/l pH 5.8) was taken in an uncoated plate to which 35µl of serum along with 11µl of reagent 2 (Reagent 2: the $ABTS^{\bullet+}$ in acetate buffer 30 mmol/l pH 3.6) was added to the each well. After that reading was taken at 560nm.

3.7.5.4. Estimation of Oxidative Stress index (OSI)

The oxidative status index was defined as the ratio of the TOS level to TAS level.

OSI (arbitrary unit) = TOS (µmol H_2O_2 Eq/L)/TAS (µmol Trolox Eq/L)

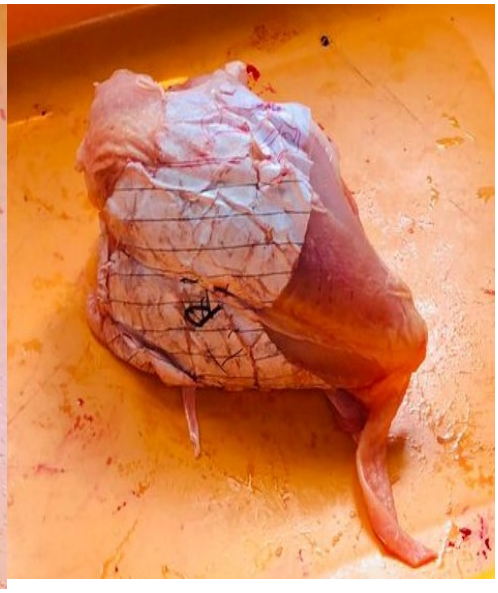
3.7.6 Carcass characteristics

At the end of each feeding trial, three birds per replicate were selected at random and utilized for the carcass evaluation study. The birds were kept off fed overnight and water was withdrawn 3-4 hours prior to slaughter. The birds were weighed before fasting. The birds were slaughtered by the *Halal* method and a bleeding time of 2 minutes was allowed. The shanks were cut off at the hock and the carcass was subjected to scalding process at 60^{o}C for 30 seconds. The feathers were removed completely using a mechanical de-featherer leaving the skin intact.

Body weight

Dressed carcass

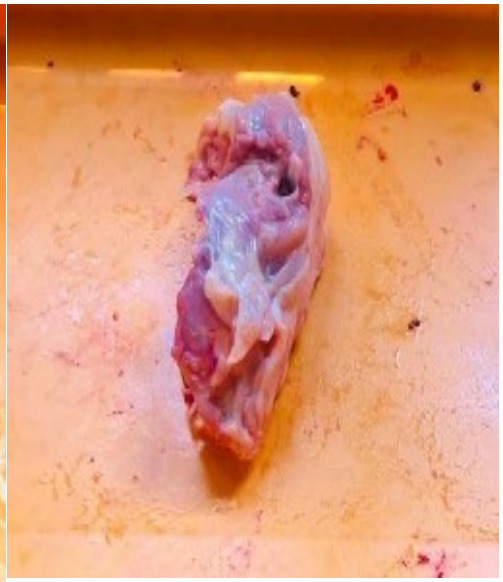
Drum stick



Breast

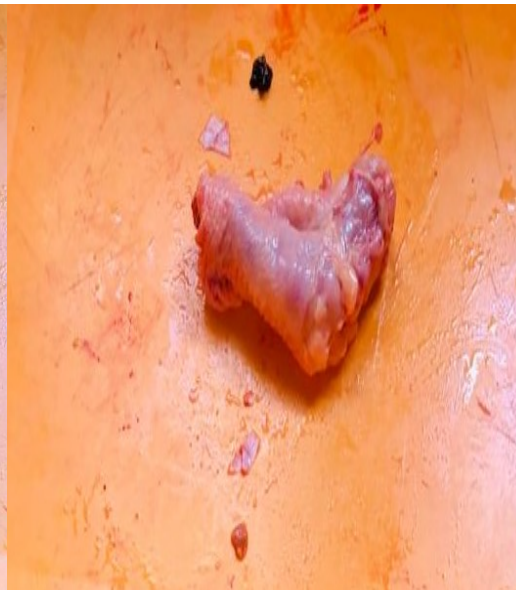
Plate 8A: Carcass parameters

Wings



Back

Thigh



Neck

Plate 8B: Carcass parameters

After that, the abdominal cavity was opened to expose the visceral organs. Slaughter characteristics, yield of giblets and cutability characteristics were calculated by the method used by Salahuddin *et al.* (2000). The following carcass parameters were recorded:

$$\text{Percent blood loss} = \frac{\text{Pre-slaughter live weight - weight after bleeding}}{\text{Pre-slaughter live weight}} \times 100$$

$$\text{Feather yield (\%)} = \frac{\text{Weight after bleeding-weight after defeathering}}{\text{Pre-slaughter live weight}} \times 100$$

$$\text{Dressing percentage} = \frac{\text{Dressed weight}}{\text{Pre-slaughter live weight}} \times 100$$

Cutability characteristics were calculated as percentage of live weight as under:

$$\text{Cut up part yield (\%)} = \frac{\text{Weight of specific cut}}{\text{Live body weight}} \times 100$$

Different carcass parameters like percent breast yield, back yield, thigh yield, drumstick yield, neck yield, wing yield and giblet yield were recorded after slaughtering three birds from each replicate.

3.8. Statistical Analysis

The analysis of data was done using one-way ANOVA. The test statistics was further referenced for p-values for its significance. Any p-value less than 0.05 ($p<0.05$) was taken as statistically significant and analysis was carried out using statistical software SPSS ver.26.00 Chicago, U.S.A for windows.

Chapter – 4

EXPERIMENTAL FINDINGS

The phytochemical studies have reported the presence of flavonoids and anthocyanins in saffron petal which showed beneficial effects as supplementary compounds. The low cost of this material encouraged its investigation to be used as a promising and sustainable feed additive for its antioxidant and coloring properties, as well as the health promoting effects. In this context, an experiment was conducted to use saffron petals as feed additive, in the broiler diet to study its effect on different productive parameters, health status and economics. One week old broiler chicks were distributed randomly into five treatment groups with four replicates of sevenchicks.All the treatment groups in the trial were reared under similar managemental conditions, provided with a thremoneutral environment (20-25°C) with the help of room heating equipment. Group T_0 was offered basal diet as per the ICAR (2013) requirement, without feed additive, and served as a control, whereas groups T_1, T_2, T_3 and T_4 was offered basal diet supplemented with grinded saffron petals as feed additive @ 0.5,1, 1.5 and 2g/kg feed on dry matter basisrespectively.The bodyweight of all experimental birds was recorded weekly, whereas feed consumption was calculated by subtracting the weight of feed residue from the weight of total feed offered. Further, in the last week of the growth trial, a 7-day metabolic trial was conducted to assess the digestibility of nutrients in different treatment groups. If any, mortality was recorded daily and the mortality rate was calculated for the experimental period.The blood samples of 3 birds from each replicate were collected on 21^{st} and 28^{th}day for humoral immunological analysis and on 35^{th} day from the jugular vein for heamto-biochemical parameters. At the end of the feeding trial, three birds per replicate were selected at random and utilized for the carcass evaluation study.

4.1 Physiological Responses:

Average in-house temperature and humidity recorded during during the experiment period are presented in table 4.1. The average weekly temperature and humidity of the experimental groups were recorded as 23.04°C and 55.50% during the day and 23.03°C and 56.20% during the night for the first week of the experiment. For the second week of the experiment, the average weekly temperature and humidity of experimental groups were recorded 23.37°C and 53.20% during the day and 23.07°C and 55.06% during the night. The average weekly temperature and humidity of experimental group was recorded 23.61°C and 56.20% during day and 23.30°C and 57.23% during night for third week of the experiment. For the fourth week of the experiment, the average weekly temperature and humidity of experimental groups was recorded 23.69°C and 55.86% during day and 23.27°C and 55.53% during night. During fifth week of

Table 4.1: In-house average weekly temperature and humidity of poultry shed during experimental period

Experimental period (weeks)	Time	Temperature (°C)	Humidity (%)
1	Day	23.04	55.50
	Night	23.03	56.20
2	Day	23.37	53.20
	Night	23.07	55.06
3	Day	23.61	56.20
	Night	23.30	57.23
4	Day	23.69	55.86
	Night	23.27	55.53
5	Day	23.79	56.16
	Night	23.77	55.83
Average	**Day**	**23.50**	**55.38**
	Night	**23.28**	**55.97**

the experiment, the average weekly temperature and humidity of experimental groups was recorded 23.79°C and 56.16% during day and 23.77°C and 55.83% during night. The overall temperature and humidity of experimental groups was recorded 23.50°C and 55.38% during day and 23.28°C and 55.97% during night throughout the experiment.

4.2 Chemical composition of experimental feed and feed additive

The chemical composition is considered as preliminary index for the assessment of quality of feed was determined for saffron petals (feed additive) as well as of complete experimental feed (presatarter, starter and finisher) is presented in Table 3.1. The prestater feed contained 91.00% DM, 23.20% CP, 11.99% EE, 2.33 % CF and 3.87% total ash. Ca and P contents were found to be 1.10% and 0.49%, respectively. The calculated ME was 3000 Kcal/kg. The starter feed contained 92.30% DM, 20.16% CP, 10.80% EE, 3.22% CF and 4.04% total ash. Ca and P contents were found to be 1.00% and 0.44%, respectively. The calculated ME was 3100 Kcal/kg. The finisher diet contained DM (92.30%), CP (18.00)%, EE (9.06%), CF (4.28%), total ash (4.16%), Ca (0.89%) and P (0.40%) with ME value of 3191 Kcal/kg.

The Saffron petals (*Crocus sativus* L.) used in the study as feed additive contained 85% DM, 11.94% CP, 5.03% EE, 7.85% CF, 52.81% NFE, 5.37% total ash, 1.33% acid insoluble ash, 36.10% NDF, 30.00% ADF, 6.10% hemicelluloses and 5.90% cellulose (Table 4.2). The Ca and P contents in Saffron petals were found to be 0.78 and 0.34 per cent, respectively. The Crocin content was found 0.785% W/W.

4.3 Production performance for broiler

4.3.1 Body Weight

The results of average weekly live body weight (g) of experimental birds subjected to different level of dietary supplements have been summarized in table

4.3 and depicted in fig.4.1. Average body weight of the chicks was 175.37, 175.90, 176.59, 175.79and 175.79g at first week of age; 346.99, 351.24, 352.22, 352.42 and 352.43g at second week of age in treatment groups T_0, T_1, T_2, T_3 and T_4 respectively,withoutany significant difference between the treatment groups. The average body weight of experimental birds at the end of 3rd week was 624.77, 637.28, 640.50, 660.50 and 662.15g for treatment groups T_0, T_1, T_2, T_3 and T_4, respectively with significantly higher body weight of birds of T_3 and T_4 than those T_0, T_1, T_2 group. At the end of 4th and 5th week, average body weight of birds was 934.09, 958.73, 964.64, 986.89 and 1008.53; 1339.41, 1368.29, 1379.79 and 1406.31g T_0, T_1, T_2, T_3 and T_4 gruoups respectively. Body weight of birds in all the treatment groups (T_1, T_2, T_3 and T_4) was significantly ($P \leq 0.01$) higher as compared to control group (T_0), with T_4 treatment group having significantly highest ($P \leq 0.05$) body weight followed by T_3, T_2 and T_1 groups.

4.3.2 Body weight gain

The average body weight gain (g) in experimental birds subjected to different treatments have been summarized in table 4.4 and depicted in fig 4.2. The average body weight gain of birds during first week was 171.61, 175.33, 175.63, 176.64, 176.65g in T_0, T_1, T_2, T_3 and T_4 groups respectively without any significant difference between the treatment groups. The mean weekly body weights were found to increase with an average of 277.79, 286.04, 288.29, 308.08 and 309.71g in T_0, T_1, T_2, T_3, T_4 treatment groups during second week respectively, with significantly ($P \leq 0.01$) higher bodyweight in T_3 and T_4 groups as compared to control (T_0) and T_1 group. The average body weight gain of birds was recorded 309.33, 321.45, 324.14, 326.39 and 346.39 at the 3rd week of age in T_0, T_1, T_2, T_3 and T_4 groups respectively with significantly ($P \leq 0.05$) higher body weight in T_4 group as compared to control (T_0). During 4thweek average body weight gain of birds in T_0, T_1, T_2, T_3 and T_4 groups

Table 4.2: Proximate, minerals and bioactive compounds in saffron petal

Proximate constituent	**Percent (%)**
Dry matter	85.00
Crude protein	11.94
Ether extract	5.03
Crude Fiber	7.85
Nitrogen free extract	52.81
Total Ash	5.37
Acid insoluble ash	1.33
NDF	36.10
ADF	30.00
Hemicellulose	6.10
Cellulose	5.90
Mineral	
Calcium	0.78
Phosphorus	0.34
Bioactive compound	
Crocin	0.6%w/w

Table 4.3: Average weekly body weight (g) of experimental broiler chicken subjected to feed additivesupplementation

Experimental Period (Weeks)	Dietary treatments						
	T_0	T_1	T_2	T_3	T_4	SEM	p value
1	175.37	175.90	176.59	175.79	175.79	0.57	0.98
2	346.99	351.24	352.22	352.42	352.43	2.20	0.94
3	624.77^{a}	637.28^{a}	640.50^{a}	660.50^{b}	662.15^{b}	4.07	0.002
4	934.09^{a}	958.73^{b}	964.64^{b}	986.89^{c}	1008.53^{d}	6.15	0.002
5	1339.41^{a}	1368.29^{b}	1379.79^{b}	1406.31^{c}	1432.10^{d}	10.53	0.003

Means with different superscripts within the same row differ significantly ($P \leq 0.01$)

was reported as 405.32, 409.56, 415.15, 419.42 and 423.57g respectively, without reaching to statisticaly significant difference.

