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SONAL THAKUR
M.V.Sc

THESIS

DOCTOR OF PHILOSOPHY

(Animal Nutrition)

||पशुधनं नित्यं सर्वलोकोपकारकम्||

2017

Department of Animals Nutrition
College of Veterinary and Animal Science
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THESIS

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the degree of

DOCTOR OF PHILOSOPHY

(Animal Nutrition)

By

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2017

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This is to certify that **Sonal Thakur** had successfully completed the **comprehensive examination** held on **12/01/2017** as required under the regulation for PhD degree.

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This is to certify that this thesis entitled **EFFECT OF SINGLE OR COMBINED DIETARY SUPPLEMENTATION OF *WITHANIA SOMNIFERA* AND SYNBIOTIC MIXTURE (PREBIOTIC AND PROBIOTIC) ON PERFORMANCE AND CARCASS CHARACTERISTICS OF BROILERS** submitted for the degree of **DOCTOR OF PHILOSOPHY** in the subject of **Animal Nutrition** embodies bonafide research work carried out by **Mrs. Sonal Thakur**, under my guidance and supervision and that no part of this thesis has been submitted for any other degree. The assistance and help received during the course of investigation have been fully acknowledged. The draft of the thesis was also approved by the advisory committee on **16/06/2017**.

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Date: Sonal Thakur

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LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

ABBREVIATIONS

%	-	Per cent
℃	-	Degree Celsius
ADG	-	Average daily gain
AOAC	-	Association of Official Analytical Chemists
b.wt.	-	Body weight
CF	-	Crude fibre
CFU	-	Colony forming units
cm	-	Centimetre
CP	-	Crude protein
d	-	Day
DCP	-	Digestible crude protein
DM	-	Dry matter
DMI	-	Dry matter intake
EDTA	-	Ethylene di amine tetra acetic acid
EE	-	Ether extract
FCR	-	Feed conversion ratio
g	-	Gram
h	-	Hour (s)
Hb	-	Haemoglobin
kg	-	Kilogram
max	-	Maximum
mg	-	Milligram (s)
min	-	Minimum
ml	-	Mililitre
N	-	Nitrogen
NFE	-	Nitrogen free extract
OM	-	Organic matter
PCV	-	Packed cell volume
rpm	-	Rotation per minute
TEC	-	Total erythrocyte count
TLC	-	Total leukocyte count
TP	-	Total protein
U	-	Unit (s)

1. INTRODUCTION

Today, the world food production is relying more and more on animal source of protein. The poultry industry has become an important economic activity in many countries for the production of high quality eggs and meat to balance the human diet. The economic and nutritional demand of our modern society for food from poultry has necessitated the raising of poultry under intensive production system. The backyard poultry farming in India is gradually transforming into commercially organized farms and has emerged as the most dynamic and fastest expanding segment of animal husbandry sector. The intensive poultry production systems have led to marked increase in the production of poultry meat throughout the world (Armstrong, 1986).

India has made tremendous progress in broiler production during the last three decades. The Indian poultry industry ranks 5th in broiler production with the total poultry population of 729.21 million in the year 2012 (Anonymous, 2014). The net meat production in India during the year 2012-13 was 5948.17 metric tons out of which the contribution from poultry meat alone was 2681.60 metric ton (2012-13) (Anonymous, 2014). The contribution of poultry meat sector in GDP at current prices was Rs. 42,041 crore in the year 2012-13 that has significantly increased nearly 3.5 times in the last decade and has contributed Rs. 3,424 lakhs in the export basket of the country in the year 2012-13 (Anonymous, 2014). It is projected that during the year 2000-2020, total poultry meat consumption is likely to expand from 687 million kilograms to 1,674 million kilograms (Samarendu and Rajendran, 2003). Poultry sector in India also plays a significant role in improvement of socio-economic status of rural masses through gainful employment and augmentation of family income. Despite this achievement, the per capita availability of poultry meat in India is only 2.96 kg which is far below the ICMR recommendation of 11 kg meat per capita per annum and world average of 10.9 kilograms of poultry meat on per capita basis (Anonymous, 2011).

The efficiency of broilers to convert feed into meat plays a key role in economics of broiler industry. The world poultry sector is facing the dual challenges of sufficiency and safer production. The production of safer poultry products without any chemical and microbial residues in an economic manner is the order of the day. The challenge of improving performance in order to ensure more net returns is more in tropical regions like India where high ambient temperature often results in serious

economic losses due to heat stress and diseases (Pervez, 1992). So it is highly essential to improve feed efficiency in broilers to produce meat economically with due consideration to food safety in an environmental sustainable manner.

The current situation has triggered the discovery and widespread use of a number of 'feed additives'. The term 'feed additive' or 'growth promoter' (Singh and Panda, 1992) is applied to all products other than feedstuffs, which could be added to the ration with the purpose of obtaining some special effects (Feltwell and Fox, 1979). Feed additive boost the performance through increase growth rate, better feed conversion efficiency, greater livability and lowered mortality in poultry birds. An ideal feed additive should be readily biodegradable, free from environmental hazards, non-toxic, involved with transferable drug resistance and improves performance effectively and economically.

Although anti-microbial feed additives such as antibiotics has achieved good performance in terms of growth and feed efficiency in broilers (Izat *et al*., 1990), it has also resulted in common problems such as emergence of drug-resistant bacteria (Sorum and Sunde, 2001) and the problem of antibiotics residues in the broilers meat (Burgat, 1999) that may have deleterious effects on human consumers and imbalance of normal microflora (Andremont, 2000). This public health concern (Donoghue, 2003) has eventually led to the ban of such poultry products especially in the western world (Nweze and Nwankwagu, 2010).

An eco-friendly substitution of antibacterial growth promoters (AGPs) with natural growth promoter in poultry diet has received much attention in the recent years (Humphrey *et al*., 2002; Botsoglou *et al*., 2004) to enhance production and prevent disease conditions. Different natural growth promoters (NGPs) such as herbs, probiotics, prebiotics and synbiotics etc. have been identified as an effective and safe alternative to AGPs to promote the natural, traditional and alternate health system (Makkar *et al*., 2007).

The use of medicinal plants or herbs as feed additives to promote growth and health is gaining popularity worldwide (Anyanwu, 2010; Owen, 2011) due to their suitability and preference, low cost of production, reduced risk of toxicity, minimum health hazards and environment friendliness (Devegowda, 1996). Traditional herbs are generally holistic in therapy and found to be more effective (Alimon, 2009) in combating adverse effects of hot weather on the growth performance of broilers. The basic strategies for using herbs in poultry diets are to influence the metabolism by combating stress and microbial activity and to regulate the hormone imbalances in

poultry. Recently field trials on certain herbs in India, Greece, UK and USA have shown encouraging results with regard to weight gain, feed efficiency, lowered mortality and increased livability in poultry birds (Kumar, 1991; Babu *et al*., 1992; Mishra and Singh, 2000; Deepak *et al*., 2002). Odoemelam *et al.* (2013) reported that herbs are now used in one third of all commercial swine and chicken rations in Europe to accelerate growth and maintain health.

Withania somnifera L. Dunal, commonly called as 'ashwagandha' or 'winter cherry' is one such well known (Singh *et al.*, 2001) and valuable subtropical herb of 60-200 cm height that belongs to Solanaceae family and grows naturally in diverse areas ranging from Africa, Mediterranean East and India. This plant is also cultivated in many dry and hilly areas of India and Pakistan. The herb is considered as 'Indian Ginseng' as it is therapeutically equivalent to Ginseng (Sangwan *et al*., 2004) and has been described as herbal tonic and health food in the famous book of Vedas (Dhuley, 2000).

W. somnifera has long been used as an anti-oxidant, adaptogen, aphrodisiac, liver tonic, anti-inflammatory agent, astringent, antibacterial and antifungal agent (Kaur *et al*., 2003; Manoharan *et al*., 2004; Punetha *et al*., 2010). The extract of this plant is a potent immune-stimulator and anticarcinogenic (Malik *et al*., 2007; Sharma *et al*., 2010). Preparations obtained from this plant have been shown to enhance circulating antibody titer, increase the activity of lysosomal enzymes and phagocytosis (Agarwal *et al*., 1999). Several reports have demonstrated the immunomodulatory (Gautam *et al*., 2004), antioxidant (Kaur *et al.,* 2003), antitumour (Agarwal *et al*., 1999), hepatoprotective (Harikrishnan *et al.*, 2008) and antibacterial (Owais *et al*., 2005) activity of *W. somnifera*. It has also reported that *W. somnifera* significantly increases hemoglobin (Hb) concentration, erythrocyte and white blood cell counts (Manish *et al*., 2004; Senthilnathan *et al*., 2006). Moreover, various parts of the plant have been reported to possess antiserotogenic and anabolic properties and have shown beneficial effects in the treatment of arthritis, stress and geriatric problems (Prakash *et al*., 2001). Several studies also support *W. somnifera* ability to increase circulating cortisol, decrease fatigue, increase physical performance, and decrease refractory depression in animals subjected to stress (Singh *et al*., 2001; Singh *et al*., 2003). Similarly, *W. somnifera* is also believed to tone up the physiological and immunological function of birds affected by stress (Dharma and Tomar, 2007).

Probiotics are also used extensively in poultry production as natural alternatives to antibiotics for growth promotion. These are live microorganisms of

nonpathogenic and nontoxic nature, which when administered through the digestive route, are favorable to the host's health (Guillot, 1998). Probiotic in broiler nutrition have a beneficial effect on broiler performance (Ashayerizadeh *et al*., 2009) through modulation of intestinal microflora and pathogen inhibition (Higgins *et al*., 2007), changes in haematobiochemical parameters (Mathivanan *et al*., 2007) and improvement in sensory characteristics of dressed broiler meat (Pelicano *et al*., 2003). Recently, it was shown that addition of probiotic to broiler diets has increased the ileal and jejunal villus height (Chichowski *et al*., 2007; Samli *et al*., 2007). However, Sanders (2000) reported gastrointestinal problems, flatulence, constipation and even death on probiotics supplementation.

Prebiotics are nondigestible selectively fermented feed additives that allow specific changes in the composition and activity of gastrointestinal microbiota and confer benefits to host well being and health (Gibson *et al*., 2004). Intake of prebiotics can either significantly modulate the colonic microbiota by increasing the number of specific beneficial bacteria such as lactobacilli and bifidobacteria (Rycroft *et al*., 2001) or reduces the undesired intestinal colonization of pathogenic bacteria by mimicking their attachment sites on the intestinal mucosa (Iji and Tivey, 1998). Several studies have shown that administration of prebiotics can improve weight gain, feed intake and feed conversion rate in broilers (Rodrigues *et al*., 2005). However in contrast, some reports indicated that prebiotic supplementation did not affect body weight gain, feed intake or feed conversion ratio (Stanczuk *et al*., 2005).

An advanced approach to maximize the utilization of feed stuff is combined use of probiotics and prebiotics. Synbiotic is the term applied to the mixture of probiotics and prebiotics that beneficially affects the host by activating the metabolism of one or a limited number of health promoting bacteria and stimulating their growth selectively, improving the host's welfare (Gibson and Roberfroid, 1995). Synbiotic products contain viable bacterial cultures that establish easily in the gut while the prebiotic present in the synbiotic serve as a source of nutrient for the probiotics. Synbiotic products impart functional benefits including resistance to gastrointestinal bacterial infection, antibacterial activity and improved immune status in broiler chicks. In addition, synbiotic increases intestinal villi height (Pluske *et al*., 1996) and has comparable potential like antibiotics to improve broiler performance (Mohnl *et al*., 2007).

Therefore keeping in view, the multiple pharmacological properties of *W. somnifera* and growth stimulating effect of synbiotic substance, the proposed study was conducted with the following objectives:

(i) To study the effect of different levels of supplementation of *Withania somnifera* (Ashwagandha) and synbiotic (prebiotic and probiotic) alone or in combination on the growth and performance of broiler chicks.

(ii) To study the effect of different levels of supplementation of *Withania somnifera* (Ashwagandha) and synbiotic (prebiotic and probiotic) alone or in combination on haemato-biochemical parameters in broiler chicks.

(iii) To study the effect of supplementation of *Withania somnifera* (Ashwagandha) and synbiotic (prebiotic and probiotic) alone or in combination on the carcass characteristics.

2. REVIEW OF LITERATURE

2.1 Effect of Climatic Variation on Growth and Performance of Broilers

The performance of broilers not only depends on the inherited capacity but also to a great extent upon the environment (Babinszky *et al*., 2011). Climate variation is one of the major threats to broiler production throughout the world (Alade and Ademola, 2013) and significantly affects the feed intake and health of the broilers during growth phase (Uzokwe and Bakare, 2013). Decrease in body weight, average daily gain and growth rate were reported in heat stressed broilers (Sohail *et al*., 2010). Short term climatic variations affect the quality and quantity of meat production in broilers (Gregory, 2010). In addition, reduced protein deposition and greater fat deposition has been reported in broilers reared under heat stress conditions (Lu *et al*., 2007). Significant mortality and state of negative energy balance was reported due to heat stress in broilers (Simmons *et al*., 1997). Although acute heat stress was shown to decrease insulin concentrations in broilers (Tang *et al*., 2013), an increase insulin level was reported during chronic heat stress in broilers (Yuan *et al*., 2008). Thus hyperglycemia and hypoglycemia were observed in birds during acute and chronic heat stress, respectively (Lin *et al*., 2000).

Thermal comfort indices such as temperature-humidity index (THI) have been developed to assess the impact of the thermal environment on thermoregulatory status. Significant impact of THI was found on the production responses of broilers and was considered as a predictor of production efficiency in broilers (Chepete *et al*., 2005). Regression analysis showed that generally, quadratic inverse relationships exist between THI and live performance parameters, specifically body weight, body weight gain and feed intake. Increased tyrosine value and malonaldihyde (MDA) level indicative of proteolysis and lipolysis, respectively have been reported in meat of broilers reared under environmental heat stress (Mujahed *et al*., 2007).

The total serum protein and albumin content of the birds exposed to heat stress were reported to decrease due to enhanced protein catabolism (Hayashi *et al.,* 1994) caused by reduced feed intake and gluconeogenesis effect of stress hormone, cortisol. An increase in level of circulating stress hormone was found to exert catabolic effect through increase in free radicals by altering oxidative metabolism and impairment of cellular functions leading to muscle wasting and retarded growth

(Sujatha *et al*., 2010). Studies have reported that serum albumin and glucose levels could be considered as reliable indicators of stress in broilers (Yalcin *et al*., 2004). Numerically higher levels of total protein, albumin and globulin were observed in birds treated with antioxidant drug during heat stress conditions (Jadhav *et al*., 2014).

The antioxidant effect of ashwagandha and the nutrient sparing role of synbiotics though not explored in conjunction with the environmental heat stress in broilers however a number of trial demonstrated significant protection of body condition through supplementation of ashwagandha (Vasanthakumar *et al*., 2014) and synbiotics substances.

2.2 Morphological Description and Distribution of *W. somnifera*

W. somnifera (L.) Dunal commonly known as “ashwagandha”, “asgandh” and “winter cherry” is an erect, grayish, stellate-tomentose under shrub (30-75 cm high) with long tuberous roots, alternate leaves, small greenish flowers and orange berry shaped fruits (Hepper, 1991) that belongs to Solanaceae family (Bano *et al*., 2015).

The herb *W. somnifera* can be seen as a wild plant in the North-Western regions of India extending from the mountainous region of Punjab, Himachal Pradesh and Jammu to an altitude of 1,500 m (Singh and Kumar, 1998). Today, this economically and medicinally significant herb is being widely cultivated (more than 4,000 ha) in drier parts of India such as Manasa, Neemuch and Jawad tehsils of the Mandsaur District (Madhya Pradesh); Punjab; and Chittorgarh district of Rajasthan (Anonymous, 1976; Sharma, 2004; Panwar and Tarafdar, 2006).

2.3 Chemical Constituents of *W. somnifera*

The chemistry of *W. somnifera* has been extensively studied and over 39 active ingredients have been identified, extracted, and isolated by different workers (Rahman *et al*., 1991; Rahman *et al*., 1993; Choudary *et al*., 1996; Kapoor, 2001; Bandyopadhyay *et al*., 2007). At present, more than 12 alkaloids, 40 withanolides, and several sitoindosides have been identified. The withanolides are a group of naturally occurring steroidal lactones that imparts distinctive earthy odour and flavour to ashwagandha (Kazutoshi *et al*., 1999). Withaferin A was the first member of this group isolated from this well-known South-Asian medicinal plant (Tursunova *et al*., 1977; Glotter, 1991). The R_f value of major Withaferin, Withanolides D and Withanolides A was reported to be 0.32, 0.50 and 0.86, respectively (Verma and Gaur, 2011).