4.3.3 Feed consumption

The results of feed consumption (g) during entire experimental period in experimental birds subjected to feed supplemented with saffron petals have been summarized in table 4.5 and depicted in fig 4.3. The average feed consumption was 171.15, 166.96, 166.66, 166.61 and 168.08 g during 1st week, 375.98, 377.33, 376.30, 379.54, 376.08g during 2nd week, 674.53, 687.49, 686.99, 675.20, 686.76g during 3rd week and 974.34, 962.07, 961.02, 964.29, 960.84g during 4th week of experimentin T_0, T_1, T_2, T_3 and T_4 treatments respectively. During entire experimental period among groups supplemented with the feed additive, feed intake were numerically higher as compared to control but could not reach tostatistically significant difference.

4.3.4 Feed conversion ratio (FCR)

Feed conversion ratio i.e. output in terms of kg feed consumed per kg body weight gain was calculated weekly for the entire period of 4 weeks of growth trial and have been presented in table 4.6 and depicted in fig 4.4. Initially during the first week of experiment average weekly FCR recorded was 0.99, 0.95, 0.95, 0.95 and 0.95 in the treatment groups T_0, T_1, T_2, T_3 and T_4, respectively with statistically non-significant difference among these experimental groups. During 2nd week of experiment the average FCR recorded was 1.36, 1.32, 1.30, 1.24, 1.22 in the treatment groups T0, T1, T2, T3 and T4, respectively with statistically ($P \leq 0.05$) higher FCR in T3 and T4 experimnetalgroups as compared to control. The difference between T_0, T_1 and T_2 were statistically similar. During 3rd week the average FCR recorded was 2.19, 2.14, 2.12, 2.07, 1.99, 2.40, 2.35, 2.32, 2.30 and 2.27 in the treatment groups T_0, T_1, T_2, T_3 and T_4, respectively without any statistically significant difference.

Table 4.4: Average weekly body weight gain (g) of broiler chicken subjected to feed additive supplementation

Experimental Period (Weeks)	Dietary treatments						
	T_0	T_1	T_2	T_3	T_4	SEM	p value
1	171.61	175.33	175.63	176.64	176.65	2.18	0.96
2	277.79^{a}	286.04^{a}	288.29^{ab}	308.08^{b}	309.71^{b}	3.97	0.01
3	309.33	321.45	324.14	326.39	346.39	4.39	0.09
4	405.32	409.56	415.15	419.42	423.57	3.09	0.36

Means with different superscripts within the same row differ significantly ($P \leq 0.01$)

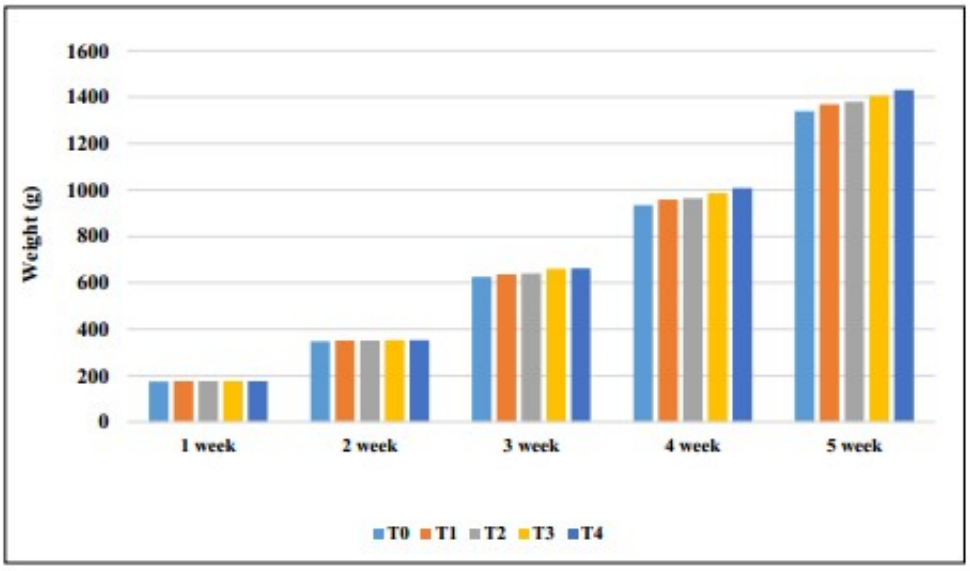


Fig 4.1: Average weekly body weight (g) of experimental broiler chicken subjected to feed additive supplementation

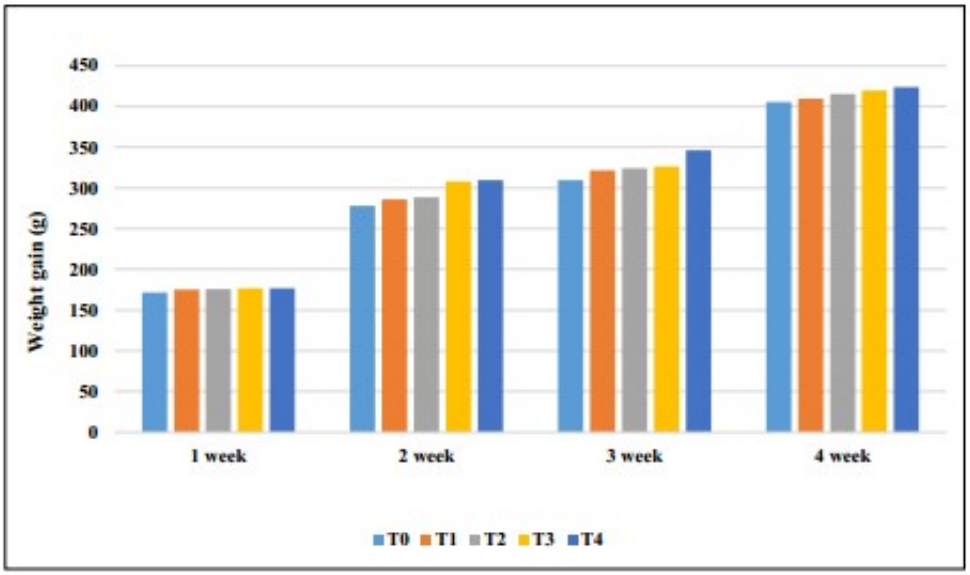


Fig 4.2: Average weekly body weight gain (g) of experimental broiler chicken subjected to feed additive supplementation

Table 4.5: Average feed intake (g) of broiler chicken subjected to feed additive supplementation.

Experimental Period (Weeks)	Dietary Treatments						
	T_0	T_1	T_2	T_3	T_4	SEM	p value
1	171.15	166.96	166.66	166.61	168.08	1.35	0.84
2	375.98	377.33	376.30	379.54	376.08	1.20	0.90
3	674.53	687.49	686.99	675.20	686.76	2.33	0.15
4	974.34	962.07	961.02	964.29	960.84	2.24	0.29

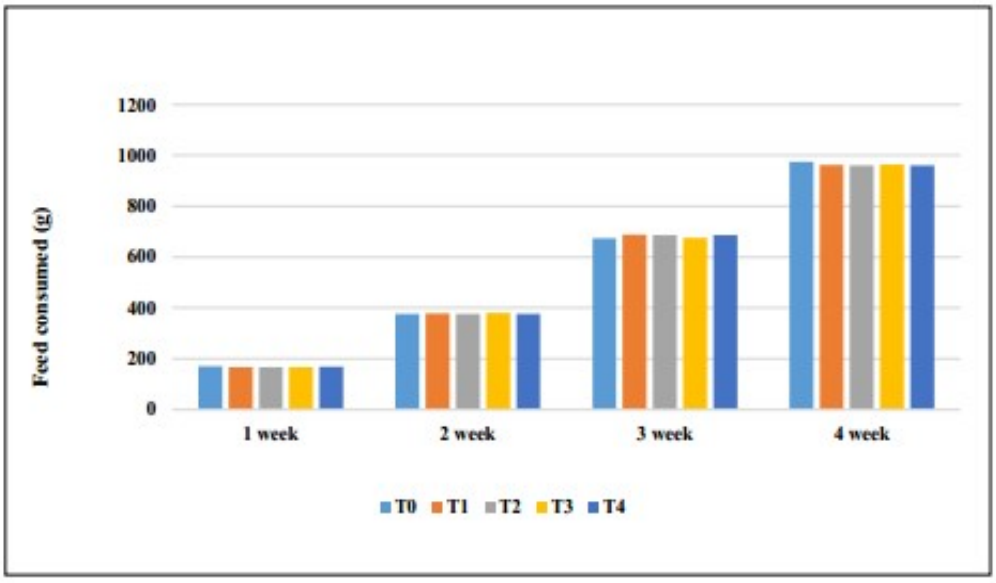


Fig 4.3: Average weekly feed consumption (g) of broiler chicken subjected to feed additive supplementation

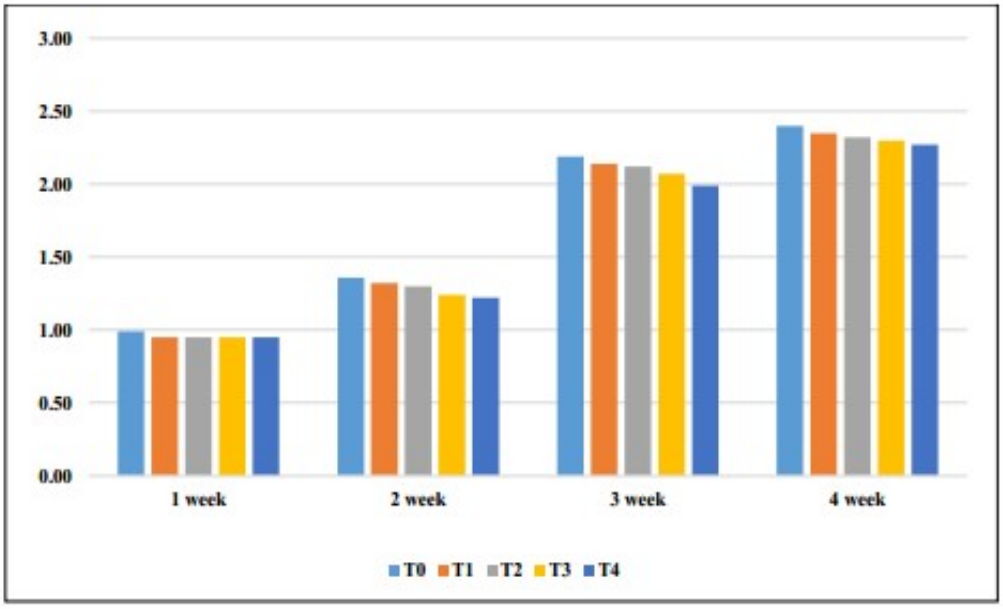


Fig 4.4: Average weekly FCR of broiler chicken subjected to feed additive supplementation

FCR of birds of T_0, T_1, T_2, T_3 and T_4 treatment groups during the 4th week was 2.40, 2.35, 2.32, 2.30 and 2.27 respectively.Statistical analysis of data revealed significantly ($P \leq 0.05$) lower FCR values in birds of T_4 group as compared to the control (T_0) group, however the FCR values among birds of T_0, T_1, T_2, T_3 treatments could no reach to statistically significant difference.

4.3.5 Feed efficiency ratio (FER)

The results of average weekly feed efficiency ratio of experimental birds subjected to feed supplemented with saffron petals under ambient conditions have been summarized in table 4.7 and depicted in fig 4.5. During the 1st week of experiment, average FER value was 1.00, 1.01, 1.06, 1.06 and 1.06; during 2nd week average FER values were 0.74, 0.76, 0.77, 0.81 and 0.83; for 3rd week FER values were 0.46, 0.47, 0.47, 0.49 and 0.50; for 4th week average FER values were 0.42, 0.43, 0.43, 0.44, 0.44 and 0.44 for T_0, T_1, T_2, T_3 and T_4 treatment groups respectively.Statistical analysis shows that during 1st, 23rd and 4th week there is nosignificant difference between the treatment groups. Howevwer during the 2nd week of the experiment birds of control group (T_0) shows significantly lower FER values as compared to T_4 treatment group. However there was no significant difference in FER values between T_1, T_2, T_3 and T_4 treatment groups.

4.3.6 Performance index (PI)

The results of weekly performance index in broiler chicken subjected to feed supplemented with saffron petals under ambient conditionss have been summarized in table 4.8 and depicted in fig 4.6. Initially during the 1nd week, the performance index values were 176.08, 184.79, 186.14, 186.29 and 185.19 in T_0, T_1, T_2, T_3 and T_4 treatments respectively withot any significant difference. The performance index values were 256.42, 266.19, 269.63, 285.68 and 290.31 during the 2rd week, 286.65, 297.84, 302.18, 319.32 and 333.55 at 3rdweek and 388.47, 408.13, 416.71, 429.20 and 444.62 at 4th week of experiment for treatment groups T_0, T_1, T_2, T_3 and T_4 respectively. Statistical analysis shows that treatment T_4 has

significantly (P≤0.005) higher performance index as compared to control (T_0), T_1 and T_2 groups but there was no significant difference among treatment groups T_1, T_2 and T_3.

Table 4.6: Average weekly feed conversion ratio of broiler chicken subjected to feed additive supplementation.