The total alkaloid content in the roots of *W. somnifera was* reported to vary between 0.13 and 0.31% though much higher yields (up to 4.3%) have been recorded (Mirjalili *et al*., 2009). The *W. somnifera* root contains low level of soluble protein (5.6 %) (Verma and Gaur, 2011).

2.4 Pharmacological Properties of *W. somnifera*

The *W. somnifera* popularly known as 'Rasayana' in Ayurveda is widely used in various ayurvedic preparations to improve strength and stamina (Dhuley, 2000). The plant was traditionally used to promote youthful vigor, endurance, strength, health; to enhance the production of vital fluids, muscle, blood, lymph, semen and to increase the capability of individual to resist environmental stress (Sharma and Dandiya, 1992). The similarity between these restorative properties and those of ginseng roots has led to ashwagandha roots being called "Indian Ginseng". It is also used as a general energy-enhancing tonic known as *Medharasayana* to promote learning and to enhance memory (Williamson, 2002). Ashwagandha is one of the main ingredients in 74 Ayurvedic, 9 Siddha, 3 Unani and 126 herbal formulations (Singh and Kumar, 1998). Roots have been regarded as a useful internal medicine in rheumatism and dyspepsia and found to be fully diuretic (Warming, 1868). In view of its varied therapeutic potential, it has also been the subject of considerable modern scientific attention. *W. somnifera* appeared in "WHO monographs on Selected Medicinal Plants" (Marderosion, 2001). A number of pharmacological studies have reported immunomodulatory, cardioprotective, neuroprotective, anti-ageing and anti-oxidant properties of *W. somnifera* (Devi *et al*., 1992).

2.4.1 Anti-oxidant property

A number of studies indicated that ashwagandha could be used as natural source of safe anti-oxidative agent (Sumathi *et al*., 2007). *W. somnifera* acts as a powerful antioxidant and increases the levels of three naturally occurring antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase (Bhattacharya *et al*., 1997; Dhuley, 2000). Similarly, oral administration of *W. somnifera* extract prevented an increase in lipid peroxidation in mice and rabbits (Dhuley, 1998). The antioxidant activity of *W. somnifera* has been demonstrated in mice (Patil *et al*., 2012) and was suggested to be imparted by withanolides, glycowithanolides and sitoindosides VII-X (Bone, 1996). A significant improvement in hemoglobin, red blood cell count, hair melanin and decreased serum cholesterol was observed in *Withania* treated individuals (Singh *et al*., 2010). *Withania* root powder

prevents cadmium- induced oxidative stress in chickens and lead induced oxidative damage in mouse (Chaurasia *et al*., 2000; Bharvi *et al*., 2010). An anti nephrocytotoxic effect was demonstrated in mice when administrated with *W. somnifera* (500mg/kg b.wt) (Jeyanthi and Subramanian, 2009).

2.4.2 Antistress / adaptogenic activity

Ashwagandha was observed as anti-depressant and antianxiety in nature when compared to drugs imipramine and lorazepam (Archana and Namasivayam, 1999). Traditionally, the herb is used to stabilize the mood of patients having behavioral disturbances (Bhattacharya *et al*., 1987; Singh *et al*., 2010) through decrease in neuronal activity. The root extracts of ashwagandha were known to produce GABA-like activity which could be responsible for its anti-anxiety effects (Mehta *et al*., 1991). Dose dependent anti-stress activity was demonstrated in mice treated with *W. somnifera* (Khare, 2007). The steroids founds in the roots of ashwagandha acts like exogenous adrenocortical steroids and lowers the ACTH secretion and consequently, endogenous steroid production. Thus, *W. somnifera* was observed as growth promoter especially during the active growth period (Mishra *et al*., 2000).

The extract of *W. somnifera* showed greater dose-dependent response in parameters like serum cortisol, creatinine, protein, pulse rate, blood pressure, hemoglobin and significantly greater responses in mean fasting blood glucose, serum lipid (Auddy *et al*., 2008). It has been demonstrated that methanolic extracts of ashwagandha reduced ulcer index, volume of gastric secretion, free acidity, and total acidity (Bhatnagar *et al*., 2005). In a rat model, withanolides were able to decrease the number and severity of chronic stress-induced ulcers and immunosuppression; and also increased peritoneal macrophage activity (Bhattacharya and Muruganandan, 2003). Both sitoindosides IX with glycowithanolides exhibits significant antistress activity and causes significant mobilization and activation of peritoneal macrophages, phagocytosis and increase activity of the lysosomal enzymes (Ghosal *et al*., 1989).

2.4.3 Immunomodulatory property

W. somnifera is an excellent immune regulator (Kuttan, 1996; Davis and Kuttan, 1999) and significantly enhances the humoral (12%) and cell mediated (19.27%) immune response (Verma *et al*., 2012) through increase in neutrophils

counts, gamma interferon (IFN-γ), interleukin (IL-2) and granulocyte macrophages colony stimulating factor (GM-CSF) factors (Grover *et al*., 2010). The withaferin A and withanolide D present in the root extract of *W. somnifera* increases the microbes killing power of immune cells by enhancing nitric oxide synthatase activity of the macrophages (Kuttan, 1996).

2.4.4 Anti-inflammatory activity

W. somnifera is a naturally occurring anti-inflammatory steroids and is as effective as hydrocortisone sodium, an anti-inflammatory drug (Khare, 2007). The extracts of *W. somnifera* have shown anti-inflammatory effects in a variety of rheumatological conditions (Anbalagan and Sadique, 1981). The extracts also caused a significant reduction in both paw swelling and bony degenerative changes in Freund's adjuvant induced arthritis in rats (Begum and Sadique, 1988). Withaferin A was found to suppress the arthritic syndrome effectively without any toxic effect. In a study (Narinderpal *et al*., 2013) on arthritic animals, individuals treated with hydrocortisone showed weight loss while the animal treated with withaferin A showed gain in weight.

2.4.5 Antibacterial activity

Withanolides possess antibacterial and antifungal activity (Singh and Kumar, 1998). Significant *in vitro* antibacterial activity of *W. somnifera* root extract against *Enterobacter aerogens*, *Bacillus subtilis*, *Klebsiella pneumoniae, Raoultella planticola* and *Agrobacterium tumefaciens* was demonstrated by Singh and Kumar (2012). The minimum inhibitory concentration (MIC) of *W. somnifera* was found to be 0.039 mg/ml against *E. aerogens*, *K. pneumoniae* and *A. tumefaciens*. Root extracts of *W. somnifera* showed maximum antifungal activity against *Fusarium solani* fungi (Ramteke *et al*., 2003). The root powder of *W. somnifera* is traditionally used in the treatment of pulmonary tuberculosis and bubonic plague in Garhwal Himalaya region (Maithani, 1973).

2.4.6 Cardioprotective activity

The alkaloids present in the *W. somnifera* possess prolonged hypotensive, bradycardic and respiratory-stimulant effect that is mainly due to its autonomic ganglion blocking action and depressant effect on higher cerebral centers (Malhotra *et al*., 1981). It was observed that ashwagandha restore the myocardial antioxidant

status and maintain membrane integrity by reducing the malonyldialdehyde levels (Mohanty *et al*., 2004) in isoprenaline induced myocardial necrosis in mice.

2.4.7 Neuroprotective and memory enhancing effect

The glycowithanolides, withaferin A (VII-X) present in the roots of ashwagandha significantly promoted the growth of nerve cell dendrites along with GABA mimetic effect during healing of brain tissue and reverted the neurotic atrophy, synaptic loss leading to dementia (Tohda *et al*., 2000). The ashwagandha root extract could increase cortical muscarinic acetylcholine receptor capacity which leads to cognition-enhancing and memory improving effect in animals and humans (Schliebs *et al*., 1997). Ashawagandha has been used traditionally as a tonic and nootropic agent. It has also been associated with improvements in scopolamine-induced memory deficits in mice (Dhuley, 2000). Methanolic extracts of this plant have been reported to induce neurite extension and the dentritic atrophy was found to be completely prevented by treatment with withanolides (Tohda *et al*., 2000).

2.4.8 Anti-cancer activity

The withanolide, withaferin A present in the roots of ashwagandha could exert great anti-tumorigenic, anti-cancerous and antiproliferative activity against various cancer cell lines (Mayola *et al*., 2011). The anti-carcinogenic effects of ashwagandha in animal and cell cultures was reported to be due to depression in expression of nuclear factor-kappa B and suppression of intercellular tumor necrosis factor; potentiation of radiation-induced apoptosis in cancerous cell lines (Ichikawa *et al*., 2006; Sindhu and Santhi, 2009; Yang *et al*., 2011). The alcoholic extract of dried root powder of *W. somnifera* was found to possess anti-tumorus and radio sensitizing activity in Chinese hamster cells and Swiss mice inoculated with Ehrlich ascites carcinoma cells (Devi *et al*., 1995; Sharada *et al*., 1996). The administration of *W. somnifera* extract was found to significantly reduce leucopenia induced by clophosmide in experimental animals (Davis and Kuttan, 1998).

2.4.9 Anti-diabetic effect

W. somnifera could be a potential source of hypoglycemic, diuretic and hypocholesterolemic drugs (Andallu and Radhika, 2000). The root extracts of *W. somnifera* was found to produce hypoglycemic and hypolipidemic effects in alloxan induced diabetic rats (Udayakumar *et al*., 2009; Sarangi *et al*., 2013). Anti-diabetic

activity may be due to increase in hepatic metabolism, increased insulin release from pancreatic β-cells or insulin sparing effect (Navinder *et al*., 2013).

2.5 Effect of *W. somnifera* on Performance of Broilers

2.5.1 Effect on feed intake

The increase in body weight invariably reflects the correlated increase in feed intake. The positive effect of ashwagandha supplementation on feed intake has been attributed to its effect on digestibility of feed. A significantly highest feed intake of 3231.27±0.44g was reported in broilers chicks when supplemented with 20 g extract of *W. somnifera* /L of water as compared to control broilers (2864.91±0.89g) (Mushtaq *et al*., 2011). The effect of *Withania* on feed consumption was found to be significantly higher during the last three weeks (4^{th} to 6^{th} week) of trial in broilers (Rindhe *et al*., 2012). Sanjyal and Sapkota (2011) reported average weekly feed consumption of 222 g, 432 g, 716 g, 764 g and 798 g, respectively from 2^{nd} to 6^{th} week on *Withania* containing diet with highest digestibility ($p<0.05$) was observed in amala+tulsi+aswagandha supplemented group. The feed intake was found to be 7.9% higher in *Withania* supplemented group than the control group (Ansari *et al*., 2008). Vasanthakumar *et al*. (2014) observed significantly higher feed intake (4580.64 g) in broilers maintained on 1% *W. somnifera* root powder based diet than nonsupplemented group (3954.22 g). Shisodiya *et al*. (2008) however observed lower feed intake in 0.5% *Withania* based diet than control broilers but with better performance.

The effect of *Withania* feeding on digestibility of the feed was also reported by Pandey *et al*. (2013) who observed significantly higher body weight in broilers with concurrent significantly low feed intake (3720.85 g/bird) on *Withania* based diet as compared to control (3916 g/bird). The average weekly feed consumption of broiler (kg/bird) from 1 to 6 week of age as a result dietary inclusion of *Withania* based indigenous herbal drug revealed significant ($p<0.05$) difference in weekly feed consumption of broiler and was reported to be 0.230, 0.370, 0.530, 0.760, 0.770, 0.960 and 0.210, 0.360, 0.510, 0.740, 0.750, 0.930 kg in control and treatment groups, respectively (Srivastava *et al*., 2012). However, it was also observed that the level of *Withania* supplementation in basal diet did not produce significant difference in overall feed intake in broilers when *W. somnifera* root powder was added either @ 1g or 2g/kg of feed (Joshi *et al*., 2015).

The feed intake of Japanese quails was also increased significantly on 1% *Withania* root powder containing basal diet (3536.35g) than control chicks (3154.18g) (Bhardwaj *et al*., 2012). Similar observations in Japanese quails were recorded by Ahmed *et al*. (2014) who revealed significant ($p \leq 0.01$) increase in feed consumption with 1g *Withania* root powder supplementation.

2.5.2 Effect on body weight

The health restorative activity and general tonic property of *W. somnifera* was described by Bhattacharya and Ghosal (1994). The results from different trials on *W. somnifera* suggested the anabolic effect and enhanced synthesis of liver proteins to increase the body weight in human and animals (Anabalagan and Sedique, 1981). Shisodiya *et al*. (2008) observed significant improvement in growth parameter such as live body weights and weekly gain in body weights with supplementation of 0.5% *W. somnifera* root powder in broiler chicks. The beneficial effect of ashwagandha recorded in the study was found to be in agreement with Arunkumar *et al*. (2000).

Ansari *et al*. (2008) tested the comparative efficacy of six medicinal plants (*Nigella sativa, Boerhavia diffusa, W. somnifera, Ipomea digitata, Azadirachta indica and Corylus avellena*) on the growth performance of 210-day old broiler chickens and observed maximum weight gain in *W. somnifera* (1819g) treated group followed by *Nigella sativa* (1805g) and *Azadirachta indica* (1800g) when herbs were added at the rate of 4g/kg of feed. The birds offered *W. somnifera* ranked first with regard to weight gain. It was concluded that medicinal plants especially *W. somnifera* could be used as growth promoters in the poultry diets for better production performance. An indigenous herbal drug formulation containing *W. somnifera, Asparagus racemosus* and *Mucuna pruriens* was tested for growth effect on body weight of Vencobb 400 broilers by Srivastava *et al*. (2012). The drug at the rate of 2% of feed imparted significant effect on body weight gain during summer season. The weekly average body weight gain (g) in treated and control groups were observed to be 100/90 (1), 290/240 (2), 340/320 (3), 540/520 (4), 450/420 (5), 450/430 (6) during different weeks, respectively. The synergistic effect of three different herbs (ashwagandha, shatavari and kapikachhu) on production performance of broilers was tried by Pandey *et al*. (2013). Ashwagandha, shatavari (*Asparagus racemosus*) and kapikachhu (*Mucuna pruriens*) powder were mixed in the ratio of 2:1:1 and added at the rate of 2% in the ration of the VenCobb-400 broiler chicks. The chicks in the herbal treated groups attained significantly ($p<0.05$) higher body weight of 2126.38±0.92 g than the control chicks. The better performance of herbs treated

group was attributed to the immunomodulatory, antioxidant and antistressor effects of *W. somnifera* (Mishra *et al*., 2000; Akotkar *et al*., 2007; Rekhate *et al*., 2010; Ram Niwas *et al*., 2011).

The feasibility of replacement of antibiotic growth promoter with herbal growth promoter was proved by Sanjyal and Sapkota (2011) in a study conducted on 192 VenCobb broiler chicks with antibiotic (chlortetracycline), probiotic (*Lactobacillus acidophilus*) and three herbal growth promoters like amla (*Emblica officinalis),* tulsi (*Ocimum sanctum)* and aswagandha (*W. somnifera)*. The maximum live weight (290g) was observed in *Withania* supplemented group during second week of trial which was found to be significantly higher than control and rest of the treatments. In addition, *Withania* supplemented group also recorded maximum weight gain during third week (194g) and fifth week of trial (412g). Rindhe *et al*. (2012) compared the efficacy of *W. somnifera* containing herbal formulation with synthetic ascorbic acid in a 42 day trial on VenCobb broilers. The mean live body weight (g) of *Withania* supplemented broiler on 42nd day was significantly ($p<0.01$) higher (2281.67±4.05) than ascorbic acid supplemented group (2173.33±4.31g) and control group (2000.00±8.35g). Kumari *et al*. (2015) in a trial found less reduction in body weight (1800±130.38 g) in 0.5% *Withania* supplemented *Salmonella* infected broilers than non supplemented *Salmonella* infected broilers (1600±70.71 g) with significantly higher body weight of 1980±66.33 g was observed in non-infected *Withania* supplemented broilers than control non-infected group.