Experimental Period (Weeks)	Dietary treatments						
	T_0	T_1	T_2	T_3	T_4	SEM	*p* value
1	0.99	0.95	0.95	0.95	0.95	0.01	0.84
2	1.36^{b}	1.32^{ab}	1.30^{ab}	1.24^{a}	1.22^{a}	0.02	0.04
3	2.19	2.14	2.12	2.07	1.99	0.03	0.28
4	2.40^{b}	2.35^{ab}	2.32^{ab}	2.30^{ab}	2.27^{a}	0.02	0.18

Means with different superscripts within the same row differ significantly (P≤0.05)

Table 4.7: Average weekly feed efficiency ratio of broiler chicken subjected to feed to feed additive supplementation.

Experimental Period (Weeks)	Dietary treatments						
	T_0	T_1	T_2	T_3	T_4	SEM	*p* value
1	1.00	1.01	1.06	1.06	1.06	0.01	0.80
2	0.74^{a}	0.76^{ab}	0.77^{abc}	0.81^{bc}	0.83^{c}	0.01	0.03
3	0.46	0.47	0.47	0.49	0.50	0.00	0.25
4	0.42	0.43	0.43	0.44	0.44	0.03	0.27

Means with different superscripts within the same row differ significantly (P≤0.05)

Table 4.8: Average weekly Performance Index of broiler birds subjected to feed additive supplementation.

Experimental Period (Weeks)	Dietary Treatments						
	T_0	T_1	T_2	T_3	T_4	SEM	*p* value
1	176.08	184.79	186.14	186.29	185.19	2.66	0.76
2	256.42^{a}	266.19^{a}	269.63^{ab}	285.68^{bc}	290.31^{c}	3.69	0.01
3	286.65^{a}	297.84^{ab}	302.18^{ab}	319.32^{bc}	333.55^{c}	4.76	0.003
4	388.47^{a}	408.13^{b}	416.71^{ab}	429.20^{bc}	444.62^{c}	5.04	0.002

Means with different superscripts within the same row differ significantly ($P \leq 0.01$)

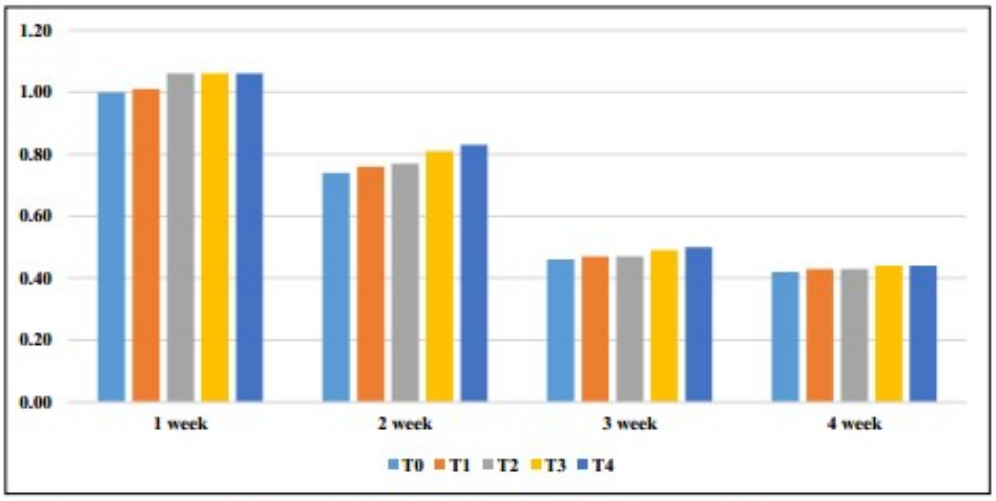


Fig 4.5: Average weekly FER of broiler chicken subjected to feed additive supplementation

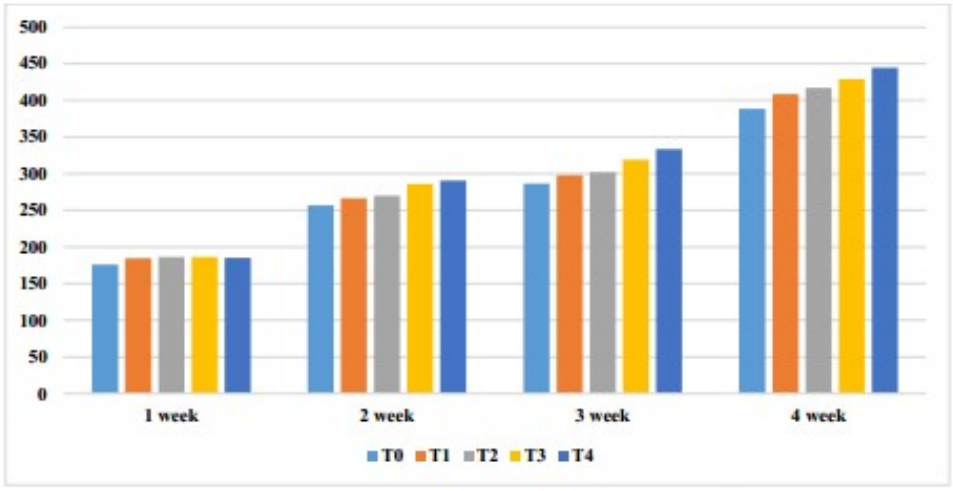


Fig 4.6: Average weekly PI of broiler chicken subjected to feed additive supplementation

4.3.7 Overall body weight gain, feed intake, FCR, FER and PI

The results of overall body weight gain, feed intake, FCR, FER and performance index in broiler chicken subjected to feed additive supplementation have been summarized in table 4.9. Treatment means of the weight gain indicates that the average weight gain of birds during the study in groups T_0, T_1, T_2, T_3 and T_4 were 1164.04, 1192.38, 1203.20, 1230.53 and 1256.32g respectively. Statistically average total weight gain of experimental birds was significantly ($P<0.01$) lower in T_0 group as compared to T_1 and T_2 treatments, which inturn was significantly lower than T_3 treatment group. Highest body weight gain was reported in T_4 treatment, significantly higher than T_0, T_1, $T_2$and T_3 treatments. The average total feed intake (g) of experimental birds in groups T_0, T_1, T_2, $T_3$and T_4 were 2196.00, 2193.86, 2190.98, 2185.65 and 2191.76g respectively, without any significant difference. The average overall FCR of experimental birds in groups T_0, T_1, T_2, T_3 and T_4 were 1.89, 1.84, 1.82, 1.78 and 1.74respectively, with significantly ($P<0.01$) higher FCR in T_0 group followed by T_1 and T_2. Significantly ($P<0.01$) lower average total FCR was observed in birds of T_3 and T_4 compared to T_0 and T_2 treatments.

The average overall FER was 0.53, 0.55, 0.55, 0.56 and 0.57 in the treatment groups T_0, T_1, T_2, T_3 and T_4 respectively. The average overall FER of birds of $T_3$and T_4 groups was significantly ($P\leq0.01$) higher than that of birds of T_2 and T_1 followed by T_0 group. The average overall performance index was 709.99, 743.73, 757.83, 791.84 and 821.05in the treatment groups T_0, T_1, T_2, T_3 and T_4 respectively, with significantly ($P\leq0.01$) higher performance index values in T_4 followed bybirds of T_3 treatment and T_1 and T_2 with statistically ($P\leq0.05$) lowest performance index values in control (T_0).

4.3.8 Nutrient Digestibility

The results of average nutrient digestibility in experimental birds subjected to feed supplemented with saffron petals has been summarized in table 4.10 and

Table 4.9: Total body weight gain, feed intake, feed conversion ratio, feed efficiency ratio and performance index of broiler birds subjected to feed additive supplementation.

Parameters	Dietary Treatments						
	T_0	T_1	T_2	T_3	T_4	SEM	*p* value
Initial body weight (g)	175.37	175.90	176.59	175.79	175.79	0.57	0.98
Final body weight (g)	1339.41^{a}	1368.29^{b}	1379.79^{b}	1406.31^{c}	1432.10^{d}	7.71	<0.05
Total weight gain (g)	1164.04^{a}	1192.38^{b}	1203.20^{b}	1230.53^{c}	1256.32^{d}	7.77	<0.05
Total feed intake (g)	2196.00	2193.86	2190.98	2185.65	2191.76	3.51	0.93
Overall FCR(kg)	1.89^{c}	1.84^{b}	1.82^{b}	1.78^{a}	1.74^{a}	0.01	<0.05
Overall FER(kg)	0.53^{a}	0.55^{b}	0.55^{bc}	0.56^{cd}	0.57^{d}	0.03	<0.05
Overall Performance Index	709.99^{a}	743.73^{b}	757.83^{b}	791.84^{c}	821.05^{d}	9.38	<0.05

Means within different superscripts in same row differ significantly (P≤0.05)

Table 4.10: Average nutrient digestibility (%) in broiler chicken subjected to feed additive supplementation.

Nutrients	Dietary treatments						
	T_0	T_1	T_2	T_3	T_4	SEM	*p* value
Dry matter	62.59^a	63.07^a	65.70^b	65.61^b	67.05^b	0.47	0.001
Crude protein	51.10	51.82	52.20	52.18	53.54	0.32	0.17
Ether extract	59.21	59.87	60.02	59.50	60.11	0.69	0.99
Crude fibre	41.85	41.07	40.66	41.39	41.77	0.39	0.89
Nitrogen free extract	55.02	55.44	55.69	56.00	56.31	0.51	0.96

Means within different superscripts in same row differ significantly ($P \leq 0.01$)

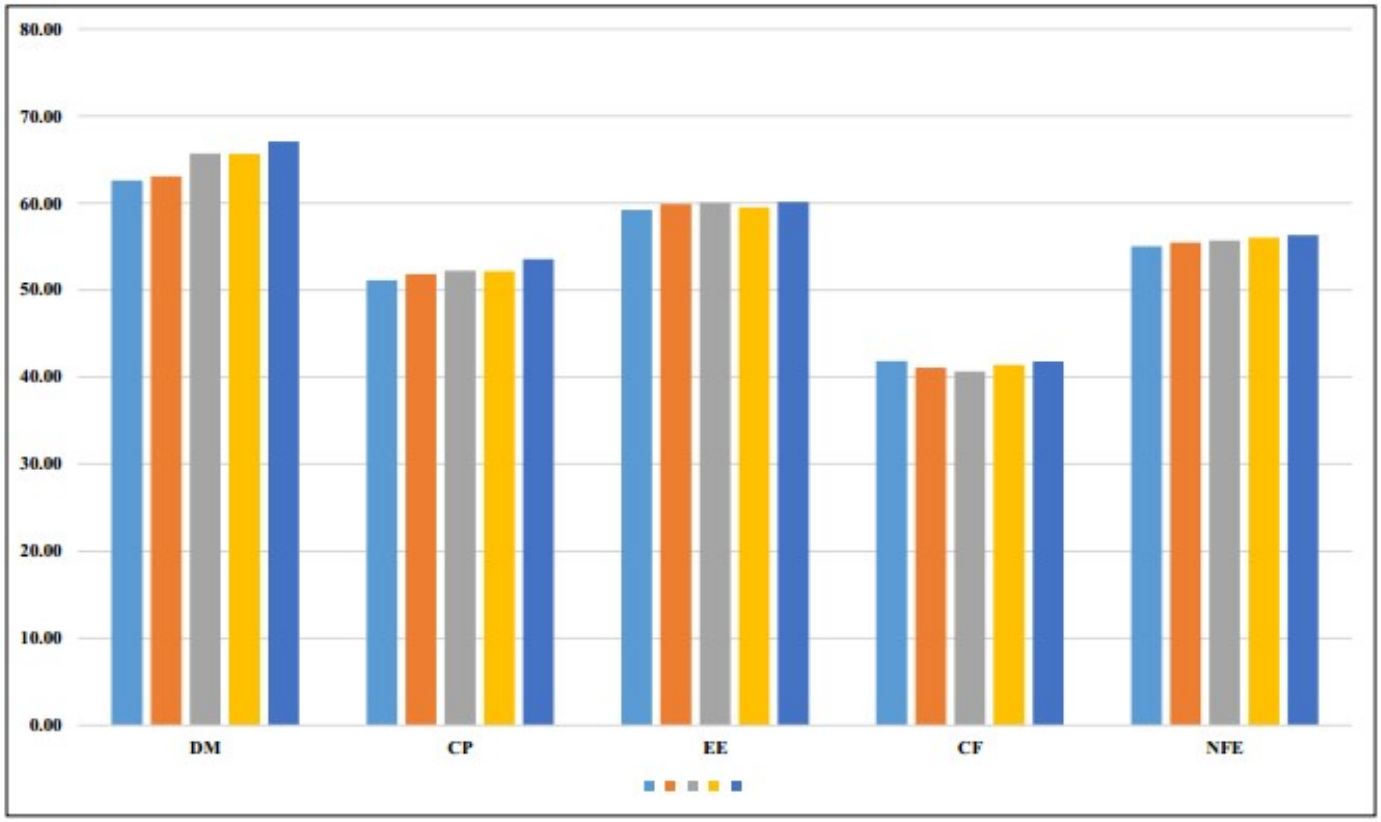


Fig 4.7: Average nutrient digestibility (%) in broiler chicken subjected to feed additive supplementation

depicted in fig 4.7. The dry matter digestibility was 62.59, 63.07, 65.70, 65.61 and 67.05% in the treatments T_0, T_1, T_2, T_3 and T_4 respectively. Significantly ($P \leq 0.05$) higher dry matter digestibility was observed in treatment T_2, T_3 and T_4 as compared to control (T_0) and T_1 treatment groups. The digestibility of crude protein was found to be 51.10, 51.82, 52.20, 52.18 and 53.54% in the treatment groups T_0, T_1, T_2, $T_3$and T_4 respectively. Statistical analysis shows that birds of treatment groupT_4 is having significantly ($P \leq 0.05$) higher digestibility of crude protein (CP) as compared to control group. However there is no significant difference among T_0, T_1, T_2 and T_3 treatment groups ..