The root extract of *W. somnifera* also exerts significant effect on body weight. Significantly higher body weight of 1736.59±0.44g was reported in broiler with 20 g extract of *W. somnifera* as compared to chicks in control group 1452.13±0.89 g (Mushtaq *et al*., 2011). Similar effect on the body weight of broiler chicks on administration of 20 g of *W. somnifera* extract was observed by Sajjad (2005) and Kakar (2006). The 0.15 % root extract of ashwagandha was found to be significantly ($p<0.05$) superior (2297.11±49.8) in increasing the body weight of broilers as compared to the control (1947.83±41.39) and 0.5% ashwagandha root powder (2214.78±57.41) fed groups (Vasanthakumar *et al*., 2014). These observations corroborated the findings of Singh *et al*. (2010) who also reported increased body weight in ashwagandha fed groups.

A dose dependent positive effect of *W. somnifera* on body weight and body weight gain in broilers was reported in a number of studies. The dose related effect of *Withania* during the different weeks of trial was indicated in a study (Ahmed *et al*.,

2015a) which observed that the body weight of Ross broiler chickens in the last two weeks of experimental trials (4 and 5 weeks) was affected more significantly ($p \leq 0.05$) by adding *W. somnifera* to basal diet compared to control group. During the period from 3-4 weeks of age, broiler chickens received 0.75 g *W. somnifera* showed significantly ($p \leq 0.05$) higher body weight gain as compared to control and other treated groups whereas the final body weight and weight gain at the last interval (4-5 weeks) was significantly ($p \leq 0.05$) more in 1.5 g *Withania* supplemented group. Thus improvement in body weight with age was related to the effect of *W. somnifera* in stimulating the thyroid gland directly and/or through the pituitary gland to secret more thyroid hormones. Similarly, Joshi *et al*. (2015) proved the anabolic effect of *W. somnifera* with two different levels (T_2:1 g/kg feed and T_3: 2 g/kg feed) and observed significant ($p<0.05$) effect on overall body weight of broilers. The chicks maintained on 2 g *Withania*/kg of feed revealed final body weight of 2199.30±40.20 g in comparison to 2138.86±34.5 g (T_2) and 2076.26±22.27 g (T_1:control). The average weekly body weight gains were found to be higher in *W. somnifera* supplemented groups than control at week first, third and for overall experimental period. The total weight gain (g) was reported to be statistically highest (2152.98±40.27) in group received 2 g *Withania*/ kg feed. However, in contrast, Thange *et al*. (2009) did not observed any effect of various doses of dietary addition of *W. somnifera* on body weights in broilers.

The supplementation of ashwagandha not only improved the body weight in thermo comfort zone but was also found to improve the body weight in extreme climate conditions. A polyherbal feed premix containing *W. somnifera* root powder significantly improved the body weight (1746.02±42.53g) of broiler after six weeks of trial in the summer months of June-July when the mean temperature-humidity index (84.74±2.51) was above the thermo comfort zone of broilers (Sujatha *et al*., 2010).

Japanese quails also exhibited improvement in performance with the supplementation of ashwagandha. Supplementation of 1% ashwagandha root powder significantly ($p<0.05$) improved the body weights of Japanese quails chicks (Bhardwaj *et al*., 2012). Similarly Ahmed *et al*. (2014) observed significant ($p \leq 0.05$) increase in body weight gain of quails supplemented with 100 mg/kg ethanolic extract of ashwagandha as compared with control group.

2.5.3 Effect on feed conversion ratio (FCR)

The FCR (amount of feed intake/unit live weight gain) ultimately decides the economics of broiler industry. Significantly low FCR was recorded by Shisodiya *et al*. (2008) in broiler chicks when basal diet was supplemented with 0.5% *Withania* root powder. A comparison of *Withania* with five different herbs (*Nigella sativa, Boerhavia diffusa, Ipomea digitata, Azadirachta indica and Corylus avellena*) in broilers also revealed significantly better FCR during most weeks in *Withania* included diet (Ansari *et al*., 2008). Rindhe *et al*. (2012) observed lowest FCR (2.05) in ashwagandha supplemented group in comparison to control and ascorbic acid supplemented broilers. The results obtained by Sanjyal and Sapkota (2011) in broilers revealed improved FCR during most weeks in a comparative study on *Withania* root powder with antibiotic and two other herbs, amla and tulsi.

Weekly FCR observed in broilers from one to sixth week of age was 2.58, 1.57, 1.67, 1.44, 1.83, 2.26 and 1.98, 1.23, 1.49, 1.37, 1.67, 2.09 kg in control and *Withania* treated groups, respectively revealed significantly better FCR in treated broilers during all the weeks (Srivastava *et al*., 2012). The overall FCR (1.74) during all the weeks was reported to be statistically very low in broilers raised on 2% herbal formulation containing 50% *Withania* powder than control broilers (2.07) by Pandey *et al*. (2013). Numerically better feed conversion efficiency was reported by Vasanthakumar *et al*. (2014) in broilers raised on 0.15% ashwagandha root extract. The graded level of *Withania* supplementation, *viz.,* 1 g/kg of feed and 2 g/kg of feed, however did not reveal any significant ($p>0.05$) difference in feed conversion ratio in broilers (Joshi *et al*., 2015). Similar nonsignificant difference in feed conversion ratio was recorded by Thange *et al*. (2009).

The improvement in feed efficiency was also observed in Japanese quails by Bhardwaj *et al*. (2012) with the supplementation of *W. somnifera*. The poorest feed efficiency was seen in control group (2.53±0.023) with improved results 2.14±0.024; 1.95 ±0.040 and 1.97±0.021 were obtained with 0.5%, 1.0% and 1.5% *Withania* root powder, respectively. The results indicated improved feed efficiency in accordance with earlier reports (Bhardwaj and Gangwar, 2011). Similarly best ($p\leq0.05$) feed conversion ratio was obtained when the quails were supplemented with ashwagandha root ethanolic extract (100 mg and 200 mg /kg feed) or with 2g/kg diet of root powder in comparison with control (Ahmed *et al*., 2014).

2.5.4 Resilient effect of ashwagandha on broilers

Considerable negative effects on livability, production, immunity, and disease susceptibility in poultry was observed following exposure to acute and chronic heat stress (Tirawattanawanich *et al*., 2011). Stress induced in response to the tropical environmental conditions might be a crucial factor contributing to the inferiority of the acquired immunity in high-meat-yielding broilers lines. Heat stress induced reduction in cell-mediated and humoral immunity in chickens could be explored through assessment of phagocytic activities and serum antibody titers (Niu *et al*., 2009).

Significant improvement ($p<0.05$) in recovery from *Salmonella gallinarum* challenged infection was observed in 28 day post infected broiler through supplementation of ashwagandha which was suggestive of adaptogenic and antistress activity of *W. somnifera* root powder (Singh *et al.,* 2003). The supplementation of antioxidant substances in Cobb male broilers resulted in linear increase in serum T3 and T4 concentrations under heat stress (Sahin *et al*., 2002).

The supplementation of herbal formulation @ 0.01% in basal diet containing *W. somnifera as* one of the main ingredient during heat stress condition of 84.74±2.51 THI significantly enhanced serum total protein and serum globulin in broilers with non significant variation in albumin content than control groups (Sujatha *et al*., 2010).

2.5.5 Effect on mortality

Ashwagandha offers significant protective effects to broilers in terms of reduction in mortality due to disease related stress and exerts considerable level of early recovery from infection. A ten times less mortality (1.42%) as compared to control (14.28%) was observed by Pandey *et al*. (2013) in broilers. Kumari *et al*. (2015) in a study conducted on broiler chickens observed considerable decrease (50%) in mortality when the broilers were raised on 0.5% *W. somnifera* root powder. The antistress and adaptogenic activity of ashwagandha reduced the severity and helped in the early recovery of the broilers from *Salmonella gallinarum* infection in experimental Salmonellosis. Similarly, the total mortality rate in broilers were reported to be 4.4, 2.2 and 2.2% in control, 0.1% and 0.2% ashwagandha supplemented broiler groups, respectively (Joshi *et al*., 2015). Similar findings (Owais *et al*., 2005; Biswas *et al.*, 2012; Srivastava *et al.*, 2012) were observed in mice when treated with *W. somnifera* during experimental salmonellosis. Thus the different studies indicated

that supplementation of *W. somnifera* might have attributed to good health in the supplemented groups.

2.6 Effect of *W. somnifera* on Haemato-serobiochemical Parameters in Broilers

2.6.1 Effect on haematological parameters

The haematinic effect of *W. somnifera* on broilers was explored by Kumari *et al*. (2015) who reported significantly higher Hb concentration, PCV, TEC values and non significant MCV and MCHC values between control and *Withania* supplemented broiler groups. The haematinic effect of *W. somnifera* root powder could be attributed to its direct and indirect action on the haematological parameters. A direct positive influence of *W. somnifera* was observed on haemopoiesis in broiler chicks through stimulation of stem cell proliferation and increase in bone marrow cellularity (Aphale *et al.,* 1998; Mishra *et al.,* 2000). In addition, *W. somnifera* root powder was found to exert significant haemoprotective effect on RBC from oxidative stress in broilers through its antioxidant activity and improvement in erythrocytic enzyme activity (Sujatha *et al.,* 2010). Daisy (2006) in broilers and Bhardwaj *et al.* (2012) in Japanese quails reported significant increase in the total erythrocytic count.

Less intense anaemia was observed in *Salmonella* challenged broilers group raised on ashwagandha root powder which induced faster recovery of chicks from the disease (Kumari *et al*., 2015). Nonsignificant variation in Hb concentrations was reported in broilers treated with the extract of *Withania* root powder (10, 20 and 30 g/L) (Mushtaq *et al*., 2011) whereas, Bhardwaj *et al.* (2012) found significant increase in Hb concentration in Japanese quails.

The PCV value of broilers in groups treated with *Withania* extract @ 10 and 20 g/L (29.16±0.00 and 26.50±0.04) were significantly higher than the values shown by their counterpart chicks in control group (23.00±0.09) (Mushtaq *et al*., 2011). Similar observations for PCV were reported in Japanese quails by Bhardwaj *et al.* (2012) who observed significant and linear increase in PCV on addition of increasing level of ashwagandha root powder (@ 0.5, 1.0 and 1.5%) as compared to non-treated group.

Significant increase in number of phagocytic cells (Davis and Kuttan, 2000; Malik *et al*., 2007) along with increase in phagocytic potential is well documented in avian species (Mishra *et al*., 2000) with the treatment of *W. somnifera.* The results

obtained by Manish *et al*. (2004) reported significant increase in white blood cell count of broilers. A significantly higher value of mean TLC in the chicks treated with 20 g/L *Withania* root extract was observed however nonsignificant difference was observed in the values of neutrophils, eosinophils, monocytes and lymphocytes in *Withania* treated groups as compared to the values shown by chicks in control group (Mushtaq *et al*., 2011). The percentage of lymphocytes in broilers supplemented with 1.5% level of ashwagandha was significantly raised up to 53.59% with absence of change in heterophil and monocyte values (Bhardwaj *et al*., 2012).

2.6.2 Effect on serobiochemical parameters

2.6.2.1 Blood glucose

The significant hypoglycemic effect (12%) of *W. somnifera* root powder observed in human subject was inconsistently verified in broilers (Andallu *et al*., 2000). An indigenous herbal preparation containing *W. somnifera* root powder supplemented @ 2% in basal feed nonsignificantly affected the serum glucose level at the end of sixth week of age in broilers (Srivastava *et al*., 2012). Similar nonsignificant role of ashwagandha on serum glucose was reported in guinea pigs (El-Boshy *et al*., 2013). The broilers raised on ashwagandha leaves also exhibited nonsignificant alteration in blood glucose level (Ahmed *et al*., 2015a).

In contrast, low plasma glucose concentration ($p \leq 0.05$) of 182.18 mg/dl on supplementation of *Withania* containing herb (@ 0.01% of feed) was observed in treated groups in comparison to control broilers, *i.e.,* 249.52 mg/dl respectively (Sujatha *et al*., 2010). The hypoglycemic effect of ashwagandha in broilers was observed mainly during the stress period (Varma *et al*., 2011).

2.6.2.2 Serum proteins

The increase in serum protein concentration could be the direct anabolic effect of ashwagandha or indirectly through increase in thyroid hormone concentration (Panda and Kar, 1997). *W. somnifera* root extract was found to effectively reverse enhanced proteolysis and lowered protein level during experimental hyperglycemia and enhanced the serum albumin as well as total protein that never deviated from the normal range throughout the trial period (Udayakumar *et al*., 2009). The serum protein regulatory effect of ashwagandha was verified by Varma *et al*. (2011) in pesticides intoxicated cockerels. 20 mg of *Withania* root extract/bird/day was found to significantly increase serum proteins concentration in

cockerels. The 0.5% ashwagandha root powder revealed significant resisting effect on reduction of serum protein and albumin concentrations in *Salmonella* infected broilers and significantly increased serum globulin level (Kumari *et al*., 2015). The leaves of ashwagandha plants, however could not contributed this protein modulating role (Ahmed *et al*., 2015a).

The significant increase in serum total protein and globulin level and numerical increase in albumin level of broilers was reported on supplementation of *W. somnifera* root powder by Dhenge *et al.* (2009). The root extract (20 mg/day/bird for 30 days) of *W. somnifera* significantly enhanced the serum total protein level to 24.42 g/100ml from 15.7 g/100ml in control cockerels (Panda and Kar, 1997). The anabolic effect of ashwagandha was observed more effective during stress period in broilers. Significant recovery was observed in enrofloxacin induced hypoproteinaemia in broilers on supplementation of ashwagandha (Arivuchelvan *et al*., 2013). Kumari *et al*. (2015) reported reduction in severity of depression in serum total protein and albumin in *S. gallinarum* infected broilers on supplementation of ashwagandha. *Withania* treated broiler group reflected higher plasma protein and total globulin concentrations (g/dl) ($p \leq 0.05$) compared to control with observed values of 3.63, 1.68 and 3.87, 1.76 in control and 4.42, 2.62 and 4.10, 2.21 in *Withania* treated group at 3rd and 5th week of age, respectively (Sujatha *et al*., 2010).

An indigenous herbal drug containing *W. somnifera*, *Asparagus racemosus* and *Mucuna pruriens* supplemented @ 2% of feed in broilers caused nonsignificant difference in serum total protein values between control and supplemented broilers with values of 6.88 and 6.81 g/dl, respectively (Srivastava *et al*., 2012).

2.6.2.3 Serum lipids

Scant information in broilers suggested that the total plasma cholesterol concentration in ashwagandha (0.01% of feed) treated broiler i.e. 86.33 and 90.21 mg/dl was significantly ($p \leq 0.01$) lower compared to untreated control 124.67 and 131.96 mg/dl, after 3rd and 5th week of age, respectively (Sujatha *et al*., 2010). 2% *W. somnifera* root powder supplementation in layer showed 30% reduction in the egg cholesterol levels and 26% reduction in egg yolk triglycerides (Qureshi *et al*., 2011). Studies in rat and human proved the hypocholesterolemic and hypolipidemic effect of ashwagandha root powder (Andallu *et al*., 2000; Udayakumar *et al.,* 2009).

2.6.2.4 Serum enzymes and minerals

The supplementation of 0.5% ashwagandha root powder was found to significantly reduced ($p<0.05$) the level of major negative hepatic health indicator enzymes, serum ALT and AST in broilers challenged with *Salmonella gallinarum* with LDH activity remained significantly higher till the end of trial, *i.e.,* 35 days and remarkably low decline in ALP was reported (Kumari *et al*., 2015). The hepatoprotective and cardioprotective effect of ashwagandha could be due to presence of alkaloids and withanolides and free radical scavenging properties of ashwagandha (Harikrishnan *et al*., 2008). The observation recorded in *E. coli* infected guinea pigs also revealed the similar decrease in ALT and AST levels (El-Boshy *et al*., 2013). Feeding of ashwagandha in pesticides intoxicated cockerels significantly ameliorated the toxic effect of pesticides in terms of reduction in ALT and AST with significant appreciation in activity of ALP related to growth (Varma *et al*., 2011). Additionally, the ALT and AST lowering effect of roots of *W. somnifera* was not observed in leaves of ashwagandha in broilers (Ahmed *et al*., 2015a).

In contrast, a study on indigenous herbal formulation containing *W. somnifera* revealed non-significant effect of ashwagandha on serum ALT and AST in broiler chicken fed @ 2% per kg of feed (Srivastava *et al.,* 2012). The calcium sparing effect of ashwagandha was reported by Varma *et al*. (2011).

2.7 Effect of *W. somnifera* on Immunological Parameters in Broilers

The findings of Manoharan *et al*. (2004) indicated an increase trend of antibody titer using the extract of *W. somnifera* in different avian models. The 10, 20 and 30 g/L concentration of *W. somnifera* extract was significantly effective in enhancement of antibody titre against IBD (Mushtaq *et al*., 2011). The immunoglobulin levels was significantly more (2.81 mg/dl) in 1.5% ashwagandha fed Japanese quail than other groups (Bhardwaj *et al*., 2012). The immune status of the broilers as assessed by RD titre values (log_2) was found to be better in 1% ashwagandha root powder (7.3) and 0.15% ashwagandha extract (7.0) treated groups as compared to control group (6.6) (Vasanthakumar *et al*., 2014). Similarly, 1% ashwagandha root powder fed broilers were proved to be significantly better than control broilers (Akotkar *et al*., 2007) in terms of RD titre. Humoral mediated immune responses of the broiler birds were improved due to ashwagandha root powder supplementation (Kumari *et al*., 2011). An improvement in total immunoglobulin concentration was reported on 0.01% *W. somnifera* supplementation (3.83) as

compared to control group (2.79) in broilers during the summer season (Sujatha *et al*., 2010). According to Okonkwo *et al*. (2015), high antibody titres could be obtained in the broiler groups raised on ashwagandha containing herbal formulation.

2.8 Effect of *W. somnifera* Supplementation on Carcass Parameters

Higher dressing percentage ($p>0.05$) in *Withania* supplemented broiler group (78%) as compared with control (76%) was observed (Sanjyal and Sapkota, 2011). Similar data had been presented by Ahmed *et al*. (2015a) that showed non-significant ($p>0.05$) increase in dressing percentage in group received 1.5 g ashwagandha leaves (76.41) as compared to control (75.23). The per cent leg weight in control (23.46%) and ashwagandha treated group (22.20%) was also observed nonsignificant in broilers (Sanjyal and Sapkota, 2011). Similar non-significant difference in breast (40.18 and 37.04) per cent and thighs cut (25.90 and 27.60) per cent was recorded in treated and control broilers (Ahmed *et al*., 2015a). However, Rindhe *et al*. (2012) reported a beneficial effect of polyherbal antistressor and antioxidant formulation containing *Phyllanthus emblica, Ocimum sanctum, Terminalia chebula* and *W. somnifera,* in improving the carcass yield, dressing percentage, fillet, tender and giblet yield. In supplemented group, carcass yield was higher by 29.64%, dressing percentage by 0.83%, fillet yield by 23.2%, tender yield by 12.88% and giblet yield by 10.8%. Similar observation for significantly high ($p<0.05$) dressing percentage, breast weight and leg weight was observed in groups supplemented with 10 ml herbal extract (62.3%) when compared with control (51.11%) (Javed *et al*., 2009).

The per cent weight of liver under control (2.69), 1% ashwagandha (2.42) and 0.15% ashwagandha extract (2.50) supplemented broiler group was observed nonsignificant (Vasanthakumar *et al*., 2014). Similarly, Sanjyal and Sapkota (2011) revealed statistically similar per cent relative weight of liver, heart and gizzard in broilers supplemented with *W. somnifera*. Ahmed *et al*. (2015a) observed significant decrease in wings cut per cent of broilers under treatment with *W. somnifera.*

Vasanthakumar *et al*. (2014) reported nonsignificant alteration in intestinal length of carcasses of broiler treated with ashwagandha as compared to control and observed intestinal length (cm) of 183.75, 213.50 and 221.33 in control, ashwagandha root powder (@ 1% of feed) and ashwagandha root extract (@ 0.15% of feed) supplemented groups, respectively.

2.9 Effect of *W. somnifera* Supplementation on Meat Quality

Supplementation of ashwagandha in basal diet of broilers was found to significantly affect the sensory qualities of broiler meat. Meat from the broilers fed herbal feed additive containing *W. somnifera* was observed significantly ($p<0.05$) superior to the control with respect to all the attributes *viz*., appearance (7.32 and 6.5), flavor (6.72 and 5.90), tenderness (7.13 and 6.14), stickiness to mouth (7.24 and 6.11), juiciness (7.30 and 7.01), and overall acceptability (7.5 and 6.03) in supplemented and control group (Pandey *et al*., 2013). Sensory evaluation of broiler meat resulted in significant improvement of organoleptic traits of broiler meat, *i.e.,* appearance (6.10 and 6.48), colour (6 and 6.81), odour (5.8 and 6.81), flavor (5.66 and 6.5), juiciness (6.1 and 6.83), texture (6 and 6.8), 6.1 and 6.8) and for overall palatability (6 and 6.6) in control and supplemented group, respectively on supplementation of herbal products AV/LAP/19 containing aswagandha (Rindhe *et al*., 2012). Improved tenderness and palatability was attributed to improvement in collagen and myofibrillar solubility of meat due to AV/LAP/19 supplementation (Rindhe *et al*., 2012).

The oxidative stability of broiler meat estimated in terms of thiobarbiturate acid (TBA) value revealed significantly ($p<0.5$) lower values in AV/LAP/19 supplemented group at the end of 15^{th}, 30^{th}, 45^{th} and 60^{th} storage day (0.31, 0.33, 0.35, 0.42, 0.54 mg malonaldihyde/kg) as compared to untreated control group (0.31, 0.39, 0.50, 0.60 and 0.66). Similarly, lower tyrosine value in broiler meat indicative of less proteolysis was observed on supplementation of AV/LAP/19 herbal product. Thus, lower TBA and tyrosine value of broiler meat observed in AV/LAP/19 supplemented group was observed to improve the shelf life of frozen raw meat (Rindhe *et al*., 2012).

2.10 Effect of *W. somnifera* Supplementation on Economic Efficiency of Broiler Farming

Mushtaq (2007) obtained better net return on supplementation of 20% root extract of *W. somnifera* in broilers. Higher net return was obtained by Javed *et al*. (2009) on combined supplementation of *Berberis lycium* and *W. somnifera* than their individual outcome which could be attributed for efficient feed utilization by the broilers at 10% extract of the studied plants. Ansari *et al*. (2008) carried out the economic evaluation and showed maximum profit per bird in *W. somnifera* root powder fed broilers (Rs. 21.44) than broilers raised on herb *Nigella sativa* (Rs.

20.60), *Azadirachta indica* (Rs. 20.38) or control. Net return was observed maximum in ashwagandha supplemented group (Rs. 48.48) followed by synthetic growth promoters (Rs. 47.92) and control (Rs. 47.34) (Shisodiya *et al*., 2008). Pedulwar (2004) also reported higher net profit per bird in broilers supplemented with ashwagandha.

In contrast, Kale *et al*. (2015) observed less net profit per bird in ashwagandha fed group (Rs. 15.60) than control (Rs. 16.55) however gross return was significantly higher in 0.25% ashwagandha supplemented (Rs. 110.10) than control group (Rs. 107.58) broilers. Higher cost of production observed for probiotic (Rs.141.8) and ashwagandha (Rs. 134.7) supplemented groups as compared to control (Rs.128.3) was attributed to the additional cost incurred on purchase of ashwagandha root powder and probiotics (Sanjyal and Sapkota, 2011).

2.11 Role of Synbiotics in Broiler Production

2.11.1 Function of probiotics

Probiotics are also used extensively in poultry production as natural alternatives to antibiotics for growth promotion. Numerous studies showed that addition of probiotics have positive effects on growth rate, feed utilization, feed efficiency and mortality rate (Sen *et al.,* 2012; Manal and El-Naga, 2012). In broiler nutrition, probiotic species belonging to *Lactobacillus*, *Streptococcus*, *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Aspergillus*, *Candida* and *Saccharomyces* have a beneficial effect on broiler performance (Ashayerizadeh *et al*., 2009) through modulation of intestinal microflora and pathogen inhibition (Higgins *et al*., 2007), changes in haematobiochemical parameters (Mathivanan and Kalaiarasi, 2007) and improvement in sensory characteristics of dressed broiler meat (Pelicano *et al*., 2003). Recently, it was shown that addition of probiotic containing *Enterococcus faecium* microorganisms to broiler diets has increased the ileal and jejunal villus height (Chichowski *et al*., 2007; Samli *et al*., 2007). However, the efficacy of probiotics depends upon the selection of more efficient strains, gene manipulation, combination of several strains and the combination of probiotics and synergistically acting components like herbs. The health promoting effect of probiotic in the gastrointestinal tract was mainly associated with their capacity to stimulate the immune response and to inhibit the growth of pathogenic bacteria (Barnes and Impey, 1972). Sanders (2000) however reported gastrointestinal problems, flatulence, constipation and even death on probiotics supplementation.

2.11.2 Function of prebiotics

Prebiotics are considered as selectively fermented nondigestible microbial modulators feed ingredients that beneficially improve the host health through selective stimulation of the growth and activity of one or more limited number of bacteria in the colon (Gibson and Roberfroid, 1995; Gibson *et al*., 2004). Intake of prebiotics significantly modulates the colonic microbiota by increasing the number of specific beneficial bacteria such as *Lactobacilli sps* and *Bifidobacteria* (Rycroft *et al*., 2001) or reducing undesired intestinal colonization of pathogenic bacteria by mimicking their attachment sites on the intestinal mucosa (Iji and Tivey, 1998). Several studies have shown that administration of prebiotics could improve weight gain, feed intake and feed convention rate in broilers (Rodrigues *et al*., 2005). However in contrast, some reports indicated that prebiotic supplementation did not affect body weight gain, feed intake or feed conversion ratio (Stanczuk *et al*., 2005).

2.11.3 Function of synbiotics

The combined use of both probiotics and prebiotics in the form of synbiotic could maximize the utilization of feed stuff and beneficially affects the host by activating the metabolism of one or a limited number of health promoting bacteria and stimulating their growth selectively, improving the host's welfare (Gibson and Roberfroid, 1995). Recent research and development of synbiotic products have been increasingly focused on functional benefits including resistance to gastrointestinal bacterial infection, antibacterial activity and improved immune status in broiler chicks. In addition, Mohnl *et al*. (2007) found that the synbiotic had a comparable potential to improve broiler performance as avilamycin antibiotics. Pluske *et al*. (1996) observed increased intestinal villi height after addition of *Bacillus subtilis* in association with prebiotics.

2.12 Effect of Synbiotics Supplementation on Broiler Performance

2.12.1 Effect on broiler performance

The nutrient sparing effect of synbiotic substances in broiler was explored in a number of studies. In a trial carried out by Saiyed *et al*. (2015), synbiotic substances were found to produce body weight gain similar to nonsupplemented broilers with significantly low quantity of feed in synbiotic supplemented group (83.87 g/bird/day) than control (91.85 g/bird/day). In an another trial, similar quantity of feed intake ($p>0.05$) in control (4457.5 g) and synbiotic supplemented broilers (4462.9 g)

resulted in significantly higher body weight (2145.4 g) and FCR (2.08) in synbiotic fed groups than control broiler's body weight (1996.6 g) and FCR (2.23) (Ashayerizadeh *et al*., 2009). A final body weight of 1,847 g was observed compared to controls (1,754 g) ($p<0.05$) in broilers with FCR value of 1.75 in broilers supplemented with synbiotic than control birds (1.89) (Awad *et al*., 2008). Significant improvement in FCR (1.85) was observed through supplementation of synbiotic substances ($p<0.05$) than control group (1.99) in broilers on similar feed intake (Dizaji *et al*., 2012).

Broiler chicks supplemented with dietary synbiotic @ 0.1% of feed exhibited greater ($p<0.01$) body weight, weight gain, and lower feed conversion into meat in comparison with birds of control group with cumulative average body weight (g), weight gain (g), feed intake (g/bird) and FCR was 2335, 2292.67, 4607 and 2.01 in control group and, 2937.7, 2892.7, 4274.6 and 1.51 in synbiotic supplemented group, respectively (Al-Sultan *et al*., 2016). A synbiotic containing probiotic *Enterococcus faecium* and prebiotic fructo-oligosaccharide resulted significant increase ($p<0.05$) in body weight gain and improvement in FCR during period of active growth phase (0 to 4^{th} week) and non-significant effect on body weight gain and FCR during finisher period (5^{th} to 6^{th} week) for statistically similar feed intake than control broiler group (Ghasemi and Taherpour, 2013). The synbiotics (TGI @ 0.025% of feed) supplemented broiler group had a greater ($p<0.05$) body weight (1702.00gm) compared with the control group at week 5^{th} and lower feed conversion ratio (FCR) for birds supplemented with synbiotics (1.75kg) than control (1.89kg) (Ahmed *et al*., 2015b). Birds supplemented with synbiotic (0.2% prebiotics and 0.3% probiotics) revealed greater ($p<0.05$) body weight (632.32 and 2299.19 g) compared with control (564.76 and 2058.10 g) at day 21 and day 42 of age, respectively (Abdel-Raheem and Abd-Allah, 2011).

The study of Bozkurt *et al*. (2009) recorded improvement of 4.2-5.1% and 1.9-2.5% in growth rate during the starter and finisher period in 0.05% synbiotic supplemented group when compared with control broilers**.** The values for feed intake (g) and FCR were 1050 and 1.54; 1061 and 1.48, for control and synbiotic group, respectively at day 21; and 3943 and 1.82; 3899, and 1.75 for control and synbiotic group, respectively at day 42. The supplementation of prebiotic component in broilers resulted in significantly higher nitrogen retention (58.43%) than control (56.65%) with nonsignificant difference in dry matter digestibility (Thorat *et al*., 2015).

The improvement in performance of broilers on dietary supplementation of synbiotic could be due to significant ($p<0.05$) increase in illial villus length (Beski and

Al-Sardary, 2015). The results were in line with the findings of Mirza (2009) who found significant increase in ileum villus height at 42 days as a result of synbiotic supplementation to the broiler diet. Al-Sultan *et al*. (2016) reported that addition of synbiotic increased the villus height (µm) and villus height/crypt depth ratio ($p<0.01$) in duodenum (1302.4 and 8.47) in comparison with the control diet (967.6 and 4.01), respectively. The experimental diet increased villus height (µm) and villus height/crypt depth ratio (952 and 7.38) compared with the control diet (845 and 6.76) at 42 day of age. The histological study of gut intestine conducted by Awad *et al*. (2008), revealed that the addition of synbiotic (Biomin @ 0.01% of starter and @ 0.005% of the grower diets) increased ($p<0.001$) the villus height/crypt depth ratio (7.13) and villus height (774 µm) in ileum compared with control (4.86 and 614 µm), respectively. However, the ileal crypt depth was decreased by synbiotic supplementation (117±2 µm) compared with control (128±2 µm).

The reported values of villus height (µm), crypt depth (µm) and villus height to crypt depth ratio in the duodenum, jejunum and ileum was 1308.53, 1086.36, 814.91; 204.93, 240.11, 144.08; 6.66, 6.16, 5.92 in synbiotic supplemented and 1178.34, 929.75, 643.18; 212.31, 203.96, 158.28; 5.44, 4.64, 4.57 in control group, respectively (Abdel-Raheem *et al*., 2012). According to Ghasemi and Taherpour (2013), the crypt depth (µm) in jejunum was not affected by dietary treatments at any stages ($p>0.05$). However, the results were in contrast with the finding of Ahmad (2004) who found an increase in crypt cell proliferation of the small intestine in broiler with the use of probiotic compared to the control.

The protein efficiency ratio (g/g) was significantly higher in synbiotic treated group (2.62 and 2.60) as compared with control (2.38 and 2.42), both at day 1-21 and day 22-42, respectively in Ross 308 broilers (Ashayerizadeh *et al*., 2011). Similarly, a study conducted by Saiyed *et al*. (2015) in broilers revealed significantly ($p<0.05$) higher European Performance Efficiency Index (EPEI) with values of 261.20, 285.76 and 231.78 in synbiotic supplemented (half and full) and control group, respectively. The European Production Efficiency Factor (EPEF) reported for synbiotic supplemented group (291) was observed higher than control group (255) in broilers (Awad *et al*., 2008). In contrast, Jung *et al.* (2008) found that addition of synbiotic containing galacto-oligosaccharides (GOS) and *Bifidobacterium lactis* had no significant effect on weight gain, feed intake and feed conversion ratio of broiler chickens when compared with control broilers.