The digestibility of ether extract (EE) was 59.21,59.87,60.02,59.50 and 60.11%; crude fibre (CF) digestibility was 41.85, 41.07, 40.66, 41.39 and 41.77% and digestibility of nitrogen-free extract (NFE) was 55.02, 55.44, 55.69, 56.00 and 56.31% in the treatment groups T_0, T_1, T_2, T_3 and T_4respectively without any significant difference.

4.3.9 Mortality

There has been no mortality reported of experimental birds during experiment.

4.3.10 Economics

In the present study attempt were made to calculate the economics of broiler production from different treatment groups (T_0, T_1, T_2, T_3 and T_4) which is presented in Table 4.11. The economics was calculated considering the purchase rate of chicks, average feed consumption of birds, feed cost, cost of feedsupplements (saffron petal), mortality, total weight gain of birds and managemental expenditure during the experimental period.

The results of present study revealed that the cost of production per kg live weight in T_0 was rupees (₹)104.13 for T_0,102.58 for T_1; 102. 56 for T_2, 101.00 for T_3 and 100.52 for T_4.The results indicate that production cost per kg live weight was least in treatment T_4 followed by T_3. The production cost

per kg live weight was highest in control (T_0), followed by T_2 andT_1 treatments.

Table 4.11: Economics of production cost perkg live weight in chicken subjected to feed additive supplementation.

S.No	Parameters	Treatment groups				
		T0	**T1**	**T2**	**T3**	**T4**
A	Chick Cost (₹) 45 Rs /chick	1260	1260	1260	1260	1260
B	Average feed cost/kg (₹) (Prestater, Starter and Finisher)	36	36	36	36	36
C	Total Feed consumption per treatment (kg)	61.49	61.43	61.35	61.20	61.37
D	Feed Cost (₹)	2213.56	2211.48	2208.60	2203.20	2209.28
E	Cost of Saffron petal (₹)	0	30	61	91	122
F	Miscellaneous cost @ 1.5 (₹) /bird	36	36	36	36	36
G	Mortality cost(₹)	0	0	0	0	0
H	Labour cost(₹)	300	300	300	300	300
Total rearing cost/treatment (A+D+E+F+G+H)		3907.06	3934.91	3962.95	3987.40	4024.65
Rearing cost /bird(₹)		139.54	140.53	141.53	142.41	143.47
Total gain in body weight/bird (kg)		1.34	1.37	1.38	1.41	1.43
Cost of production/kg live weight (₹)		**104.13**	**102.58**	**102.56**	**101.00**	**100.52**

4.4 Heamato-Biochemical Parameters

4.4.1 Heamatological Parameters

To ascertain the effect of supplementation of saffron petal as feed adiitive under on physiological health status, various blood biochemical parameters were studied in treatment groups (T_0, T_1, T_2, T_3 and T_4) at the end of the experimental period. Results of the haematological parameters ofexperimentalbirds have been summarized in table 4.12 and depicted in fig 4.8.

The blood heamoglobin (Hb) concentration of T_0, T_1, T_2, T_3 and T_4 birds was 9.01, 9.19, 9.41, 10.61 and 11.13(g/dl) respectively. Statistical analysis shows that birds of T3 and T4group was having significantly ($P \leq 0.01$) higher Hb concentration as compared to control (T0) and T1 group. However there was no significant difference in Hb concentration among birds of T_1,$T_2$and T_3 treatment groups. The PCV values of 28.58, 28.20, 28.94, 28.43 and 28.25%; RBC count of 2.40, 2.49, 2.46, 2.47 and 2.47 ($x10^6$) and WBC count of 6.40, 6.49, 6.46, 6.47 and 6.47 ($x10^3$) was reported from the birds of treatment groups T_0, T_1, T_2, T_3 and T_4respectively without any significant difference. The MCH value of the birds was 32.58, 32.20, 32.94, 32.43 and 32.25(pg); MCV values were 99.50, 101.67, 100.21, 100.18 and 99.91 FI with MCHC values of 30.71, 30.84, 30.21, 30.88 and 30.47 g/dl respectively of treatment groups T_0, T_1, T_2, T_3 and T_4 respectively without any significant difference.

4.4.2. Biochemical Parameter

The results of the serum biochemical parameters of experimental birds subjected to saffron petals as feed additive have been summarised in table 4.13 and figure 4.9 and 4.10. The blood glucose values of the experimental birds were 242.88, 243.30, 264.79, 264.94 and 268.21 mg/dL in the treatment groups T_0, T_1, T_2, T_3 and T_4 respectively. The experimental birds of group T_0 and T_1 showed significantly lower ($P \leq 0.01$) blood glucose levels than the birds in T_2, T_3 and T_4, with no significant difference between T_2, T_3 and T_4 treatment groups.

Table 4.12: Average heamtological parameters in broiler chicken subjected to feed additive supplementation.

Heamatological Parameters	Dietary treatments						
	T_0	T_1	T_2	T_3	T_4	SEM	*P* value
Hb (g/dl)	9.01^{a}	9.19^{a}	9.41^{ab}	10.61^{bc}	11.13^{c}	0.25	0.01
PCV (%)	28.58	28.20	28.94	28.43	28.25	0.49	0.99
RBC ($\times 10^{6}$)	2.40	2.49	2.46	2.47	2.47	0.027	0.93
WBC($\times 10^{3}$)	6.40	6.49	6.46	6.47	6.47	0.027	0.93
MCH (pg)	32.58	32.20	32.94	32.43	32.25	0.49	0.99
MCV (FI)	99.50	101.67	100.21	100.18	99.91	0.83	0.96
MCHC(g/dl)	30.71	30.84	30.21	30.88	30.47	0.65	0.99

Means within the same row with different superscripts differ significantly (P≤0.01)

The serum creatinine concentration of experimental birds were 0.5, 0.49, 0.45, 0.47 and 0.47mg/dL with statistically non significant difference between the treatment groups . The serum cholesterol values of the experimental birds of treatment groups T0, T1, T2, T3 and T4 were 135.39, 126.79, 125.36, 123.71and 116.87. Statistical analysis of the data revealed that birds of T4 treatment group is having significantly ($P \leq 0.05$) lower cholesterol levels as compared to control group (T0) with non-significant difference between T1, T2 and T3 treatment groups. The serum triglycerides of the birds of treatment groups T0, T1, T2, T3 and T4 were 29.24, 27.06, 26.94, 26.43 and 22.75 respectively with significantly ($P \leq 0.05$) lower values in T4as compared to control (T0). However no significant difference was observed in plasma triglyceride values of $T_{0,}$ $T_{1,}$ T_2 and $T_{3.}$

The serum HDL values were 25.24, 25.09, 24.63, 24.17, 24.03 and serumLDL values were 101.89, 76.32, 100.03, 98.52, 93.68 of birds of treatment groups $T_{0,}$ $T_{1,}$ $T_{2,}$ T_3 and T_4 respectively with non significant difference between the treatment groups. The serum aspartate transaminase (AST) activities in the experimental birds were 142.89, 143.30, 142.29, 142.44 and 140.72 mg/dL with serum alanine transaminotransferase (ALT) activity of 27.96, 27.84, 27.21, 27.67 and 27.47 U/Lin the treatment groups T_0, T_1, T_2, T_3 and T_4 respectively with statistically non-significant difference between the treatment groups.

4.5 Immune parameters

4.5.1 Humoral Immune parameters

The results of mean values of haemagglutination inhibition titres of the birds of treatments $T_{0,}$ $T_{1,}$ $T_{2,}$ T_3 and T_4 at day 21 and 28^{th} post Newcastle Disease vaccination subjected to feed supplemented with saffron petals have been summarized in table 4.14 and depicted in fig 4.11 and 4.12. At 21^{st} and 28^{th} day, the mean of HI titre was 53.04, 58.85, 90.24, 97.95, 107.16 units and 100.57, 109.91, 144.94, 155.55, 168.01 units in the treatments $T_{0,}$ $T_{1,}$ $T_{2,}T_{3,}$ T_4

Table 4.13: Serum biochemical parameters in broiler chicken subjected to feed additive supplementation.

Biochemical Parameters	Dietary treatments						
	T_0	T_1	T_2	T_3	T_4	SEM	*p* value
Glucose (mg/dL)	242.88[a]	243.30[a]	264.79[b]	264.94[b]	268.21[b]	2.98	<0.05
Creatinine (mg/dL)	0.50	0.49	0.45	0.47	0.47	0.03	0.99
Cholestrol (mg/dl)	135.39[b]	126.79[ab]	125.36[ab]	123.71[ab]	116.87[a]	2.03	0.04
Triglycerides (mg/dL)	29.24[b]	27.06[b]	26.94[b]	26.43[ab]	22.75[a]	0.71	0.04
HDL(mg/dL)	25.24	25.09	24.63	24.17	24.03	0.41	0.88
LDL(mg/dL)	101.89	76.32	100.03	98.52	93.68	5.07	0.54
Enzyme Activity (U/L)							
SGOT/AST	142.89	143.30	142.29	142.44	140.72	3.02	0.99
SGPT/ALT	27.96	27.84	27.21	27.67	27.47	0.64	0.99

Means within the same row with different superscripts differ significantly (P≤0.05)

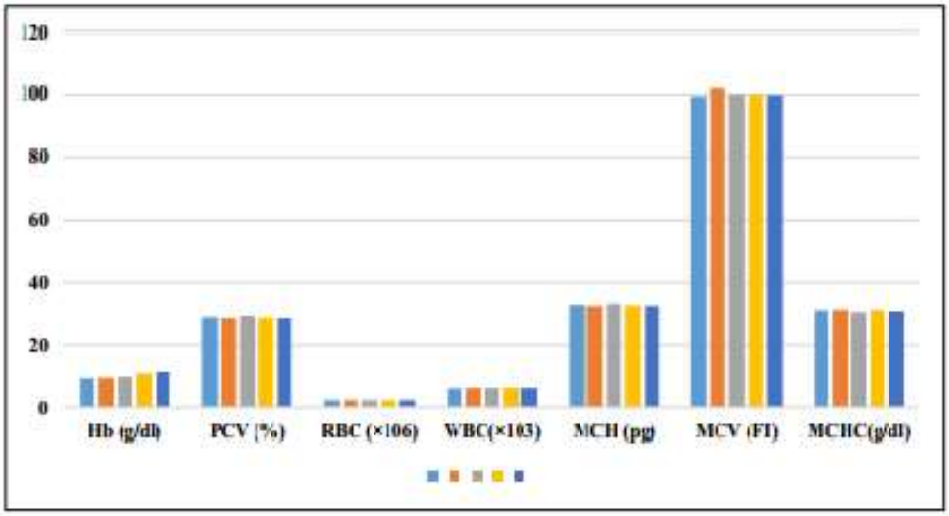


Fig 4.8: Haematological parameters in broiler chicken subjected to feed additive supplementation

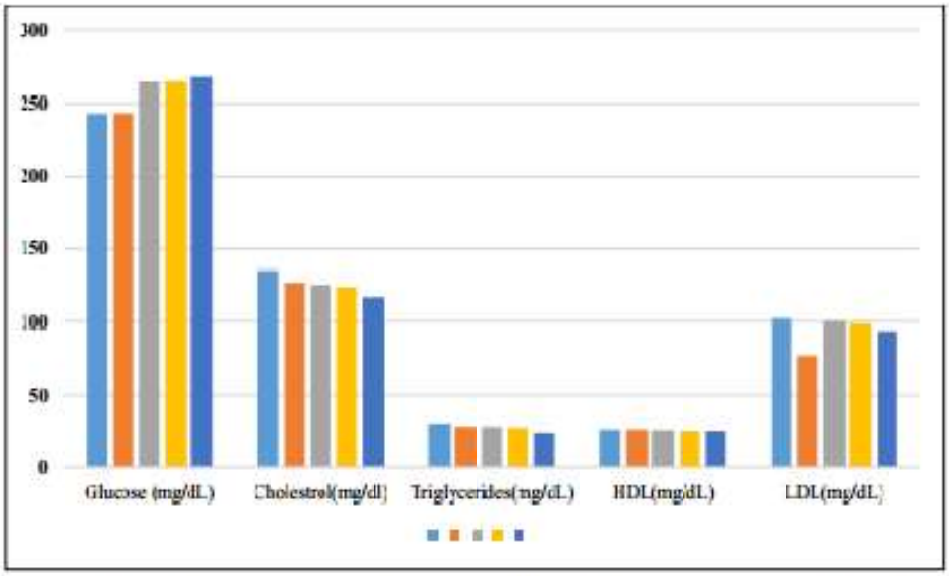


Fig 4.9: Serum biochemical parameters in broiler chicken subjected to feed additive supplementation.

Table 4.14: Humoral immune response in broiler chicken subjected to feed additive supplementation.