2.12.2 Effect of Synbiotics on performance of heat stressed broilers

Sohail *et al*. (2013) reported a significant ($p<0.05$) difference between heat stressed synbiotic (0.5% MOS+0.1% probiotic mixture) supplemented broilers and heat stressed control group in body weight gain (g) and FCR (g/g) at day 21 and 42, while feed consumption was non-significant at day 21 but significant ($p<0.05$) at day 42. The value in synbiotic group and heat stressed control group was 605.7 and 564.8; 995.2 and 1002.4; 1.65 and 1.78 at day 21 and, 1659.4 and 1510.7; 3436.5 and 3390.2; 2.07 and 2.24 at day 42 for average body weight gain (g), average feed consumption (g) and FCR, respectively.

2.12.3 Effect of synbiotics on mortality of broilers

Significant reduction in mortality rate (1.33%) was observed as compared to control (4.11%) in initial active growth phase of broilers by Abdel-Raheem and Abd-Allah (2011). In contrast, numerically lower mortality percentage was recorded for the synbiotic supplemented group (0%) as compared to control (6.66%) (Al-Sultan *et al*., 2016). Similarly, non-significant difference in mortality between synbiotic treated (9.37%) and control broilers (6.25%) (Dizaji *et al*., 2012). Nonsignificant difference ($p<0.05$) in mortality was also observed by Bozkurt *et al*. (2009) in broilers raised on 0.05% synbiotic substances than control group.

2.13 Effect of Synbiotics on Haemato-serobiochemical Parameters of Broilers

2.13.1 Effect on haematological parameters

An experiment in male Ross 308 chicks demonstrated significantly ($p<0.05$) higher Hb concentration and total erythrocyte count on diets containing synbiotic (@ 0.25% of feed) than the control group while PCV values was statistically non-significant between both group (Beski and Al-Sardary, 2015). The results were in line with the findings of Al-Kassie *et al*. (2008) who found that the supplementation of probiotic to the broiler diet at a rate of 10 g/kg significantly increased Hb concentration in 42 days old chicks compared to the control.

In disagreement, Silvia *et al*. (2008) found that the addition of synbiotic to broiler diet had no significant effects on RBC count at 42 days. Similarly, Nyamagonda *et al*. (2009) in broilers revealed that mean total erythrocyte counts, Hb and PCV were statistically non-significant ($p>0.05$) in broilers on day 21 and 42 with

supplementation of synbiotics. These findings were in agreement with the observations made by Ahmed *et al*. (2015b) who stated that there was non-significant ($p>0.05$) difference among the mean TEC between groups and the observed value was 2.37±0.08 million/mm^3 for synbiotics (TGI @ 0.025% feed) supplemented and 2.165±0.20 million/mm^3 for control group. However, the effect of synbiotics TGI was detected significant for packed cell volume in broilers in contrast to the findings of Beski and Al-Sardary (2015). Similar increase in PCV without alteration in RBC and Hb was reported in broilers by Al-Saad *et al.* (2014) on supplementation of probiotics.

Nyamagonda *et al*. (2009) reported remarkable increase ($p<0.05$) in total leukocyte count (TLC) in broiler groups treated with synbiotic as compared to control group with TLC values observed in synbiotic supplemented and control group was 13.25 and 15.39 thousands/ µl, respectively. The present results were in concurrence with the observation of Shoeib *et al*. (1997) who reported an increase in total leukocyte count on supplementation with a probiotic containing viable lactic acid bacteria. Stimulation of the mucosal immune system and increased cellularity of Peyer's patches was observed in birds raised on probiotic *Lactobacillus* organism (Perdigon *et al*., 1995). The contrast results were presented by Al-Saad *et al.* (2014) and Shahir *et al.* (2014) who reported no effect of supplementation of probiotic and prebiotic to two separate groups of chickens on TLC, lymphocyte, heterophil and monocyte counts. In the study of Capcarova *et al*. (2008), a non-significant decrease in TLC was observed in the two groups of turkey supplemented with graded doses of probiotic. Lower TLC value along with similar H:L ratio, lymphocyte, heterophil and monocyte counts was also reported in synbiotic treated guinea fowls than control (Habibu *et al*., 2016).

2.13.2 Effect on serobiochemical parameters

2.13.2.1 Blood glucose

Non-significant value of serum total glucose (mg/dl) was observed on supplementation of synbiotic (213.33) as compared to control (203.50) (Ashayerizadeh *et al*., 2009). Similar nonsignificant difference in serum glucose values was observed by Beski and Al-Sardary (2015) between control (227.7 mg/dl) and synbiotic supplemented (232.0 and 228.7 mg/dl) groups @ 0.25 and 0.50% of feed, respectively.

Probiotic present in synbiotic mixtures were known to exert non-significant effect on concentration of serum T3 hormone and significant difference in T4 concentration with serum value of T4 hormone as 5.90 and 6.87 ng/ml in control and probiotic supplemented broilers (Khan *et al*., 2013).

2.13.2.2 Serum proteins

Birds fed diet supplemented with synbiotic revealed significantly higher value of serum total protein compared with control guinea fowl, *i.e.,* 5.56 and 6.10 g/dl in control and supplemented group (Habibu *et al*., 2016). Broilers raised on synbiotic (MOS @ 2% and *Saccharomyces cerevisiae* @ 3% of feed) produced significantly higher serum total protein 3.36 g/dl than control 2.5 g/dl on day 21 but nonsignificant difference was observed between groups on day 42 (Abdel-Raheem *et al.*, 2011). In correspondence, Beski and Al-Sardary (2015) also observed non-significant difference in serum total protein values between control (3.1 g/dl) and synbiotic supplemented (3.0 and 2.8 g/dl) groups @ 0.25 and 0.50% of feed, respectively. A feeding trial on broilers chicken revealed nonsignificant effect of synbiotic on total protein, serum albumin and serum globulin included in the ration at the rate of 2.9 kg/ton of feed (Ashayerizadeh *et al*., 2009).

Ahmed *et al*. (2015b) reported that the value of creatinine concentration for synbiotics supplemented broilers were increased significantly ($p<0.01$) than the control group. The creatinine level in synbiotics supplemented group and control was (0.71 and 0.60 mg/dl), respectively.

2.13.2.3 Serum lipids

Beski and Al-Sardary (2015) reported significantly ($p<0.05$) lower concentration of cholesterol and LDL in chickens received synbiotic (@ 2.5 or 5 g/kg diet) in their diets than those fed on control diet while the serum triglyceride and HDL cholesterol values were nonsignificantly different between groups. Age dependent effect of synbiotic on serum lipid profile was observed by Abdel-Raheem *et al.* (2011) who observed nonsignificant but numerically lower values of total cholesterol (mg/dl) and triglyceride (mg/dl) serum concentration at day 21 and significant decrease in serum total cholesterol (93 mg/dl) and triglycerides (101.67 mg/dl) at day 42 of experimental trial than control (160.70 and 125 mg/dl). The serum cholesterol and LDL lowering effect of synbiotic was also observed in broilers by Ghasemi and Taherpour (2013) although no significant difference was observed in triglyceride and VLDL-cholesterol levels during the experiment. A study conducted by Mohamed *et al*.

(2014) revealed that the concentration of total lipid, triglyceride, total cholesterol and LDL-cholesterol decreased significantly ($p<0.05$) and HDL-cholesterol increased significantly ($p<0.05$) in synbiotic fed group as compared to synbiotic non-fed group which were subjected to 'repeated fasting and refeeding cycles' under heat stress from 28th day till the end of experiment. Addition of prebiotic MOS @ 0.05% in basal diet of broilers significantly lowered serum total cholesterol concentration (71.6 mg/dl) than the broilers in the control group (Yalcinkaya *et al*., 2008).

2.13.2.4 Serum enzymes and minerals

Numerical decrease in serum ALT (µ/l) and AST (µ/l) in synbiotic supplemented broiler in comparison with the control at day 21 and 42 was observed by Abdel-Raheem *et al.* (2011) who reported values for ALT as 12.10 and 16.40, and AST as 43.00 and 50.30 in synbiotic treated and control group, respectively. Ahmed *et al*. (2015b) also observed that there was no significant ($p>0.05$) difference for synbiotics supplemented group than control group for ALT and AST in broilers. The probiotic protexin also failed to demonstrate its significant effect on ALT and AST in broiler breeder after moulting. The study of Yalcinkaya *et al*. (2008) reported similar nonsignificant effect of 0.15% MOS prebiotic on serum AST values, however the values reported for serum ALT were significantly ($p<0.05$) higher in the control group than MOS fed broilers.

The serum calcium and phosphorus levels was numerically higher in synbiotic (MOS @ 2% and *Saccharomyces cerevisiae* @ 3% of feed) supplemented group in comparison with the control at 21 and 42 day of age (Abdel-Raheem *et al*., 2011). Similar non-significant difference in serum calcium and magnesium level was observed by Khan *et al*. (2013) between control and probiotic (protexin @ 50 mg/L of water) supplemented broiler group.

2.14 Effect of Synbiotic on Immunological Parameters of Broilers

Ghasemi and Taherpour (2013) reported that dietary inclusion of synbiotic could increase the antibody-mediated immune response at both 28th and 42nd day of age. The effect of feed additives on the humoral antibody titer post Newcastle disease virus (NDV) vaccination was significantly ($p<0.01$) higher in synbiotic (0.1% in feed) supplemented group as compared with the control group. The $\log_{10}$ NDV antibodies titre value observed after vaccination was 2.86 and 3.87 in control and supplemented group, respectively (Al-Sultan *et al*., 2016).

2.15 Effect of Synbiotic Supplementation on Carcass Parameters of Broilers

Significant increase ($p<0.05$) in the carcass weight and dressing percentage (1616.66 g and 70.68%) in synbiotic supplemented broilers compared with (1450.66 and 67.96%) control group was observed with non-significant difference in breast (388.33 and 301.33g), thigh yield (151 and 127.66g) and other giblet weights among both treated and control broilers (Abdel-Raheem *et al*., 2011). Greater ($p<0.05$) carcass percentage (66.77±13.45%) in synbiotic fed broilers and highly significant difference in carcass yield (6-7%) for synbiotic group was observed by Ahmed *et al*. (2015b). Nonsignificant difference in dressed weight, liver, heart, gizzard and total giblet weight was observed in broilers on supplementation of synbiotic (Probiotic @ 0.005%+Prebiotic @ 0.025% of feed) (Saiyed *et al*., 2015). The results reported by Ashayerizadeh *et al*. (2009) reflected a significantly higher thigh meat weight (%) in group fed with synbiotic (primalac+biolex-MB) as compared with control while the same study could not established the effect of synbiotic on breast meat weight (%) and percentage carcass yield.

The relative weight of spleen and caeca increased significantly ($p<0.05$) in heat stressed broilers on inclusion of synbiotic (0.5% MOS+0.1% probiotic mixture) in diets (Sohail *et al*., 2013). An increase ($p<0.05$) in absolute weight of the immune organs (bursa-2.3g and thymus-13.5g) and liver (49.66g); and numerical increase in spleen weight (3.83g) in synbiotic supplemented broilers was observed than control broilers having values of 1.43, 8.03, 3.6 and 43.33g for bursa, thymus, spleen and liver, respectively (Abdel-Raheem *et al*., 2011). Numerically highest intestinal length was found in the synbiotic supplemented group (80.25 inch) than control (79.50 inch) broilers (Saiyed *et al*., 2015). However, the investigation of Bozkurt *et al*. (2009) could not found the significant effect of dietary synbiotic (0.05% probiotic+0.05% prebiotic of feed) supplementation on the weight of liver and intestine in broilers. The synbiotic (Amax4x @ 0.1% of feed) contributed no difference ($p>0.05$) in mean weight of proventriculus, gizzard, liver and bursa of broilers (Dizaji *et al*., 2012). Similar observation for gizzard, bursa and spleen weight was reported on addition of synbiotic in broiler diets (Awad *et al*., 2008).

The sensory analysis data declared that there was no significant difference in Hedonic scale values between the control sample and those from chicken fed synbiotic concerning tenderness, juiciness and flavor (Abdel-Raheem *et al*., 2011).

2.16 Effect of Synbiotic Substances on Intestinal Health of Broilers

Caecum is considered as an area of high microbial activity in the intestine of chicks. The caecal microflora of the alimentary tract was found to exert significant effect on the health and performance of poultry through prevention of establishment of microbial pathogens belongs to coliforms group (Barrow, 1992). Modulation of intestinal bacteria towards a “healthy community” by feeding probiotics and prebiotics was observed to improve gastrointestinal health by favoring beneficial microflora and suppressing pathogenic bacteria (Apajalathi *et al*., 2004). Bonomi *et al*. (1995) reported that the addition of probiotics in the diet of poultry enhanced the development and activity of intestinal microflora through increase in number of LAB, including lactobacilli and decrease of number of coliforms, particularly *E. coli*. Many other investigators have studied the *in vitro* and *in vivo* inhibitory potentials of probiotics and prebiotics on enteric microorganisms (Edens, 2003). The direct antimicrobial effect of ashwagandha on harmful enteric bacteria (Singh and Kumar, 2012) and competitive exclusion of pathogenic bacteria by synbiotic substances in the intestine of broilers affects the pH and microbial load of intestine and caecum. Significant decrease in pH of caecum was also observed to suppress the growth of coliforms (Denev, 2006).

The synbiotic supplementation was reported to significantly decrease the caecal *E. coli* count (6.21 $\log_{10}$ cfu/g digesta) as compared to control broilers (6.97 $\log_{10}$ cfu/g digesta) (Al-Sultan *et al*., 2016). In contrast, Abdel-Raheem *et al*. (2012) observed nonsignificant variation in total *E. coli* count in caecal digesta of control and synbiotic supplemented broilers, *i.e.,* 5.93 and 5.31 $\log_{10}$ cfu/g digesta in synbiotic fed group as compared to control (7.53 and 6.93 $\log_{10}$ cfu/g digesta) at day 21 and 42, respectively. Similar inconsistent results were observed in synbiotic treated and heat stressed broilers with total coliform count of 7.41 and 7.39 $\log_{10}$ cfu/g digesta in treated and control broilers (Sohail *et al*., 2013).

2.17 Effect of Synbiotic Supplementation on Economic Efficiency of Broiler Farming

Saiyed *et al*. (2015) calculated the average return over feed cost (ROFC) income in terms of Rs./bird and %/bird from selling of the birds and was observed significantly ($p<0.05$) higher in synbiotic groups than the control group with non-significant difference between different level of synbiotics supplementation. Feed cost during whole experimental period was significantly ($p<0.05$) lower in synbiotic

supplemented groups (full and half) than other groups. ROFC of the control group found significantly ($p<0.05$) lower than all treatment groups. In terms of percentage, highest ROFC (Rs/kg live weight and %/bird) was in synbiotic-half (34.52 and 29.48) than synbiotic-full (34.06 and 26.87) as compared to control (28.43 and 30).

3. MATERIALS AND METHODS

Feed is one of the major inputs in poultry production. Various feed additives are used in broiler production to increase the feed utilization and to get maximum output through increase in performance of the broilers. In this present trial, a holistic approach was adopted to observe the effect of different levels of natural feed additives, *W. somnifera* and synbiotics either alone or in combination on overall performance and carcass characteristics of broilers.

A brief account of the experimental procedures and analytical techniques adopted during the course of the present study, are presented under the following headings:

3.1. Procurement and chemical evaluation of basal feed, *W. somnifera* and synbiotic

3.1.1 Procurement of basal feed, *W. somnifera* and synbiotic
3.1.2 Analysis of basal feed, *W. somnifera* and synbiotic
3.1.3 Estimation of alkaloid content of *W. somnifera*

3.2 Collection and analysis of meteorological information

3.3. Feeding trial using day old broiler chickens

3.3.1 Location
3.3.2 Experimental chicks
3.3.3 Experimental design
3.3.4 Preparation of treatment diets
3.3.5 Housing and general management
3.3.6 Feeding trial

3.4 Performance parameters

3.4.1 Feed intake
3.4.2 Body weight and body weight gain
3.4.3 Feed conversion ratio (FCR)
3.4.4 Protein efficiency ratio and performance index
3.4.5 Mortality

3.5 Digestion/Metabolism trial

3.5.1 Digestibility/Metabolizability of nutrients

3.5.2 Nitrogen, calcium and phosphorus retention

3.6 Haemato-serobiochemical Parameters

3.6.1 Determination of blood Hb, erythrogram and leucogram

3.6.2 Estimation of different serum biochemical parameters

3.7 Immunological parameters

3.8 Carcass parameters

3.8.1 Carcass yield and meat yield

3.8.2 Weight of different organs (offals and giblets)

3.8.3 Shank length, intestine length and caecal length

3.9 Evaluation of gut health

3.9.1 Intestinal pH

3.9.2 Total coliforms count in caecal content

3.10 Meat quality evaluation

3.10.1 Proximate analysis of broiler meat

3.10.2 pH and water holding capacity

3.10.3 Sensory evaluation of broiler meat

3.11 Statistical analysis

3.1. Procurement and Chemical Evaluation of Basal Feed, *W. somnifera* and Synbiotic

3.1.1 Procurement of basal feed, *W. somnifera* and synbiotic

The ISO certified basal feed in the form of broiler starter and broiler finisher was procured from reputed feed manufacturer “Venkys India Limited”, Pune (Maharashtra) in sufficient quantity.

W. somnifera commonly called as ‘ashwagandha’ is a well known subtropical herb, grows naturally in diverse areas and is well distributed in several parts of country. The roots of *Withania somnifera* is considered as potential source of biologically active substances and is widely used for its health promoting effect. Good quality 'A' grade solid cylindrical roots having minimum length of 7 cm and diameter of 1-1.5 cm with smooth external surface and pure white from inside were procured from reputed firm of Bikaner (Rajasthan) in sufficient quantity. The roots were then sun-dried and ground to pass through 1mm sieve and were stored in air tight plastic containers for further use (**Plate 1)**.

The commercially available synbiotic formulation “TGI” consisting of prebiotic and probiotic mixture, was procured from “Polchem Laboratories, Pune”. The ingredient composition of synbiotic and their concentration are represented in **Table 3.1**.

3.1.2 Analysis of basal feed, *W. somnifera* root powder and synbiotic

The proximate analysis of broiler starter, broiler finisher, *W. somnifera* root powder and synbiotic sample was carried out in triplicate according to the standard methods of analysis (AOAC, 2005) (**Table 3.2**).

3.1.3 Estimation of alkaloid content of *Withania somnifera* root powder

The representative root powder sample of *Withania somnifera* was sent in airtight sealed polypropylene container to R&D Laboratory of Ayurvet Limited, Baddi (Himachal Pradesh) for the estimation of total alkaloid content.

Table 3.1 Ingredient composition of synbiotic used in the experimental trial

Ingradients	Active constituents	Concentration
Prebiotic	Mannon-oligosachharide	14-16%
Probiotics	*Lactobacillus acidophilus*	10^9 CFU/g
	Lactobacillus bulgaricus	
	Lactobacillus plantarum	
	Streptococcus faecium	
	Bifidobacterium bifidus	

.

Table 3.2 Proximate composition[1] of broiler starter, broiler finisher, *W. somnifera* root powder and synbiotic

Chemical Composition (DM basis)	Broiler Starter	Broiler Finisher	*Withania somnifera*	Synbiotic Mixture
Proximate principles (%)				
Moisture	7.0	7.0	4.88	3.96
Organic matter	93.0	93.0	95.12	96.04
Crude protein	22.87	20.1	5.97	23.43
Ether extract	5.8	6.9	0.65	0.5
Nitrogen free extract	61.18	62.74	73.36	34.42
Crude fibre	3.95	4.36	13.8	3.5
Total ash	6.2	5.9	6.22	38.15
Mineral composition (%)				
Calcium	1.02	1.06	1.17	0.83
Phosphorus	0.96	0.81	0.63	1.28

[1]Average of the values determined on samples compounded on three occasions.

3.2 Collection and Analysis of Meteorological Information

The weather information related to ambient temperature and relative humidity for the period between Feb 29, 2016 to April 10, 2016 was collected from the Meteorological Department of Agricultural Research Station, Swami Keshwanand Rajasthan Agricultural University, Bikaner, Rajasthan. The temperature humidity index (THI) value for different weeks was calculated as per formula suggested by Kelly and Bond (1971).

$$THI = T_a - (0.55-0.55XRH) X (T_a-58.8)$$

where, T_a = ambient temperature (^oF)

RH = relative humidity divided by 100

3.3. Feeding Trial using Day Old Broiler Chickens

3.3.1 Location

The experimental trial that was carried out in the present study included feeding trial and the laboratory analysis. The feeding trial of the current study was carried out at the Experimental Poultry Unit, located in Poultry Farm of College of Veterinary and Animal Science, Bikaner. The estimation and chemical analysis of different parameters were carried out primarily in the laboratory of Department of Animal Nutrition and in different departments of College of Veterinary and Animal Science, Bikaner.

3.3.2 Experimental chicks

360-day old VenCobb 400 broilers chicks were procured from commercial reputed hatchery. The experimental broiler chicks were wing banded and weighed individually before starting of feeing trial.

3.3.3 Experimental design

The completely randomized design was adopted for the present feeding trial. The 360 experimental broiler chicks were equally and randomly divided into eight dietary treatments groups (T_1-T_8) and each dietary group was replicated to 3 sub-groups (R_1-R_3) to make the initial body weight uniform and nonsignificant (**Table 3.3**). Thus each dietary group consists of 45 chicks distributed into 3 replicated pens of 15 chicks each. The layout of the experiment is shown in **Table 3.4**.

3.3.4 Preparation of treatment diets

Eight different treatment diets were prepared for the feeding of broilers under different dietary groups. The herbal feed additive *W. somnifera* (ashwagandha) root powder and synbiotic was supplemented in basal broiler starter and broiler finisher ration either alone or in combination. The description of different treatment diets used for feeding broiler chicks is described in **Table 3.4**. The proximate analysis of different treatment mixtures was also carried out to find any significant variation in proximate principles if exist.

3.3.5 Housing and general management

Deep litter system of housing was adopted for the feeding trial in the present study with an objective to provide maximum comfort to the broilers. The pens were thoroughly cleaned and disinfected before starting of experiment. Fresh and dried wheat straw and wood husk was used as a bedding material. All the chicks were maintained under standard managemental regimen of brooding and lighting. Proper ventilation and biosecurity measures were ensured throughout the trial. The chicks were vaccinated against Marek's disease, Ranikhet Disease (F1 strain) and Infectious Bursal Disease on 0, 4^{th} and 14^{th} day, respectively. *Ad libitum* clean and fresh *water* was provided throughout the trial (**Plate 2 and Plate 3**).

3.3.6 Feeding trial

A 42 day feeding trial was carried out from Feb 29, 2016 to April 10, 2016. Differently prepared broiler starter and broiler finisher ration were provided to broiler chicks from day 1 to day 21 and day 22 to day 42, respectively. *Ad libitum* supply of feed was ensured throughout the trial. The feeding trial was aimed to assess the pattern of feed intake of various levels of ashwagandha and synbiotic based diets as well as the pattern of growth performance in broiler chickens (**Plate 4**).

Table 3.3 Randomization and initial body weight (g) of broiler chicks in different experimental groups

Treatment Groups	Body weights (g)			Mean^{NS}
	Replications			
	1	2	3	
1	42.67±0.25	42.73±0.25	42.60±0.22	42.67±0.13
2	42.93±0.23	42.53±0.24	42.87±0.25	42.78±0.14
3	42.87±0.24	42.60±0.25	42.60±0.29	42.69±0.15
4	42.93±0.28	42.53±0.24	43.00±0.22	42.82±0.14
5	42.93±0.18	42.67±0.21	42.87±0.26	42.82±0.12
6	42.93±0.18	42.67±0.21	42.93±0.21	42.84±0.11
7	42.80±0.28	42.67±0.25	42.67±0.23	42.71±0.14
8	43.27±0.25	42.67±0.21	42.78±0.21	42.40±0.14
Mean^{NS}	42.92±0.083	42.63±0.082	42.74±0.083	42.76±0.048

NS: non-significant ($P > 0.05$)

Table 3.4 Experimental design for different treatment groups

S.N.	Treatment Groups		No. of Broiler Chicks/ Replication			Total No. of Broiler Chicks in Each Group
			R_1	R_2	R_3	
1	T_1	Basal diet (Control)	15	15	15	45
2	T_2	Basal diet supplemented with 0.5% *Withania somnifera*	15	15	15	45
3	T_3	Basal diet supplemented with 1.0% *Withania somnifera*	15	15	15	45
4	T_4	Basal diet supplemented with 1.5% *Withania somnifera*	15	15	15	45
5	T_5	Basal diet supplemented with 0.025% Synbiotic (Probiotic and Prebiotic Mixture)	15	15	15	45
6	T_6	Basal diet supplemented with 0.050% Synbiotic (Probiotic and Prebiotic Mixture)	15	15	15	45
7	T_7	Basal diet supplemented with 0.25% *Withania somnifera* and 0.025% Synbiotic (Probiotic and Prebiotic Mixture)	15	15	15	45
8	T_8	Basal diet supplemented with 0.50% *Withania somnifera* and 0.050% Synbiotic (Probiotic and Prebiotic Mixture)	15	15	15	45

3.4 Performance Parameters

3.4.1 Feed intake

Weighted amount of the designated type and quantities of feed according to dietary groups were fed to the experimental chicks during the whole trial. Enough care was taken to offer the quantity of feed required by each replicate which was by and large decided almost every day. Left over residue by each replicate was noted if any. Weekly feed consumption in units of g/broiler chick was calculated as the total feed consumed in each replication divided by the number of birds available in that replicate.

3.4.2 Body weight and body weight gain

The body weight of the experimental broilers was recorded at beginning of the experiment as well as on weekly basis to assess the body weight change and the growth pattern due to dietary regimens. The weighing of the birds was done in the early hours of the day before feeding, using an electronic scale (**Plate 5**).

Live weight gain (g/broiler chick) at weekly interval was calculated from difference in body weight attained between the two consecutive weeks. Average daily body weight gain (ADG) (g/day/chick) was estimated by dividing total body weight gain through number of days.

3.4.3 Feed conversion ratio (FCR)

The weekly FCR of broilers chicks under different treatments was calculated by dividing the weekly feed intake through body weight gain/loss during the individual week of the experimental period.

3.4.4 Protein efficiency ratio (PER) and performance index (PI)

PER is one of the oldest measures of assessment of efficiency of conversion of dietary protein into body mass. PER values were calculated during each studied weekly growth period according to equations of Ali (1999) as follows:

$$PER = \frac{\text{Body weight gain (g)}}{\text{Crude protein consumed}} \times 100$$

The productive performance of broilers in term of performance index under different treatments on weekly interval was estimated by as per equation reported by North (1981).

$$PI = \frac{\text{Body weight (kg)}}{\text{FCR}} \times 100$$

3.4.5 Mortality

Regular observation was carried out to record mortality in broiler chicks if any. Mortality rate (per cent) was calculated from the records of dead birds up to end of the study against total number of birds on treatment basis.

3.5 Digestion/Metabolism Trial

A digestion/metabolic trial of five days duration was conducted in the last week of feeding trial from 37th day to 42nd day to assess the digestibility of different dietary principles and nutrient retention of nitrogen, calcium and phosphorus in different dietary groups. Three birds per replicate under each treatment were randomly selected and shifted to metabolic cages (**Plate 6**). A three day adaptation period was provided before metabolic trial. During the five day metabolic period, the group wise daily feed intake and quantum of excreta voided were recorded. The total collection method was adopted to study nutrient metabolizability and the retention of nitrogen, calcium and phosphorus. Dropping trays covered with aluminum foil paper were used for total excreta collection on daily basis for five days. Utmost care was taken to collect droppings devoid of contaminants, *viz.,* feed and feathers, if any. The representative samples of droppings from each replicate were oven dried at temperature of about 80ºC for 24hrs to remove the moisture content and to bring the faeces to a constant weight. The five day collection of such oven dried excreta of each group was mixed and ground to pass through 1mm sieve and was stored separately in air tight plastic containers for further analysis. Representative samples of treatment mixtures used for feeding were also collected and oven dried to obtain dry matter content of feed consumed. For nitrogen estimation, a representative sample of the fresh excreta voided by each group everyday was transferred to a bottle containing 15ml of concentrated sulphuric acid to make pooled sample.

3.5.1 Digestibility/metabolizability of nutrients

Samples of feed offered under different treatments and voided excreta were analysed for proximate principles as per AOAC (2005). The treatment wise digestibility/metabolizability of dry matter (DMD) of diet was determined using the following formula:

$$\text{DMD (\%)} = \frac{\text{Weight of dry matter consumed (g)} - \text{Weight of excreta voided on dry matter basis (g)}}{\text{Weight of dry feed consumed (g)}} \times 100$$

Similarly, the digestibility/metabolizability of organic matter (OMD), crude protein (CPD), ether extract (EED), nitrogen free extract (NFED) and crude fibre (CFD) was determined using the formula given below:

$$\text{Digestibility/Metabolizability coefficient of nutrients (\%)} = \frac{\text{Unit nutrient intake} - \text{Unit nutrient outgo}}{\text{Unit nutrient intake}} \times 100$$

3.5.2 Nitrogen, calcium and phosphorus retention

The total nitrogen content of feed and excreta was determined through Kjeldahl's method using Kel plus Automatic Nitrogen Analyzer equipment. The calcium and phosphorus of feed and excreta samples was estimated using procedures described by Talpatra *et al*. (1940). The per cent nitrogen, calcium and phosphorus retention under different treatments was calculated through per cent difference in intake and outgo of nutrient.

3.6 Haemato-serobiochemical Parameters

About 3 ml blood samples was collected aseptically from wing vein of each of the three randomly selected birds from each replication (nine birds/treatment) at 28th and 42nd day of experiment for the estimation of different haemato-serobiochemical parameters (**Plate 7**). Half of the blood was transferred into ethylenediamine tetra acetic acid (EDTA) containing vacutainer tubes for estimation of blood Hb, erythrogram and leucogram. The remaining blood sample was transferred to non-EDTA tubes for preparation of serum. Subsequently, the serum was harvested through centrifugation of sample at 3000 rpm for 15 min and stored at -20℃ until further analysis.

3.6.1 Determination of blood Hb, PCV, erythrogram and leucogram

Blood haemoglobin and PCV was estimated by Sahli's haemoglobinometer and micro- haematocrit methods, respectively. Total erythrocytes count (TEC) and total leukocytes count (TLC) was carried out manually through haemocytometer as per standard method of Benjamin (1978). The proportion of different leucocytes in blood was estimated through differential leukocytes count (DLC) method. A thin blood film was prepared, stained with Wright Giemsa stain and at least 100 cells were examined to estimate the proportion of different leucocyte cells.

3.6.2 Estimation of different serum biochemical parameters

The treatment wise serum samples were analysed for glucose, total protein, albumin, creatinine, triglycerides (TG), cholesterol, high density lipoprotein cholesterol (HDL), calcium, phosphorus, magnesium and enzymes like alanine transaminase (ALT) and aspartate transaminase (AST) through Idexx spectrophotometer using commercial test kits as per manufacturer's protocol. Serum globulin content was calculated through subtraction of serum albumin concentration from total serum protein levels.

The very low density lipoprotein cholesterol (VLDL) and low density lipoprotein cholesterol (LDL) was calculated as per method of Friedwald *et al*. (1972) using formula:

$$\text{VLDL} = \frac{\text{Triglycerides concentration}}{5}$$

LDL cholesterol = Total cholesterol - HDL - VLDL

3.6.3 Estimation of serum thyroid stimulating hormone (TSH), triiodothyronine (T3) and thyroxin (T4) hormones

The TSH, T3 and T4 serum hormones levels of broilers in different treatments were estimated at the end of the trial using commercial kits (MP Biomedicals ELISA Kit) as per method of Tietz (2005). The microplate ELISA methodology was carried out using standard calibrators and enzyme conjugate in streptavidin coated microwells. The absorbance of different hormones was read at 450 nm in microplate reader. The concentration of hormones was calculated by preparing standard graph using calibrators.

3.7 Immunological Parameters

The hemagglutination inhibition (HI) titer for RD virus was estimated as per the method of Alexander (1988). An indirect ELISA was carried out using micro ELISA plate method as described by Nandapalan *et al*. (1981) and Adeniran and Oyejide (1995) with slight modifications to estimate serum antibody titre for Infectious Bursal Disease (IBD) virus. The absorbance of IBD titre was read at 492 nm wavelength using spectrophotometer. The ELISA values for IBD antibodies of the chickens were then recorded with the help of standard curve.

3.8 Carcass Parameters

At the end of 42nd day of trial, three birds from each replicate having body weight close to the group average were selected for estimation of carcass characteristics. The selected birds were weighed individually and allowed to fast for 12 hour to empty gut contents before sacrifice. The broilers were sacrificed as per standard procedure (Panda, 1995) by severing the occipito-atlantal joint and allowed to bleed completely. The birds were de-feathered manually and carcasses were eviscerated to measures various parameters of carcass. All the estimates were expressed as per cent of live weight (**Plate 8**).