Parameters	Dietary treatments						
	T_0	T_1	T_2	T_3	T_4	SEM	*p* value
HI titre (21 days)	53.04^{a}	58.85^{a}	90.24^{b}	97.95^{bc}	107.16^{c}	5.26	<0.05
HI titre (28 days)	100.57^{a}	109.91^{a}	144.94^{b}	155.55^{bc}	168.01^{c}	6.53	<0.05
DNCB (mm)	1.09^{a}	1.37^{ab}	1.51^{bc}	1.78^{c}	1.69^{bc}	0.07	0.004

Means with different superscripts in same row differ significantly (P≤0.05)

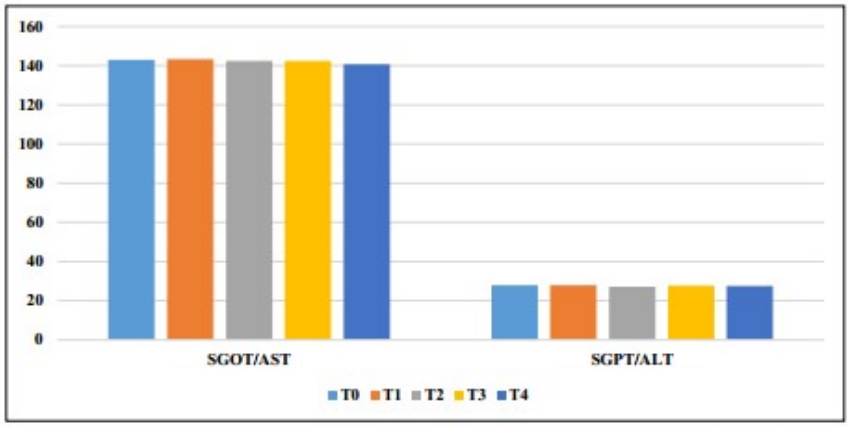


Fig 4.10: Serum liver enzyme status in broiler chicken subjected to feed additive supplementation.

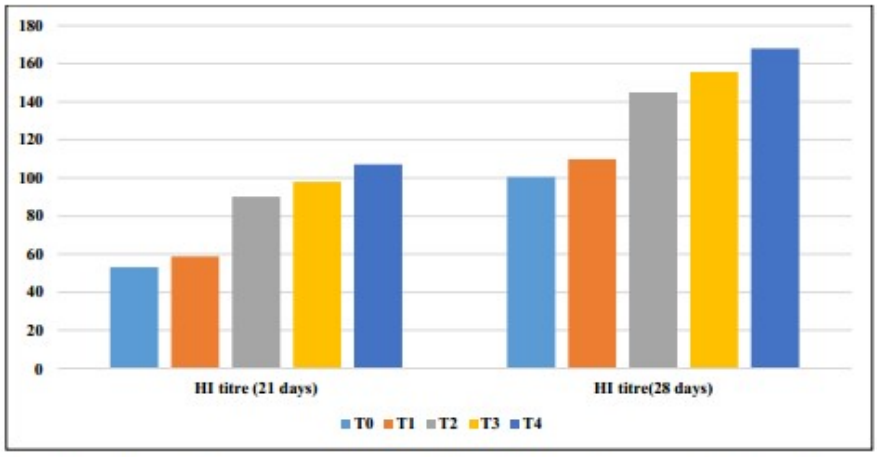


Fig 4.11: Humoral immune status in broiler chicken subjected to feed additive supplementation.

respectively. The birds of group T4 produced a significantly (P≤0.05) higher antibody response than the birds of control (T0), T1 and T2 groups with no significant difference was found among the immune responses of the birds of T4 and T3 groups. The mean of DNCB values were, 1.09, 1.37, 1.51, 1.78 and 1.69 mm in the treatments T0, T1, T2, T3 and T4 respectively. Significantly (P≤0.05) lower DNCB values were reported in birds of control (T0) group as compared to birds of T2, T3 and T4 treatments with non-significant difference with birds of T2 group.

4.5.2 Immune organ weight

The summary of mean weights (g) of different immune organs of the birds of group T_0, T_1, T_2, T_3 and T_4 subjected to saffron petal supplementation are presented in table 4.15 and depicted in fig.4.13. No significant difference was observed in average weights of bursa, caecal tonsils and ileum among all treatment groups. However, a significantly (P≤0.05) lower mean spleenweight wasrecorded in birds of T_0 as compared toT_4 treatment, with non-significant difference between T_1, T_2, T_3 and T_4groups.

Table 4.15: Immune organ weight of broiler chicken subjected to feed additive supplementation.

Organs (g)	Dietary treatments						
	T_0	T_1	T_2	T_3	T_4	SEM	*p* value
Spleen	1.49^{a}	1.60^{ab}	1.62^{ab}	1.74^{ab}	1.90^{b}	0.06	0.02
Bursa	2.17	2.20	2.24	2.34	2.50	0.06	0.34
Caecal tonsils	0.68	0.68	0.74	0.75	0.76	0.72	0.73
Ileum	10.41	11.26	11.56	11.66	11.66	0.22	0.37

Means with different superscripts in same row differ significantly (P≤0.05)

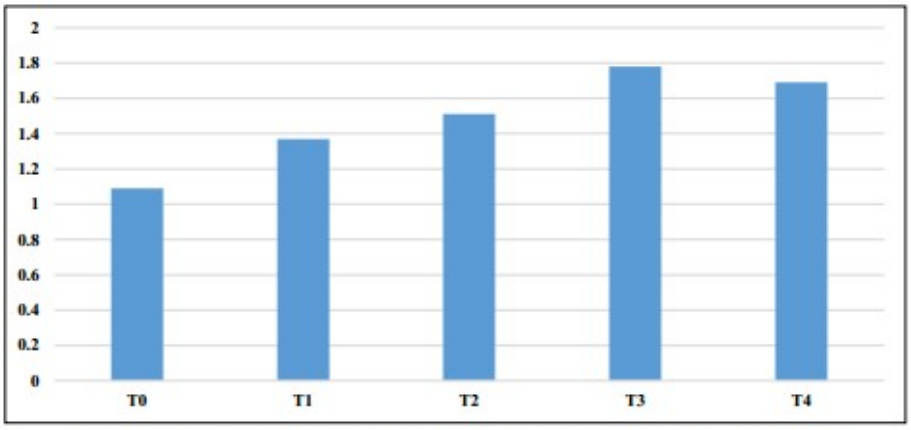


Fig 4.12: Cell mediated immune status in broiler chicken subjected to feed additive supplementation.

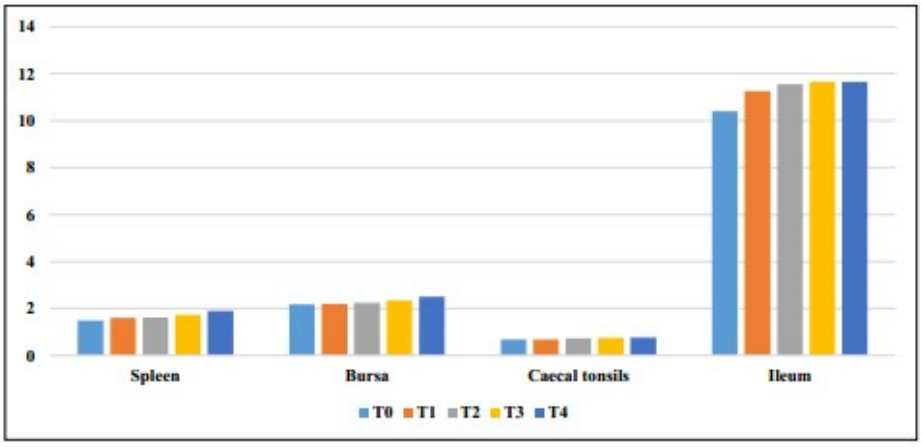


Table 4.13: Immune organ weight of broiler chicken subjected to feed additive supplementation.

4.6 Serum Oxidative status

Results of the oxidative parameters of experimental birds subjected to saffron petal as feed additive supplementation have been summarized in table 4.16 and depicted in fig 4.14A and 4.14B. The total oxidative status (TOS) of experimental birds were 7.45, 7.22, 6.88, 6.65 and 6.30 in the T_0, T_1, T_2, T_3 and T_4 treatments respectively. Significantly ($P \leq 0.05$) lowermean TOS values were recorded in birds of T_2, T_3 and T_4 treatments as compared to control (T_0), with non-significant difference between T_0 and T_1 group.The total antioxidant status (TAS) values in the experimental birds were 1.15, 1.30, 1.30, 1.37 and 1.46 in the treatment groups T_0, T_1, T_2, T_3 and T_4 respectively, with treatment groups (T_1, T_2, T_3 and T_4) having significantly ($P \leq 0.01$) higher values as compared to control (T_0) without any significant difference between T_1, T_2 and T_3 treatments. The oxidative stress index (OSI) values in the experimental birds were 6.53, 5.55, 5.26, 4.87 and 4.31 in the treatment groups T_0, T_1, T_2, T_3 and T_4 respectively, with significantly ($P \leq 0.05$) lower values in T_3 and T_4 treatments as compared to control (T_0). However there was no significant difference in OSI in T_1, T_2 and T_3 treatments.

The T-BARS (Breast) values in the experimental birds were 0.84, 0.84, 0.82, 0.80 and 0.74 in the T_0, T_1, T_2, T_3 and T_4 treatments respectively, withsignificantly ($P \leq 0.01$) lower valuesin T_4 treatment as compared to T_0, T_1, $T_2$and T_3. There was no significant difference among control (T_0) and T_1, T_2 and T_3treatments. The T-BARS (Thigh) in the experimental birds were 0.68, 0.53, 0.45, 0.40 and 0.32 in the T_0, T_1, T_2, T_3 and T_4 treatments respectively, with significantly ($P \leq 0.01$) higher values in control (T_0) followed by T_1, T_2, T_3 and T_4 treatments respectively.

4.7 Carcass parameters

4.7.1 Dressing parameters

The results of carcass parameters (%) of the birds of group T_0, T_1, T_2, T_3 and T_4 subjected to saffron petal as feed additive supplementation have been summarized in table 4.17 and depicted in fig. 4.15. The dressing percentage of the

Table 4.16: Average oxidative parameters in broiler chicken subjected to feed additive supplementation.

Oxidative parameters	Dietary treatments						
	T_0	T_1	T_2	T_3	T_4	SEM	*P* value
TOS (µmol H_2O_2 Eq/L)	7.45^{d}	7.22^{cd}	6.88^{bc}	6.65^{ab}	6.30^{a}	0.11	0.02
TAS(µmol Trolox Eq/L)	1.15^{a}	1.30^{b}	1.30^{b}	1.37^{bc}	1.46^{c}	0.03	0.001
OSI (arbitrary unit)	6.53^{c}	5.55^{b}	5.26^{b}	4.87^{ab}	4.31^{a}	0.19	<0.05
T-BARS Breast (µM/g)	0.84^{b}	0.84^{b}	0.82^{b}	0.80^{b}	0.74^{a}	0.01	0.001
T-BARS Thigh (µM/g)	0.68^{e}	0.53^{d}	0.45^{c}	0.40^{b}	0.32^{a}	0.03	0.001

Means with different superscripts in same row differ significantly (P≤0.01)

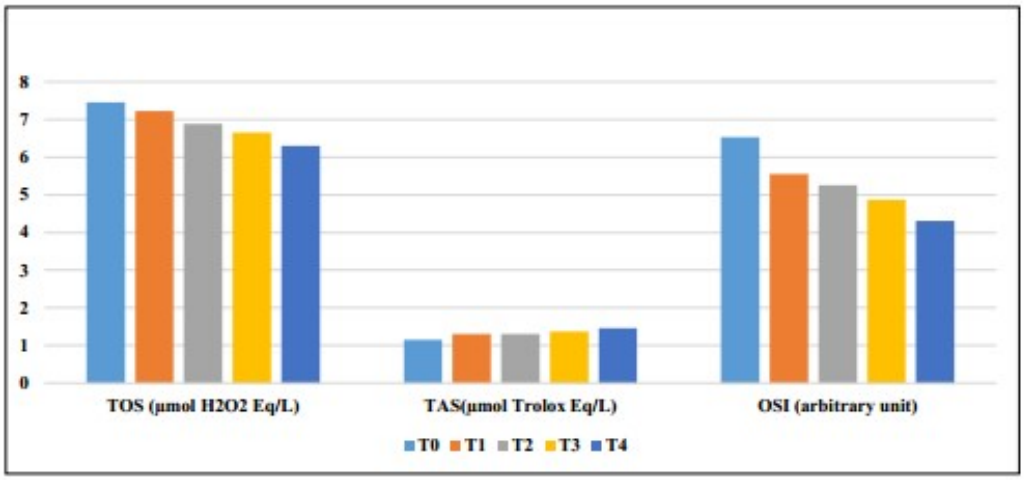


Fig 4.14A: Average oxidative parameters in broiler chicken subjected to feed additive supplementation.

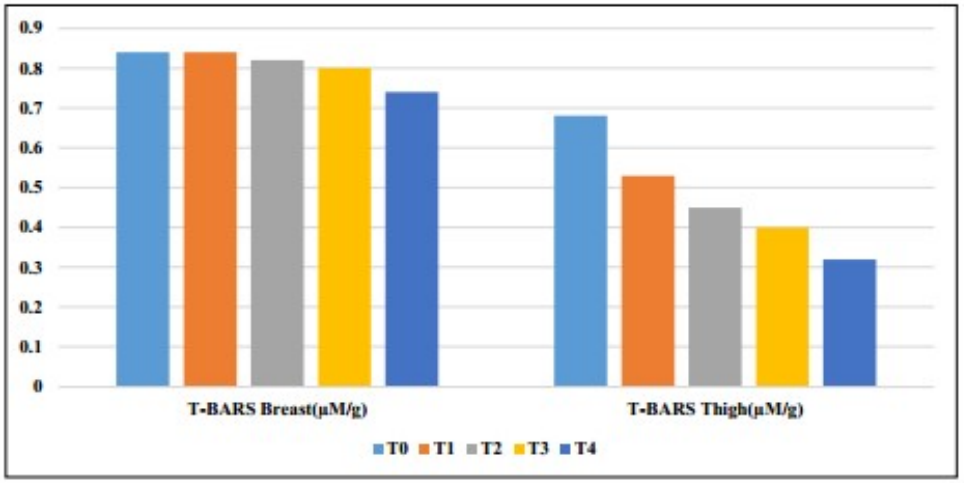


Fig 4.14B: Average oxidative parameters in broiler chicken subjected to feed additive supplementation.

experimental birds in the treatment groups T_0, T_1, T_2, T_3 and T_4 was71.26, 72.73, 73.36, 73.58 and 74.28 respectively depicting a significantly ($P \leq 0.05$) higher dressing percentage in birds of group $T_3$and T_4 compared to that of control (T_0).