3.8.1 Carcass yield and meat yield

The dressing percentage of the carcass was estimated as total edible portion of the broilers including giblets such as liver, heart and gizzard. The eviscerated weight was calculated as the remaining weight of the carcass after removal of visceral organs and other offal such as feathers, head and shank. The dressed weight and eviscerated weight were also expressed as per cent of live weight. Lastly,

the breast of the carcasses were deboned, weighed and were packaged in labeled airtight low density polyethylene (LDPE) bags for meat quality evaluation (**Plate 9 and Plate 10**). The liver of the respective carcasses were also packed in the LDPE bags with similar objective. The meat samples were immediately transferred to refrigerated box at 4˚C for 24 hour and later on stored in deep freezer at -18±2˚C for further analyses.

3.8.2 Weight of different organs (offals and giblets)

The weight of different external offals such as head, feathers and shank; internal offals like lung, crop, proventriculus, pancreas, gallbladder, whole intestine (without content), caeca, spleen and bursa of fabricus; and giblets (gizzard, heart and liver) were recorded individually after removal of all the attachments with the help of electronic balance. The weight of blood lost was also recorded.

3.8.3 Shank length, intestine length and caecal length

The feet and shanks were removed at the tibio-tarsus joint. Shank length was taken as distance from foot pad to hock joint. The whole intestinal tract was removed and washed properly with normal saline solution. The intestinal length was measured from the pyloric end of proventriculus to anus with the help of measuring tape. The caeca was cut at the junction of ileocaecal point and accordingly the length was measured.

3.9 Evaluation of Gut Health

3.9.1 Intestinal pH

Representative samples of duodenal and caecal content from the intestine of the broilers were collected after slaughter and stored in the laboratory at refrigerated temperature of 4˚C till further analysis. The pH of the intestinal contents was measured with the help of digital pH meter equipped with a combined glass electrode.

3.9.2 Total coliforms count in caecal content

The total coliforms count in caecal content was estimated as colony forming unit (cfu) on Mac Conkey agar plate. One gram caecal content was diluted in sterile 0.9% normal saline solution in ratio of 1:10. Similarly, tenfold serial dilution up to 10^5 of each sample was prepared in 9 ml of 0.9% sterile normal saline solution. The Mac

Conkey agar plates were prepared and sterilized in autoclave. The media plates in triplicates were inoculated with 100 µl of the diluted samples from each serial dilution and were incubated aerobically at 37℃ in incubator . The cfu count was carried out after 24hr of incubation. The coliforms colonies were identified with the pinkish red colour on media plates. The numbers of cfu in each plate was counted manually and expressed as $\log_{10}$ cfu/g of intestinal content (**Plate 11**).

3.10 Meat Quality Evaluation

The qualitative evaluation of meat quality was carried out strictly as per standard norms. The test for keeping quality of meat was also done to evaluate the residual effect of ashwagandha and synbiotic on meat.

3.10.1 Proximate analysis of broiler meat

The chemical evaluation of breast muscle was carried out through proximate analysis as per standard methods of AOAC (2005). Each sample was analysed in triplicate to reduce systematic error. The samples were oven dried at 105℃ for the estimation of moisture content. The protein, ether extracts and total ash of the meat sample was estimated through Kjeldahl, solvent extraction and muffle furnace (550℃/8h), respectively. The proximate composition was expressed as per cent of dry matter.

3.10.2 pH and water holding capacity

The pH of fresh meat is considered as an indicator of efficient animal handling and ante mortem stress. It also determines the suitability of meat for consumption and long term storage. The pH measurement of the breast meat was carried out as per method of Trout *et al*. (1992) with the help of digital pH meter equipped with a combined glass electrode. The pH meter was calibrated with standard buffer solutions of pH 4, 7 and 9. About 10g meat sample after homogenization in 50 ml distilled water for 1 minute were used for pH measurement. The reading was recorded at 25ºC after the electrode was stabilized.

The water holding capacity of the meat determines the functional characteristics of meat because of its key role in determining the sensory quality of meat and for successful product formulation. The water holding capacity of breast meat was estimated according to Petracci *et al.* (2012) with slight modifications

through pressing the weighed meat sample and expressed as the difference between weights.

3.10.3 Sensory evaluation of broiler meat

Nine consumer based sensory panel was constituted to evaluate the organoleptic quality of breast meat of broilers raised under different dietary treatments. Deep frozen (-18±2℃) breast meat wer e thawed at 4℃ for 24h before sensory testing. Cleaned thawed coded meat samples were cooked in microwave oven at 80℃/45 minutes, cooled and cut into small parts before presented to sensory panel. The order in which the samples were presented to the panelists was randomized to avoid sampling bias. Each panelist was then asked to evaluate the cooked chicken breast meat separately for appearance, flavor, tenderness, stickiness to mouth, juiciness and overall acceptability using a 9-point Hedonic Scale in which scale 1 indicates extreme dislike, 5 indicates neither like nor dislike and 9 indicates extreme likeness (Meilgaard *et al*., 2007).

3.12 Statistical Analysis

The experimental data were subjected to statistical analysis (SPSS *Ver.* 20.0) using one way analysis of variance as described by Snedecor and Cochran (2004) to test for significant variation between treatment groups. Probabilities values of less than 0.05 ($p<0.05$) were considered significant. Comparison of mean values was carried out by Duncan's Multiple Range Test (Duncan, 1955). The results were interpreted and expressed as means ± pooled SEM.

4. RESULTS AND DISCUSSION

The data on various parameters recorded during the present investigation have been statistically analyzed and the observed results are presented and discussed under the following headings:

4.1 Chemical Evaluation of Broiler Starter, Broiler Finisher, *W. somnifera* Root Powder and Synbiotic

4.1.1 Proximate composition and mineral constituents

4.1.2 Alkaloid content of *W. somnifera*

4.2 Meteorological Pattern during Experimental Trial

4.3 *In vivo* Evaluation of *W. somnifera* and Synbiotic in Broiler Chicks (Feeding Trial)

4.3.1 Proximate composition of treatment mixtures

4.3.2 Feed Intake

4.3.3 Body weight gain

4.3.4 Feed conversion ratio

4.3.5 Protein efficiency ratio and performance index

4.3.6 Mortality

4.4 Metabolism/Digestibility Trial

4.4.1 Digestibility of proximate principles

4.4.2 Balance of nitrogen, calcium and phosphorus

4.5 Haemato-serobiochemical Parameters

4.5.1 Haemoglobin and erythrogram

4.5.2 Leucogram

4.5.3 Blood glucose

4.5.4 Serum TSH, triiodothyronine and thyroxin hormones

4.5.5 Serum protein profile

4.5.6 Serum lipid profile

4.5.7 Serum mineral profile

4.5.8 Serum enzyme profile

4.6 Immunological Parameters

4.7 Carcass evaluation

4.7.1 Carcass yield and meat yield

4.7.2 Giblet and offals yield

4.7.3 Shank length, intestine length and caecal length

4.8 Evaluation of Gut Health

4.8.1 Intestinal pH

4.8.2 Total coliforms count

4.9 Meat Quality Evaluation

4.9.1 Proximate composition of broiler meat

4.9.2 pH and water holding capacity

4.9.3 Sensory characteristics of broiler meat

4.10 Economics of Single and Combined Use of *W. somnifera* and Synbiotic in Ration of Broiler Chicks

A growth study involving feeding trial, digestion cum metabolic trial, haemato-serobiochemical profile, immunological parameters, carcass and meat quality evaluation was carried out during 42-day trial to study and assess the effect of different levels of supplementation of *W. somnifera* (ashwagandha) and synbiotic alone or in combination in the diets of broiler ration.

4.1 Chemical Evaluation of Broiler Starter, Broiler Finisher, *W. somnifera* and Synbiotic

4.1.1 Proximate composition and mineral constituents

Proximate composition and mineral constituents of plants provides valuable information about its medicinal and nutritional quality (Hameed and Hussain, 2015). The roots of ashwagandha contain low quantity of protein than other parts of the plants (Khanna *et al.,* 2006). The present investigation also observed low quantity of protein in analyzed plant roots. The crude protein content of ashwagandha estimated in the present study was 5.97% (**Table 3.2**) which is in close agreement with values (5.6%) reported by Verma and Gaur (2011). Relatively higher total soluble protein concentration of 6.8 mg/gm was found in the whole plant of *W. somnifera* (Sharma *et al*., 2014). Hameed and Hussain (2015) reported higher values of CP (6.60%) and ether extract (3.51%); lower values of ash (3.62%) and an analogous NFE (72.62%) and CF (13.65%) content in roots of *W. somnifera* on DM basis than observed in the present study. In contrast, remarkably lower values of CP (4.21%), EE (0.32%), NFE (53.91%), ash (4.76%); and higher values of CF (34.90%) and calcium (2.3%) were reported by Kumari and Gupta (2016). The variability in nutritional composition of *W. somnifera* could be ascribed to phenological stage of the plants (Hameed and Hussain, 2015).

4.1.2 Alkaloid content of *W. somnifera*

The content of total alkaloid in ashwagandha root powder has been reported to vary between 0.13 to 0.31% and up to 4.3% value have been recorded (Anonymous, 1976). The total alkaloid estimated in the *W. somnifera* root sample in the present study was 3.4%. The higher alkaloid content obtained in the present study might be related with the superior quality grade ‘A’ roots procured for investigation. It was reported that size and diameter of the ashwagandha roots affects the alkaloid content (Rao *et al*., 2012) that ultimately affects the pharmacological activity of ashwagandha (Chaudhari *et al*., 2013). The qualitative

assessment of ashwagandha roots by Verma and Gaur (2011) highlighted the presence of higher alkaloid content in the roots than seeds. The distinctive earthy odour and flavour to the ashwagandha root powder was also contributed by its alkaloids content (Bhatnagar, 1976).

4.2 Meteorological Pattern during Experimental Trial

Huge economic losses often occur in broiler farming due to higher ambient temperature and erratic climatic pattern. Climate induced heat stress is a worldwide problem particularly in broiler lines and affects the growth and performance through direct effects on organ and muscle metabolism when the ambient temperature reaches above 27ºC. Broilers chickens are particularly more sensitive to temperature-associated environmental challenges as they are devoid of sweat glands and are fully covered with feathers. Reduced feed intake (16.4%), impaired growth performance, higher feed conversion ratio (25.6%), reduced dietary digestibility, decreased plasma protein and calcium levels have been reported in broilers that were subjected to chronic heat stress (Sohail *et al*., 2012). VenCobb strain of broiler developed for faster growth and production are particularly more vulnerable to environmental stress due to their greater metabolic activity and more body heat (Deeb and Cahaner, 2002).

The semiarid region of Bikaner (Rajasthan) is known for its harsh, extreme climate with scanty and erratic rainfall. The impact of global warming has further worsened the situation. Weekly mean ranges of several climatic variables during experimental periods are considered in the present study **(Table 4.1)**. The values and trends of mean weekly temperature, relative humidity and temperature humidity index (THI) are depicted in **Fig 4.1**. The mean weekly temperature was observed to be higher in all the weeks except second week. The temperature fluctuation was found to be erratic in nature. Higher ambient temperature indicated the heat stress condition. The boilers were exposed to gradually declining relative humidity till fourth week that suddenly dipped in fifth week and again increased in the last week of study. The erratic pattern of climate change in terms of temperature and humidity particularly during the fifth and sixth week suggest the sudden stress on the body condition of broilers. However, the continuous high THI value above thermo comfort zone except second week of trial indicated the high level of environmental stress during the whole trial period. It was reported that higher ambient temperatures >34ºC after three weeks of age results in significant deterioration of meat quality in broilers (Yalcin *et al*., 1999).

Table 4.1 Ambient temperature, relative humidity and THI range observed during different weeks of experimental trial

Week of Trial	Ambient Temperature (°C)			Relative Humidity (%)			THI		
	Min	Max	Avg	Min	Max	Avg	Min	Max	Avg
Week-1	13.00	36.60	33.00	45.00	96.00	83.43	56.28	91.81	88.18
Week-2	12.50	34.60	29.83	47.00	87.00	80.28	55.28	90.42	82.73
Week-3	13.40	36.60	34.59	22.00	83.00	76.14	62.31	91.94	89.55
Week-4	11.00	39.40	35.12	13.00	82.00	63.86	64.07	90.41	87.60
Week-5	16.40	42.40	38.40	14.00	58.00	43.28	60.31	93.34	87.96
Week-6	18.20	37.40	36.57	23.00	67.00	54.71	63.19	90.18	88.11

4.3 *In vivo* Evaluation of *W. somnifera* and Synbiotic in Broiler Chicks (Feeding Trial)

4.3.1 Proximate composition of treatment mixtures

The proximate composition of different treatment mixtures were chemically evaluated on three different occasions to test for significant difference in crude protein and other nutrients due to inclusion of test materials in basal diets. The proximate compositions of different treatments are represented in **Table 4.2**. The treatments were found to be statistically similar and same level of nutrients were estimated under different treatments.

4.3.2 Feed intake

The average feed intake of broilers under different treatments on weekly basis has been presented in **Table 4.3**. The feed intake of broilers in control group (T_1) was found to be significantly ($p<0.05$) lower compared to one or more treatments in any particular week. However, a non-significant variation in feed intake among all treatments was observed in 4^{th} week. During 3^{rd} week of experiment, feed intake in all the treatments was statistically similar except control broilers (T_1). The feed intake performance of T_5 and T_6 group showed downward trend in the present study with the increase in ambient temperature. The treatment group T_8, exhibited remarkably higher feed intake ($p<0.05$) during period of extreme stress (5^{th} week) with non-significant difference with T_4 group. Similarly feed intake values of T_4 and T_8 groups were significantly deviated from control in 6^{th} week of feeding trial. The total feed intake over all the weeks was found to be similar ($p>0.05$) in T_1, T_2, T_3, T_5, T_6, T_7 groups except T_4 and T_8 groups (**Fig. 4.2**). The failure of 0.25% level of *W. somnifera* present in T_7 group to produce any significant variation in feed intake of broilers corresponds to observation made by Joshi *et al*. (2015) in broilers raised on 0.1% or 0.2% ashwagandha root powder.

Synbiotic was found to be more effective in enhancing the feed intake during initial half of the trial (Abdel-Rehman *et al*., 2011) in which the relatively favourable environment conditions were present. Sohail *et al*. (2013) also observed increase feed intake in broilers raised on synbiotic supplemented feed.