The dressing percentage of birds of T_0, T_1 and T_2 groups could not reach to statistically significant difference. The feathering loss and bleeding loss of the experimental birds was 3.57, 3.68, 3.68, 3.81, 3.85% and 3.03, 3.51, 3.51, 3.56, 3.64% in the T_0, T_1, T_2, T_3 and T_4 treatment groups respectively, withoutany statistically significant difference between control and treatment groups.

Table 4.17: Carcass parameters of broiler chicken subjected to feed additive supplementation.

Carcass parameters (%)	Dietary treatments						
	T_0	T_1	T_2	T_3	T_4	SEM	*P* value
Dressing	71.26^{a}	72.73^{ab}	73.36^{ab}	73.58^{b}	74.28^{b}	0.35	0.05
Feathering loss	3.57	3.68	3.68	3.81	3.85	0.16	0.99
Bleeding loss	3.03	3.51	3.51	3.56	3.64	0.12	0.52

Means with different superscripts in same row differ significantly ($P \leq 0.05$)

4.7.2 Cutability Characteristics

The percent cutability characteristicsof the birds of group T_0, T_1, T_2, T_3 and T_4 subjected to saffron petal as feed additive supplementation have been summarized in table 4.18 and depicted in fig.4.16. The breast percentage of experimental birds in T_0, T1, T_2, T_3 and T_4 treatments was 23.76, 23.89, 23.91, 23.84 and 24.19% respectively. The back percentage of the experimental birdsin the treatments T_0, T_1, T_2, T_3 and T_4 was recorded 10.01, 10.18, 10.20, 10.28 and 10.30% respectively. Drumstick and thigh percentage was 10.50, 10.63, 10.66, 10.58,

10.63 and 11.26, 12.00, 12.49, 12.86, 12.58 in the treatments T_0, T_1, T_2, T_3 and T_4 respectively. The wing, neck and giblet percentage of the experimental birds was 9.56, 10.51, 10.54, 10.44, 10.51; 5.48, 5.55, 5.57, 5.58 and 5.13, 5.49, 5.50, 5.55, 5.84% in T_0, T_1, T_2, T_3 and T_4 treatments respectively. Breast, back, drumstick, thigh, wing, neck and total giblet percentage of experimental birds of all treatment groups could not reach to significant difference statistically.

Table 4.18: Cutability parameters of broiler chicken subjected feed additive supplementation.

Cutability Parameters (%)	Dietary treatments						
	T_0	T_1	T_2	T_3	T_4	SEM	*p* values
Breast	23.76	23.89	23.91	23.84	24.19	0.12	0.86
Back	10.01	10.18	10.20	10.28	10.30	0.07	0.80
Drumstick	10.50	10.63	10.66	10.58	10.63	0.09	0.99
Thighs	11.26	12.00	12.49	12.86	12.85	0.26	0.27
Wings	9.56	10.51	10.54	10.44	10.51	0.16	0.29
Neck	5.48	5.55	5.54	5.57	5.58	0.10	0.99
Total Giblet	5.13	5.49	5.50	5.55	5.84	0.10	0.31

Means with different superscripts in same row differ significantly ($P \leq 0.05$)

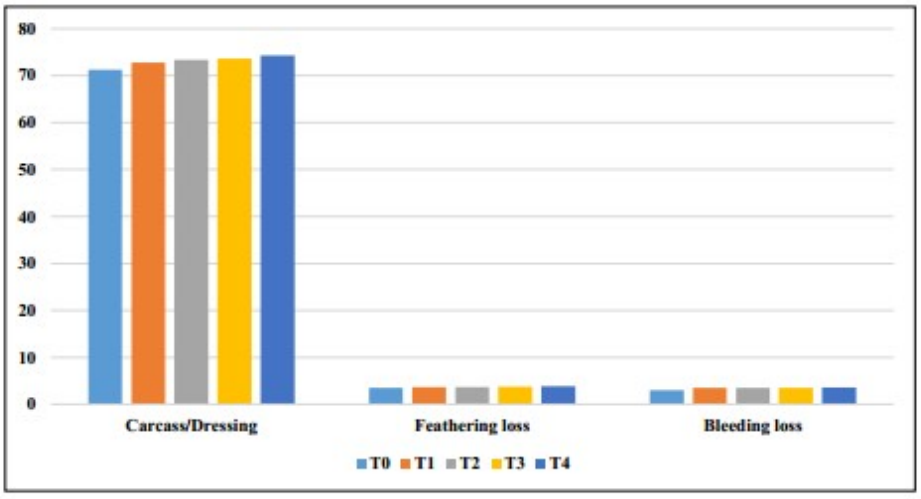


Fig 4.15: Carcass parameters in broiler chicken subjected to feed additive supplementation.

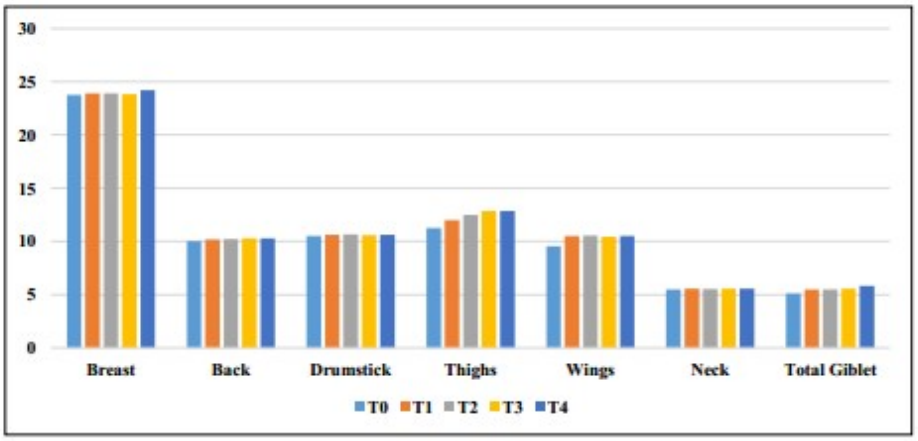


Fig 4.16: Cutability parameters in broiler chicken subjected to feed additive supplementation.

Chapter – 5

DISCUSSION

The present study was conducted on effect of saffron petal as feed additiveon growth performance, immune response, antioxidant status, carcass quality, retention of nutrients and economics of broiler chickens, which is discussed hereunder:

5.1 Chemical composition of saffron petals:

The pre-stater feed contained 91.00% DM, 23.20% CP, 11.99% EE, 2.33 % CF and 3.87% total ash. Ca and P contents were found to be 1.10% and 0.49%, respectively. The calculated ME was 3000 Kcal/kg. The starter feed contained 92.30% DM, 20.16% CP, 10.80% EE, 3.22% CF and 4.04% total ash. Ca and P contents were found to be 1.00% and 0.44%, respectively. The calculated ME was 3100 Kcal/kg. The finisher diet contained DM (92.30%), CP (18.00) %, EE (9.06%), CF (4.28%), total ash (4.16%), Ca (0.89%) and P (0.40%) with ME value of 3191 Kcal/kg. The Saffron petals (*Crocus sativus* L.) used in the study as feed additive contained 85% DM, 11.94% CP, 5.03% EE, 7.85% CF, 52.81% NFE, 5.37% total ash, 1.33% acid insoluble ash, 36.10% NDF, 30.00% ADF, 6.10% hemicelluloses and 5.90% cellulose. The Ca and P contents in Saffron petals were found to be 0.78 and 0.34 per cent, respectively. The Crocin content was found 0.785% W/W. Similarly, Fahim *et al.,*(2012) reported that saffron petals contain 10.20% crude protein, 5.3% ether extract, 8.80%crude fiber and 7.00% ash. Being rich source of minerals, saffron petals contain sodium (25.75 mg/100 g), potassium (542.13 mg/100 g), calcium (486.25 mg/100 g), copper (0.87 mg/100 g), iron (17.99 mg/100 g), magnesium (2.93 mg/100 g), zinc (1.80 mg/100 g) and phosphorus (209.90 mg/100 g). Also, it is composed of carotenoidcrocin, @ 0.6%w/w (Eka *et al.,* 2015).

5.2 Production performance for broiler

5.2.1 Body Weight

For judging the dietary status,bodyweight is an important feature of consideration. The average weekly live body weights (g) of experimental birds subjected to different levels of saffron petal as feed additive in dietary treatment groups (T_0, T_1, T_2, T_3 and T_4) had non-significant differences at the first and second weeks of age. However, the average body weight of birds at the end of 3^{rd}, $4^{th}$and 5^{th}week was significantly lower ($P \leq 0.05$) in the T_0(control) group as compared to feed additive supplemented groups (T_1, T_2, T_3 and T_4), with T_4 treatment group having significantly highest ($P \leq 0.05$) body weight followed by T_3,T_2 and T_1 groups. These results are as per the reports of Hosseini-Vashan *et al.,* (2018)who investigated the effect of hydroethanolic saffron petals extract (HSPE) in Japanese quail and reported that inclusion of hydroethanolic saffron petals' extract to quail diets improved the body weight and FCR. Zeweil *et al*., (2015) conducted a study on effect of vitamin E and phytogenic feed additives including saffron on performance of broiler chicks and reported better growth performance on chicks supplemented with saffron alone or in combination with rosemary. Several studies have been conducted on the effect of dietary herbs alone or in combinations on the performance of animals but with varying and often conflicting results. While some reports suggested that dietary herbal essential oils improved growth performance in poultry (Alcicek *et al.,* 2003; Basmacioglou *et al.,* 2004), others showed no such effect (Botsoglou *et al.,* 2002a; Lee *et al.,* 2003; Papageorgiou *et al.,* 2003).The use of fenugreek seeds as feed additive in broiler diets @ 5.33 kg per ton (Abdel-Rahman *et al.,* 2014), @ 0.3% level (Aksa *et al*., 2012; Rabia, 2010), @ 0.5% level (Abdel-Azeem 2006), @ 1% level (Mamoun *et al*., 2014; Weerasingha and Atapattu, 2013; Yatoo *et al*., 2012), @ 1.5% level (Magda, 2012), 2% level (Hind *et al*., 2013), 4% level resulted improvement in the live body weight, feed intake and feed efficiency with reduced mortality. Similarly, Raziq (2012), Abaza (2001) Guo *et al*., (2004)and Farman Ullah *et al.*,

(2009) reported improved body weight and feed efficiency with reduction in feed cost when the diets of broiler chicken were supplemented with Fenugreek seeds as natural feed additives. Qureshi *et al.,* (2016) reported that diets supplemented with either Dandelion leaves @ 5g/kg feed or Fenugreek seeds@ 10g/kg feed alone or in combination with or without enzyme addition resulted into an improvement in the body weight, feed conversion ratio and economicsof broiler chicken. Supplementation of Dandelion @ 650 mg/kg feed in broiler chicken showed improved weight gain of broiler with no significant ($p>0.05$) difference in feed intake and FCR (Alhussianyinyi *et al*., 2012).Galib *et al.* (2010) reported that the supplementation of Dandelion in broiler chicken @ 0.5% of diet had showed no effect on feed intake, improved body weight gain and a significant ($p\leq0.05$) reduction in the mortality rate with better feed conversion ratio (FCR). The live weight of broilers increases with the inclusion of clove essential oil at a rate from 300 to 500 mg per 1 kg of feed (Valenzuela-Grijalva *et al.,* 2017).

5.2.2. Feed consumption

During entire experimental period among groups supplemented with the feed additive, feed intake werenumerically higher as compared to controlbut could not reach tostatistically significant difference. In support of present resultsBotsoglou *et al*. (2005b) investigated the effect of feeding rosemary, oregano, saffron or *a*-tocopheryl acetate on hen performance and egg quality and reported no significant differences in feed intakeamong treatments.Similarly, Hosseini-Vashan *et al.,* (2018) who investigated the effect of hydroethanolic saffron petals extract (HSPE) in Japanese quail and reported that inclusion of hydroethanolic saffron petals' extract to quail diets had no effect on feed intake.Galib *et al.* (2010) reported that the supplementation of Dandelion in broiler chicken @ 0.5% of diet had showed no effect on feed intake. Similarly, supplementation of Dandelion @ 650 mg/kg feed in broiler chicken showed no significant difference in feed intake (Alhussianyinyi *et al*., 2012).

5.2.3 Feed conversion and Feed efficiency ratio (FCR & FER)

Initially, during the first week of the experiment there was no significant difference among the FCR values and the FER values in the treatments T_0, T_1, T_2, T_3 and T_4; however, as the age progressed, a significantly ($P \leq 0.05$) better feed efficiency (lower FCR and higher FER) was recorded in the birds of T_3 (1.5% supplemented) and T_4(2% supplemented)groups as compared to birds of the control group (T_0). The overall FCR and FER of the experimental birds during entire experimental period was significantly better in supplemented (T_1, T_2, T_3 and T_4) groups as compared to control group (T_0). In accordance with the present findings, Hosseini-Vashan *et al.,* (2017)reported that inclusion of hydroethanolic saffron petals' extract to quail diets improved FCR.The use of fenugreek seeds as feed additive in broiler diets resulted improvement feed intake and feed efficiency (Abdel-Rahman *et al.,* 2014; Aksa *et al*., 2012; Rabia, 2010; Abdel-Azeem 2006; Mamoun *et al*., 2014; Weerasingha and Atapattu, 2013; Yatoo *et al*., 2012; Magda, 2012; Hind *et al*., 2013.Similarly, Raziq (2012), Abaza (2001) Guo *et al*., (2004), Azoua, (2001) and Farman Ullah *et al.*, (2009) reported improved feed efficiency when the diets of broiler chicken were supplemented with Fenugreek seeds. Qureshi *et al.,* (2016) reported that diets supplemented with either Dandelion leaves or Fenugreek seeds alone or in combination with or without enzyme addition resulted into an improvement feed conversion ratio of broiler chicken. Galib *et al.* (2010) reported that the supplementation of Dandelion in broiler chicken @ 0.5% of diet had better feed conversion ratio (FCR).

5.2.4 Performance index (PI)

The results of weekly performance index in broiler chicken subjected to saffron petal as feed additive depicted that treatments T_3andT_4 has significantly ($P \leq 0.05$) higher performance index as compared to control (T_0), T_1 and T_2 groups. The overall performance index of broiler chicken was significantly ($P \leq 0.01$)

higher values in T_4 followed by T_3 treatment. T_2 and T_1 with statistically ($P \leq 0.05$) lowest performance index values in control (T_0). Hosseini-Vashan *et al.,* (2017) also reported improved body weight and FCR in quail fed hydroethanolic saffron petals extract supplemented diets. Similarly, Zeweil *et al*., (2015) reported that phytogenic feed additives including saffron improved performance of broiler chicks. Alcicek *et al.,* 2003; Basmacioglou *et al.,* 2004; Abdel-Rahman *et al.,* 2014; Aksa *et al*., 2012 and Rabia, 2010also reported that dietary herbal essential oils improved performance in poultry. Similarly, Raziq (2012), Abaza (2001) Guo *et al*., (2004), Azoua, (2001) and Farman Ullah *et al.*, (2009) reported improved body weight and feed efficiency with reduction in feed cost when the diets of broiler chicken were supplemented with Fenugreek seeds as natural feed additives.

5.2.5 Nutrient Digestibility

The results of average nutrient digestibility in experimental birds subjected to feed supplemented with saffron petals showsignificantly ($P \leq 0.05$) higher dry matter digestibility in treatment T_2,T_3 and T_4 as compared to control (T_0) and T_1 treatment groups. Birds of treatment groupT_4 also havesignificantly($P \leq 0.05$) higher digestibility of crude protein (CP) as compared to control group with no significant difference among T_0, T_1, $T_2$and T_3 treatment groups. There was nosignificant effect of feed additive supplementation on digestibility of ether extract (EE), crude fibre (CF) and nitrogen-free extract (NFE). The present study revealed there had been better nutrient digestibility in the birds of the treatments with higher level of supplementation of feed additive (saffron petal) possibly because of itsstomachic, eupeptic and laxative nature with protective role on pancreatic and hepatic tissue against oxidative damage which may help to maintain the proper functionality of the pancreas, stimulating the secretion of digestive enzymes, thus improving the digestibility of nutrients(Mortazavi *et al.,* 2001; Hosseinzadeh *et al.,* 2008b; Pitsikas *et al.,* 2007).The essential oils intensify the replacement of cells in the villi of the

intestines, which blocks the intensity of the development of pathogenic microflora in broiler chicken (Adaszyńska-Skwirzyńska and Szczerbińska, 2017).

5.2.6 Mortality

There has been no mortality reported of experimental birds during experiment. Studies related to the influence of saffron and its byproducts on health are due to multitude of bioactive agents present(Zarinkamar *et al.,* 2011). The different health benefits of saffron and its by-products on health has been aphrodisiac agent, anticatarrhal, laxative, eupeptic, stimulant, antispasmodic, antidepressant, a respiratory decongestant, nerve sedative, stomachic, expectorant, emmenagogue, carminative, diaphoretic, anodyne, gingival sedative, galactogogue, amenorrhea, dysmenorrhea and in treatment of bladder, liver, kidney, eye, acne, and several skin diseases (Hosseinzadeh *et al.,* 2008b; Pitsikas *et al.,* 2007, 2008). Other pharmaceutical use of saffron are treatment of ascites, naevus, freckles, ulcer, headache, cold, smallpox, scarlet fever, bronchitis, pharyngopathy, vomiting, asthma, myopia, discomfort of teething infants, cholera, inflammation, melancholia, epilepsy, menstruation disorder, painful labor, strengthening the heart and memory enhancer (Nilkashi *et al.,* 2011). There have reports by Adaszyńska-Skwirzyńska and Szczerbińska, (2017) that use of lavender oil (*Lavandula stoechas L.*) in broiler chicken @ 24 mg perkg of feed led to an increase in live weight and a decrease in mortality in the experimental group.Also, Galib *et al.* (2010) reported that the supplementation of Dandelion in broiler chicken had showed a significant ($p \leq 0.05$) reduction in the mortality.The use of fenugreek seeds as feed additive in broiler diets resulted improvement in the live body weightwith reduced mortality (Abdel-Rahman *et al.,* 2014; Mamoun *et al*., 2014; Weerasingha and Atapattu, 2013; Hind *et al*., 2013).

5.2.7 Economics

The economics of broiler production from different treatment groups (T_0, T_1, T_2, T_3 and T_4)was calculated considering the purchase rate of chicks, average

feed consumption of birds, feed cost, cost of feed supplements (saffron petal), mortality, total weight gain of birds and managemental expenditure during the experimental period.The results of the present study depict that supplementation of the diets with feed additive (saffron petal) has a significant effect on economics. The cost of production per kg live weight in T_0 was rupees (₹)104.13,102.58 for T_1; 102. 56 for T_2, 101.00 for T_3 and 100.52 for T_4.The results indicate that production cost per kg live weight was least in treatment $T_4$and height in T_0. In support of present findings, Qureshi *et al.,* (2016) reported that diets supplemented with either Dandelion leaves or Fenugreek seeds alone or in combination with or without enzyme addition resulted into an improvement in the body weight, feed conversion ratio and economicsof broiler chicken.Similarly, Hosseini-Vashan *et al.,* (2017)investigated the effect of hydroethanolic saffron petals' extract on performance of Japanese quail and reported that inclusion of hydroethanolic saffron petal extract to quail diets improved the body weight and FCR with better economical returns.Raziq (2012), Abaza (2001) Guo *et al*., (2004), Azoua, (2001) and Farman Ullah *et al.*, (2009) also reported improved body weight and feed efficiency with reduction in feed cost when the diets of broiler chicken were supplemented with Fenugreek seeds as natural feed additives.

5.3 Haemato-biochemical Parameters

To ascertain the effect of saffron petal as feed additive in the diet of broiler birds on health status, different haemato-biochemical parameters were analysed at the end of experiment. All the parameters were within normal range indicating no deleterious effect of saffron petal as feed additive in the diet of broiler birds.The blood heamoglobin (Hb) concentration of experimental birds shows that birds of T_3 and T_4group were having significantly ($P \leq 0.01$) higher Hb concentration as compared to control (T_0) and T_1 group.However, there was no significant difference in Hb concentration among birds of T_1,$T_2$and T_3treatment groups. Other parameters like RBC and

WBC count, PCV, MCH, MCV and MCHC values of birds of control (T_0) and treatment groups (T_1, T_2, T_3 and T_4) could not reach to statistically significant difference.Zeweil *et al*., (2015) conducted a study on effect of vitamin E and phytogenic feed additives (Rosemary and Saffron) on blood constituents of broiler chicks, and reported no significant effects. Alipour*et al.,* (2019) conducted a study to investigated the effects of the administration of ethanolic saffron petal extract (SPE) and vitamin E (Vit E) on blood metabolites in Baluchi lambs and reported no significant effect of supplementation was observed on haematological parameters. In a study conducted on rats received graded doses of saffron petal extract with no difference in hematological parameters such as red blood cells, hemoglobin, hematocrit, and platelet has been observed between treated groups with control (Babaei *et al.,* 2014).

The results of the serum biochemical parameters of experimental birds subjected to saffron petals as feed additive have been found within normal range. The blood glucose values of the experimental birds of group T_0 and T_1 showed significantly lower ($P \leq 0.01$) levels than the birds in T_2, T_3 and T_4, with no significant difference between T_2, T_3 and T_4 treatment groups.The present study witnessed the higher blood glucose levels in the supplemented groups, indicating better nutrient utilization in birds if these groups. Significantly ($P \leq 0.05$) lower cholesterol and serum triglycerides levels were found in birds of T_4 treatment group as compared to control group (T_0) with non-significant difference between T_1, T_2 and T_3 treatment groups. There was no significant difference in serum creatinine, HDL and LDL values among birds of control and treatment groups. Also,the serum aspartate transaminase (AST) and serum alanine transaminotransferase (ALT) activity of the control (T_0)and treatment groups (T_1, T_2, T_3 and T_4)were statistically non-significant.These findings are also supported by Zeweil *et al*., (2015) whoreported significantly ($P \leq 0.05$) lower cholesterol and triglyceride in birds fed phytogenic feed additives (Saffron and Rosemary). Similarly, Hosseini-Vashan *et al.,* (2017) investigated the effect of hydroethanolic

saffron petal extract (HSPE) on blood biochemical parameters of Japanese quail. The results revealed that inclusion of hydroethanolic saffron petalextract decreased serum cholesterol and triglyceride of quail with no effect on HDL and LDL concentration. Alipour*et al.,* (2019)reported that administration of ethanolic saffron petal extract in Baluchi lambs has no significant effect on plasma creatinine, AST and ALT enzyme concentration, with significantly ($p< 0.01$) lowerplasma cholesterol.Ardalan *et al.,*(2012) reported that extracts of saffron petal at doses of 500, 1000 and 1500 mg/kg for 15 days, did not change the levels of creatinine, AST, ALTand other indexes in lambs.

5.4 Immune parameters

5.4.1 Humoral Immune parameters

The birds of group T_4 produced a significantly ($P\leq0.05$) higher antibody response than the birds of control (T_0), T_1 and T_2 groups with no significant difference was found among the immune responses of the birds of T_4 and T_3 groups.Significantly ($P\leq0.05$) lower DNCB values were reported in birds of control (T_0)group as compared to birds of T_2, T_3 and T_4 treatments with non-significant difference with birds of T_2 group. The better immune status of supplemented birds is possibly because tepals of *Crocus sativus L.*flower, are a rich source of biologically active compounds; such as, phenols, flavonoids, tannins and anthocyanins (Kanakis *et al.,* 2006; Srivastava *et al.,* 2010) which have high antioxidant properties. Babaei *et al.,*(2014) reported that rats received graded doses of saffron petal extract have increased IgG levels in comparison with control group.

5.4.2 Immune organ weight

The summary of mean weights (g) of different immune organs of the birds of group T_0, T_1, T_2, T_3 and T_4 subjected to saffron petal supplementation are have no significant difference in average weights of bursa, caecal tonsils and ileum.

However, a significantly (P≤0.05) highermean spleenweight wasrecorded in birds of T_4treatment as compared to control (T_o), with non-significant difference between T_1, T_2, T_3 and T_4groups. There has been little literature available about the effect of sffron and its constituents on immune organ weight in poultry. However, Hosseini-Vashan *et al.,* (2017) reported that relative weight of fabricus bursa was increased when Japanese quail birds received the hydroethanolic saffron petals extract.

5.5 Serum Oxidative stress enzymes

The production of 'reactive oxygen species' (ROS) or free radicals is directly related to the rate of metabolism, which results in the occurrence of oxidative damage to the body tissues, posing a high risk of the development of chronic diseases due to membrane and DNA damage and inhibition of the immune system (Rahman *et al.,* 2014). Antioxidants combat ROS and protect the body from the harmful effects of ROS in various ways (Witztum, 1994; Rao and Agarwal, 1999), which acts as the body's self defence mechanism. Significantly (P≤0.05) lowermean TOS values were recorded in birds of T_2, T_3 and T_4treatments as compared to control (T_0), with non-significant difference between T_0 andT_1group.The total antioxidant status (TAS) values in the treatment groups (T_1, T_2, T_3 and T_4)have significantly (P≤0.01) higher values as compared to control (T_0)without any significant difference between T_1, $T_2$and T_3 treatments. These results indicate improved oxidative status in broilers supplemented saffron petals as feed additive assaffron petals are rich in phenolic and biologically active compounds; such as, flavonoids (kaempferol, rutin, quercetin, luteolin, hesperidin, and bioflavonoids), tannins and anthocyanins (Kanakis *et al.,* 2006; Srivastava *et al.,* 2010).The T-BARS (Breast)values were significantly (P≤0.01) lower in T_4 treatment as compared to T_0, T_1, $T_2$and T_3. There was no significant difference among control (T_0)and T_1, T_2 and T_3treatments. The T-BARS (Thigh)values were significantly (P≤0.01) higher values in control (T_0) followed by T_1, T_2, T_3andT_4 treatments

respectively.These results are in accordance with findings of Zeweil *et al*., (2015) who reported that birds fed diet supplemented with phytogenic feed additives (Rosemary and Saffron) have significantly (P≤0.05) higher total antioxidant capacity with significantly (P≤0.05) lower MDA compared to unsupplemented group.Similarly, Botsoglou *et al*. (2005a) reported that feed supplementation with red stigmas of saffron reduces lipid oxidation in shell eggs, as measured by malondialdehyde (MDA) formation. Pham *et al.* (2000) have shown that crocins, the main biologically active constituents of saffron, could prevent the oxidation of linoleic acid *in vitro* as their anti- oxidative activity was found to be comparable to that of butylated hydroxyanisole.Florou-Paneri *et al.* (2006) reported that incorporation of saffron in poultry feeds at the level of 20 mg/kg feed was more effective in retarding lipid oxidation in both raw and cooked samples compared to the level of 10 mg saffron /kg feed. Antioxidant activity of saffron petal in lambs has been evaluated by Ardalan *et al.,*(2012) using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free-radical method. The extracts of saffron petal were gavaged at doses of 500, 1000 and 1500 mg/kg for 15 days, reported increased anti-oxidant status at all doses.

5.6 Carcass parameters

The results of carcass parameters (%) of the birds of group T_0, T_1, T_2, T_3 and T_4 subjected to saffron petal as feed additive supplementation have depicted that dressing percentage was significantly (P≤0.05) higher in birds of $T_3$and T_4 groups compared to that of T_0 group. The dressing percentage of birds of T_0, T_1 and T_2 groups could not reach to statistically significant difference. The other dressing parameters like feathering loss andbleeding loss of the experimental birds were withoutany statistically significant difference. The breast, back, drumstick, thigh, wing, neck and total giblet percentage of experimental birds of all treatment groups also could not reach to significant difference statistically. These results are in accordance with findings of Zeweil *et al*., (2015) who reported that phytogenic

feed additives (Rosemary and Saffron) have no significant effects on carcass characteristics in broiler chicks.Similarly, Hosseini-Vashan *et al.,* (2017) reported that inclusion of hydroethanolic saffron petals' extract to quail diets did not affected relative weight of breast, thigh, pancreases, and heart. Alipour*et al.,* (2018)reported that administration of ethanolic saffron petal extract have no significant effect carcass parameters of lambs.Qureshi *et al.,* (2016) reported that broiler diets supplemented with either Dandelion leaves or Fenugreek seeds have no effect on carcass parameters with only higher dressing percentage in supplemented groups as compared to control.

Chapter – 6

SUMMARY AND CONCLUSION

The present study is aimed to evaluate the effect of dietary supplementation of saffron petal as feed additive on the growth performance, feed intake, nutrient utilization, economics, immune status, oxidative status and carcass traits of broilers. The study was undertaken in the Division of Animal Nutrition, FVSc & AH, and Instructional Poultry farm of the Division of Livestock Production and Management, FVSc & AH, SKUAST- Kashmir, Shuhama, Alusteng (J&K). Petalsare procured from saffron growing farmers, freed from impurity, dried under shade and grinded to fine powder for further use.

In the experiment, 140-day-old chicks were randomly distributed in five treatment groups having four replicates of seven chicks each.Birds of treatment group T0 were offered basal diet without feed additives. Birds of treatment group T1, T2 T3 and T4 were offered basal diet supplemented with grinded saffron petals as feed additive @ 0.5,1, 1.5 and 2g/kg feed on dry matter basis. The experimental diets were formulated to contain 3000 Kcal ME/kg and 23.20% CP for pre-starters, 3100 Kcal ME/kg and 20.16% CP for starters and 3191 Kcal ME/kg with 18.00% CP for finishers. The daily temperature and humidity of experimental groups were recorded. The bodyweight of experimental birds was recorded at weekly intervals, whereas feed intake was calculated by subtracting leftover feed from total feed offered within a week. A metabolic trial was conducted to assess the digestibility of nutrients in different treatment groups, with mortality recorded on a daily basis.The blood samples of 3 birds from each replicate were collected on the 21^{st} and 28^{th}day from the wing vein for oxidative and immunological analysis

and on the 35th day from the jugular vein for heamato-biochemical parameters. At the end of the feeding trial, three birds per replicate were selected at random and utilized for the carcass evaluation study.

The pre-stater feed contained 91.00% DM, 23.20% CP, 11.99% EE, 2.33 % CF and 3.87% total ash. Ca and P contents were found to be 1.10% and 0.49%, respectively. The calculated ME was 3000 Kcal/kg. The starter feed contained 92.30% DM, 20.16% CP, 10.80% EE, 3.22% CF and 4.04% total ash. Ca and P contents were found to be 1.00% and 0.44%, respectively. The calculated ME was 3100 Kcal/kg. The finisher diet contained DM (92.30%), CP (18.00) %, EE (9.06%), CF (4.28%), total ash (4.16%), Ca (0.89%) and P (0.40%) with ME value of 3191 Kcal/kg. The Saffron petals (*Crocus sativus* L.) used in the study as feed additive contained 85% DM, 11.94% CP, 5.03% EE, 7.85% CF, 52.81% NFE, 5.37% total ash, 1.33% acid insoluble ash, 36.10% NDF, 30.00% ADF, 6.10% hemicelluloses and 5.90% cellulose.

The average weekly temperature and humidity of the experimental groups were recorded as 23.04°C and 55.50% during the day and 23.03°C and 56.20% during the night for the first week of the experiment. For the second week of the experiment, the average weekly temperature and humidity of experimental groups were recorded 23.37°C and 53.20% during the day and 23.07°C and 55.06% during the night. The average weekly temperature and humidity of experimental group was recorded 23.61°C and 56.20% during day and 23.30°C and 57.23% during night for third week of the experiment. For the fourth week of the experiment, the average weekly temperature and humidity of experimental groups was recorded 23.69°C and 55.86% during day and 23.27°C and 55.53% during night. The overall temperature and humidity of experimental groups was recorded 23.43°C and 55.19% during day and 23.17°C and 56.01% during night throughout the experiment.

The average weekly live body weights (g) of experimental birds subjected to different levels of saffron petal as feed additive in dietary treatment groups (T_0, T_1, T_2, T_3 and T_4) had non-significant differences at the first and second weeks of age. However, the average body weight of birds at the end of 3rd, 4th and 5th week was significantly lower ($P \leq 0.01$) in the T_0 (control) group as compared to feed additive supplemented groups (T_1, T_2, T_3 and T_4), with T_4 treatment group having significantly highest ($P \leq 0.01$) body weight followed by T_3, T_2 and T_1 groups.

During entire experimental period among groups supplemented with the feed additive, feed intake were numerically higher as compared to control but could not reach to statistically significant difference.

Initially, during the first week of the experiment there was no significant difference among the FCR values and the FER values in the treatments T_0, T_1, T_2, T_3 and T_4; however, as the age progressed, a significantly ($P \leq 0.05$) better feed efficiency (lower FCR and higher FER) was recorded in the birds of T_3 (1.5% supplemented) and T_4 (2% supplemented) groups as compared to birds of the control group (T_0). The overall FCR and FER of the experimental birds during entire experimental period was significantly better in supplemented (T_1, T_2, T_3 and T_4) groups as compared to control group (T_0).

The results of weekly performance index in broiler chicken subjected to saffron petal as feed additive depicted that treatments T_3 andT_4 has significantly ($P \leq 0.05$) higher performance index as compared to control (T_0), T_1 and T_2 groups. The overall performance index of broiler chicken was significantly ($P \leq 0.01$) higher values in T_4 followed by T_3 treatment. T_2 and T_1 with statistically ($P \leq 0.05$) lowest performance index values in control (T_0).

The results of average nutrient digestibility in experimental birds subjected to feed supplemented with saffron petals show significantly ($P \leq 0.05$) higher dry matter digestibility in treatment T_2, T_3 and T_4 as compared to control (T_0) and

T_1treatment groups. Birds of treatment group T_4 also have significantly($P \leq 0.05$) higher digestibility of crude protein (CP) as compared to control group with no significant difference among T_0, T_1, $T_2$and T_3 treatment groups.There was nosignificant effect of feed additive supplementation on digestibility of ether extract (EE), crude fibre (CF) and nitrogen-free extract (NFE).

There has been no mortality reported of experimental birds during experiment.

The economics of broiler production from different treatment groups (T_0, T_1, T_2, T_3 and T_4) was calculated considering the purchase rate of chicks, average feed consumption of birds, feed cost, cost of feed supplements (saffron petal), mortality, total weight gain of birds and managemental expenditure during the experimental period. The results of the present study depict that supplementation of the diets with feed additive (saffron petal) has a significant effect on economics. The cost of production per kg live weight in T_0 was rupees (₹)104.13,102.58 for T_1; 102. 56 for T_2, 101.00 for T_3 and 100.52 for T_4.The results indicate that production cost per kg live weight was least in treatment T_4 and height in T_0.

To ascertain the effect of saffron petal as feed additive in the diet of broiler birds on health status, different haemato-biochemical parameters were analysed at the end of experiment. All the parameters were within normal range indicating no deleterious effect of saffron petal as feed additive in the diet of broiler birds. The blood heamoglobin (Hb) concentration of experimental birds shows that birds of T_3 and T_4 group were having significantly ($P \leq 0.01$) higher Hb concentration as compared to control (T_0) and T_1 group. However, there was no significant difference in Hb concentration among birds of T_1, T_2 and T_3 treatment groups. Other parameters like RBC and WBC count, PCV, MCH, MCV and MCHC values of birds of control (T_0) and treatment groups (T_1, T_2, T_3 and T_4) could not reach to statistically significant difference.

The results of the serum biochemical parameters of experimental birds subjected to saffron petals as feed additive have been found within normal range. The blood glucose values of the experimental birds of group T_0 and T_1 showed significantly lower ($P \leq 0.01$) levels than the birds in T_2, T_3 and T_4, with no significant difference between T_2, T_3 and T_4 treatment groups. The present study witnessed the higher blood glucose levels in the supplemented groups, indicating better nutrient utilization in birds if these groups. Significantly ($P \leq 0.05$) lower cholesterol and serum triglycerides levels were found in birds of T_4 treatment group as compared to control group (T_0) with non-significant difference between T_1, T_2 and T_3 treatment groups. There was no significant difference in serum creatinine, HDL and LDL values among birds of control and treatment groups. Also, the serum aspartate transaminase (AST) and serum alanine transaminotransferase (ALT) activity of the control (T_0) and treatment groups (T_1, T_2, T_3 and T_4)were statistically non-significant.

The birds of group T_4 produced a significantly ($P \leq 0.05$) higher antibody response than the birds of control (T_0), T_1 and T_2 groups with no significant difference was found among the immune responses of the birds of T_4 and T_3 groups. Significantly ($P \leq 0.05$) lower DNCB values were reported in birds of control (T_0)group as compared to birds of T_2, T_3 and T_4 treatments with non-significant difference with birds of T_2 group.

The summary of mean weights (g) of different immune organs of the birds of group T_0, T_1, T_2, T_3 and T_4 subjected to saffron petal supplementation are have no significant difference in average weights of bursa, caecal tonsils and ileum. However, a significantly ($P \leq 0.05$) higher mean spleen weight was recorded in birds of T_4 treatment as compared to control (T_o), with non-significant difference between T_1, T_2, T_3 and T_4 groups.

Significantly ($P \leq 0.05$) lower mean TOS values were recorded in birds of T_2, T_3 and T_4 treatments as compared to control (T_0), with non-significant difference between T_0 and T_1 group. The total antioxidant status (TAS) values in

the treatment groups (T_1, T_2, T_3 and T_4) have significantly ($P \leq 0.01$) higher values as compared to control (T_0) without any significant difference between T_1, T_2 and T_3 treatments. The T-BARS (Breast) values were significantly ($P \leq 0.01$) lower in T_4 treatment as compared to T_0, T_1, T_2 and T_3. There was no significant difference among control (T_0) and T_1, T_2 and T_3treatments. The T-BARS (Thigh) values were significantly ($P \leq 0.01$) higher values in control (T_0) followed by T_1, T_2, T_3 and T_4 treatments respectively.

The results of carcass parameters (%) of the birds of group T_0, T_1, T_2, T_3 and T_4 subjected to saffron petal as feed additive supplementation have depicted that dressing percentage was significantly ($P \leq 0.05$) higher in birds of T_3 and T_4 groups compared to that of T_0 group. The dressing percentage of birds of T_0, T_1 and T_2 groups could not reach to statistically significant difference. The other dressing parameters like feathering loss and bleeding loss of the experimental birds were without any statistically significant difference. The breast, back, drumstick, thigh, wing, neck and total giblet percentage of experimental birds of all treatment groups also could not reach to significant difference statistically.

CONCLUSION

Based on the overall findings and the ensuing discussion in light of the available literature, the followings are the salient conclusions emanated from the current study:

- Based on the performance of broiler birds subjected to the feeding of experimental diets supplemented with saffron petal as feed additive with respect to growth performance, feed intake, nutrient utilization efficiency, immune status, oxidative status and carcass parameters, it appears that use of saffron petal as feed additive in poultry diets is a viable proposition with better results at higher levels (2g/kg of feed).
- The use of saffron petal as such or its extract in broiler ration can be used by poultry farmers to reduce the chick mortality for better economic

returns. Though the findings of the present study are encouraging, a long-term feeding trial on different strains of birds with higher dose level seems to be required to arrive at a recommendation for poultry farmers.

SUGGESTIONS FOR FURTHER WORK

1. Studies on Saffron petal as feed additive should be conducted in different age group of egg laying strains of birds and in various other species of animals.
2. The organoleptic properties of meat and shelf life of finished products from birds fed diets supplemented with Saffron petal as feed additive should be conducted to further validate the use of organic feed additives in poultry feeding.
3. Some other aspects like effect of Saffron petal as feed additive on histomorphology, antibacterial, anticoccidial, anti-inflammatory,hepatoprotective, renoprotective and antitumor activityin various species of animals needs to be studied.

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CERTIFICATE

Certified that all the corrections/ amendments as suggested by the external examiner **Dr. Raman Malik** during viva voce examination held on **27-01-2022** has been incorporated in the manuscript entitled **"Effect of saffron (*Crocus sativus L.*) petals as feed additive on the performance of broiler chicken"** submitted by **Naveed Ahmed Malik (Regd No. MSV-2019-442)**.

(Dr.Gowher Gull Sheikh)

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