Fig. 4.1 Temperature, relative humidity and THI trend during different weeks of experimental trial

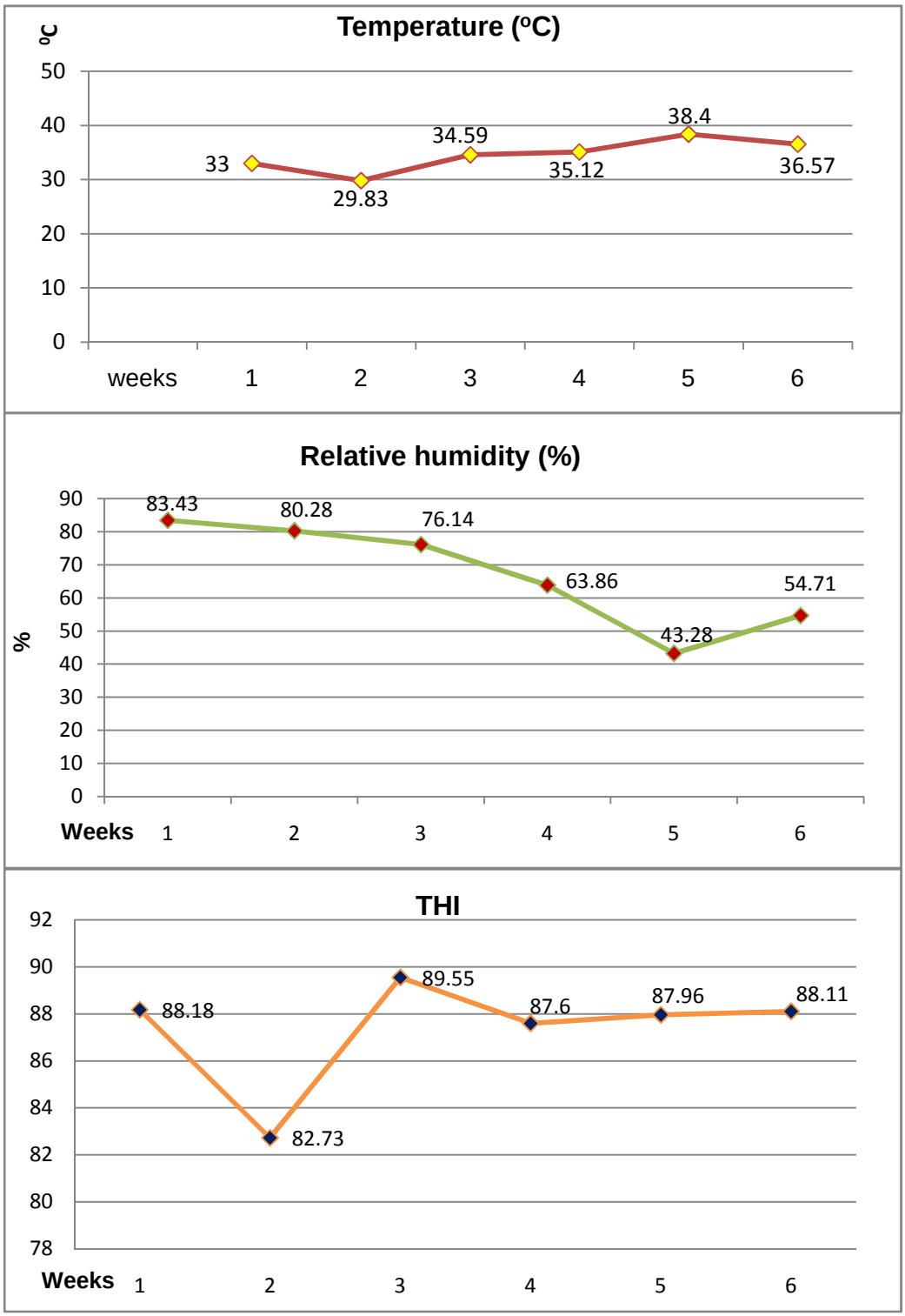


Table 4.2 Proximate composition[1] of different treatment mixtures (DM basis) used during feeding trial

Parti-culars	Treatment Mixtures							
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
	C	0.5% WS	1.0% WS	1.5% WS	0.025% Syn	0.05 % Syn	0.25% WS + 0.025% Syn	0.5% WS +05% Syn
Broiler starter								
Proximate principles (%)								
DM	92.69	94.28	93.19	93.38	93.45	93.65	93.5	93.71
CP	22.87	22.71	22.30	22.57	22.49	22.73	22.74	22.88
EE	5.82	6.33	5.76	5.47	5.55	6.05	6.50	5.70
CF	3.96	4.2	4.32	4.75	4.17	4.22	4.23	4.74
TA	6.20	6.17	6.17	6.10	6.23	6.10	6.27	6.30
NFE	61.14	60.59	61.45	61.12	61.56	60.91	60.03	60.38
AIA	0.92	0.99	0.88	0.82	1.03	0.87	0.89	0.82
Mineral composition (%)								
Ca	1.02	1.01	1.02	1.01	1.03	1.08	1.04	1.04
P	0.97	0.92	0.92	0.94	0.92	0.95	0.89	0.93
Broiler finisher								
Proximate principles (%)								
DM	94.37	94.81	94.29	94.09	94.26	93.71	94.52	94.14
CP	20.10	20.07	19.79	19.70	19.88	20.45	19.60	20.10
EE	6.90	7.50	7.55	7.39	7.82	7.60	6.86	7.40
CF	4.17	4.30	4.00	4.23	4.10	4.13	4.13	4.23
TA	5.90	6.07	6.50	6.00	6.23	6.53	6.00	6.03
NFE	62.93	62.06	62.16	63.28	61.96	61.28	63.41	62.23
AIA	1.00	1.00	0.90	1.00	1.00	1.00	1.10	0.90
Mineral composition (%)								
Ca	1.04	1.05	1.05	1.05	1.06	1.07	1.05	1.06
P	0.81	0.91	0.91	0.92	0.97	0.96	0.81	0.86

[1] Average of the values determined on samples compounded on three occasions

C Control WS *Withania somnifera* Syn synbiotic

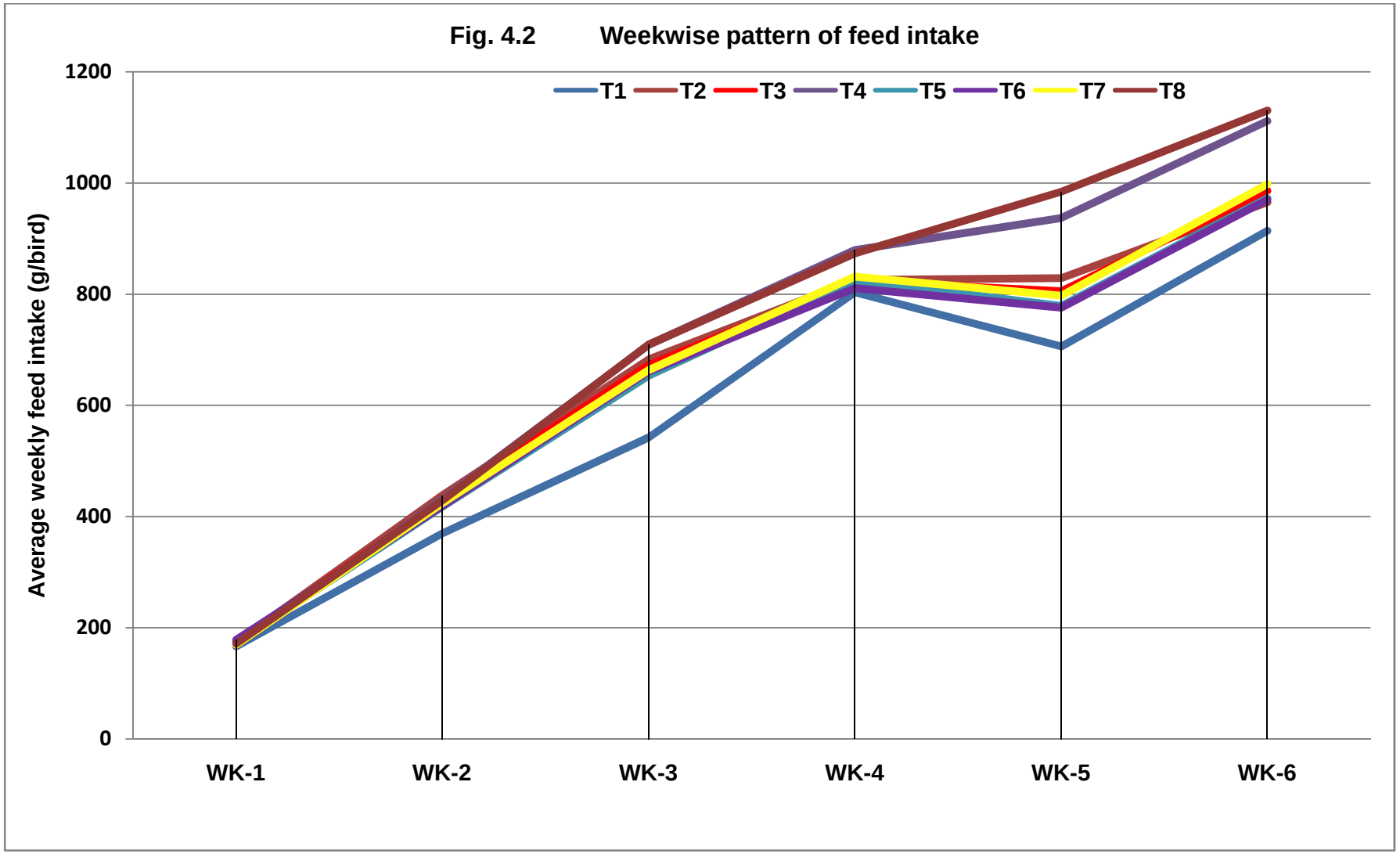
Table 4.3 Weekly feed intake (g/bird) of broilers under different treatments

Weeks	Treatment Groups								SEM
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	
	C	0.5% WS	1% WS	1.5% WS	0.025% Syn	0.05% Syn	0.25% WS +0.025% Syn	0.5% WS +0.05% Syn	
Week- 1	166.58^{a}	173.33^{ab}	172.67^{ab}	167.78^{a}	172.89^{ab}	178.45^{b}	169.11^{ab}	171.77^{ab}	1.00
Week- 2	369.81^{a}	438.23^{c}	430.11^{bc}	431.03^{bc}	417.39^{b}	419.11^{b}	423.67^{bc}	428.76^{bc}	1.99
Week- 3	542.27^{a}	683.11^{b}	672.69^{b}	709.06^{b}	653.65^{b}	660.99^{b}	663.67^{b}	709.60^{b}	8.13
Week- 4^{NS}	803.51	825.64	823.53	879.35	824.58	811.07	831.58	873.29	11.57
Week- 5	705.93^{a}	828.91^{ab}	805.61^{ab}	936.96^{bc}	779.12^{ab}	776.09^{ab}	797.18^{ab}	984.23^{c}	18.62
Week- 6	914.22^{a}	965.43^{ab}	986.01^{ab}	1111.62^{b}	972.67^{ab}	970.07^{ab}	997.84^{ab}	1130.12^{b}	20.11
Cumulative Feed Intake	3651.27^{a}	3966.82^{ab}	3890.61^{a}	4235.80^{bc}	3847.8^{a}	3864.98^{a}	3933.20^{ab}	4297.78^{c}	35.77

Means in the same row bearing different superscripts are significantly different ($p<0.05$).

C Control W S *Withania somnifera* Syn Synbiotic

Fig. 4.2 Weekwise pattern of feed intake



In difference with the findings of the present trial, few studies (Bozkurt *et al*., 2009; Dizaji *et al*., 2012; Saiyed *et al*., 2015) have failed to report any significant effect of inclusion of synbiotics on the feed intake of broilers. The variation in response for feed intake could be due to broiler strain, composition of synbiotic employed and the level of thermal stress /comfort experienced by the broiler under different studies.

The present study observed a dose dependent effect of ashwagandha on feed intake with level of climatic stress. Low quantity of ashwagandha was found to be sufficient to raise feed intake in broilers during period of low environmental stress in earlier weeks whereas higher level of ashwagandha (1.5%) was required to maintain the feed intake during period of high heat stress. The level of ashwagandha required to maintain feed intake depends on the climatic conditions prevailing in a particular geographical region. The present study observed a 1.5% level of *W. somnifera* to be optimum for the adverse climate of semi arid region to induce similar difference (584.53 g) in feed intake as observed by Vasanthakumar *et al*. (2014) for 1% level of *Withania* inclusion in broilers diets in Southern parts of India.

The use of ashwagandha root powder was proved to be more efficacious in enhancing the level of feed intake in broilers in the present study than the root extract of *W. somnifera* used by Mushtaq *et al*. (2011). The average weekly feed consumption of broiler (g/bird) from 1 to 6 week of age as a result of dietary inclusion of *W. somnifera* were observed to be higher than values reported by Sanjyal and Sapkota (2011) and Srivastava *et al*. (2012). An increase in feed intake of around 8.64% (T_2), 7.72% (T_7), 16% (T_4) and 17.71% (T_8) than control broilers was observed in the present study than value (7.9%) reported by Ansari *et al*. (2008) in 0.4% *Withania* treated broilers. The significantly enhanced feed consumption on *Withania* supplemented diet observed by Rindhe *et al*. (2012) in last three weeks (4^{th} to 6^{th} week) of trial in broilers was not observed in current trial except at higher level of incorporation, *i.e.,* 1.5%, or in combined approach adopted in T_8 group.

4.3.3 Body weight gain

The genetically regulated embryonic and early growth is greatly influenced by nutritional regimen to exploit the full genetic potential of broilers particularly in fast growing strains such as VenCobb 400 broilers. The average body weight and body weight gain of broilers fed diets supplemented with different source and level of

growth promoters on the basis of individual weeks has been presented in **Table 4.4** and **Table 4.5**.

The average weekly body weight was found to be higher in supplemented groups than control for overall experimental period. The performance of control broilers was found to be statistically lower in comparison to treatment groups receiving either *Withania* or synbiotic or their combinations in all the weeks. The graded level of *Withania* supplementation (T_2-T_4) was found to affect body weight in a similar fashion during initial three weeks of experiment which suggest that 0.5% level is an optimum dose of *Withania* for growth of broilers in initial stages of growth in broilers. However, higher level of *Withania* (1.5%) in broiler diet was able to produce significant difference in body growth with advancement in age from 3rd week onwards and was found to be most successful in negating the effect of consistently higher temperature without compromising the growth of broilers. The body weight gain achieved under T_2 to T_4 treatments also revealed non-significant variation ($p>0.05$) among themselves except in 6th week in which 1.5% *Withania* fed broilers gained significantly higher body weight than 0.5% *Withania* supplemented broilers.

The T_2 and T_3 graded level of either *Withania* or T_5 and T_6 level of synbiotic failed to demonstrate any statistical difference between them during whole trial. Thus 0.025% level of synbiotic in diet was observed to be sufficient to achieve comparable body weight than broilers raised on 0.05% synbiotics. Similar to body weight statistics, no statistical difference between the two levels of synbiotic on weekly body weight gain was observed in the present study. The growth promoting effect of synbiotic in T_5 and T_6 treatment groups were most pronounced during initial stages of life when the mean weekly ambient temperature was lower and the growth related features like feathering were less developed to prevent heat dissipation from the body. The 0.5% *Withania* fed broilers (T_2) exhibited comparable performance to 0.025% synbiotic fed broilers (T_5) during the whole trial except in 5th week which supports the antistressor effect of *Withania* in broilers and failure of synbiotic fed broilers to resist the sudden rise in ambient temperature. The combined feeding approach at low level of supplementation of *W. somnifera* and synbiotic (T_7 group) demonstrated equivalent performance with all other treatments except 1.5% *Withania* supplemented broilers (T_4) and combined treatment group (T_8).

Table 4.4 Weekly body weight (g/bird) of broilers under different treatments

Week	Treatment Groups								SEM
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	
	C	0.5% WS	1.0 % WS	1.5% WS	0.025% Syn	0.05% Syn	0.25% WS +0.025% Syn	0.5% WS +0.05% Syn	
0- Day^{NS}	42.67	42.78	42.69	42.82	42.82	42.84	42.71	42.78	0.048
Week-1	160.78^{a}	167.22^{b}	168.96^{bc}	170.42^{bcd}	173.24^{bcd}	175.09^{cd}	172.20^{bcd}	176.7^{d}	0.762
Week-2	386.53^{a}	438.98^{b}	440.29^{bc}	448.29^{bc}	450.04^{bc}	455.02^{bc}	452.69^{bc}	456.91^{c}	1.95
Week-3	708.8^{a}	881.07^{bc}	881.33^{bc}	912.96^{c}	852.00^{b}	870.62^{b}	871.51^{b}	914.62^{c}	3.96
Week-4	1140.89^{a}	1374.22^{b}	1375.11^{b}	1450.44^{c}	1332.67^{b}	1348.89^{b}	1358.89^{b}	1453.78^{c}	6.70
Week-5	1481.81^{a}	1810.68^{c}	1803.33^{c}	1963.11^{d}	1722.05^{b}	1748.05^{bc}	1776.14^{bc}	1988.89^{d}	8.83
Week-6	1882.76^{a}	2299.89^{b}	2311.67^{b}	2548.33^{c}	2177.63^{b}	2219.48^{b}	2269.66^{b}	2578.87^{c}	18.14

Means in the same row bearing different superscripts are significantly different ($p<0.05$).

NS Non significant C Control W S *Withania somnifera* Syn Synbiotic

Table 4.5 Weekly body weight gain (g/bird) of broilers under different treatments

Weeks	Treatment Groups								SEM
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	
	C	0.5% WS	1.0 % WS	1.5% WS	0.025% Syn	0.05% Syn	0.25% WS +0.025 % Syn	0.5% WS +0.05% Syn	
Week-1	118.07^{a}	124.40^{b}	126.20^{bc}	127.49^{bcd}	130.40^{bcd}	132.18^{cd}	129.53^{bcd}	133.98^{d}	0.76
Week-2	225.76^{a}	271.76^{b}	271.33^{b}	277.87^{b}	276.80^{b}	279.93^{b}	280.49^{b}	280.16^{b}	1.94
Week-3	322.27^{a}	442.09^{cd}	441.04^{cd}	464.67^{d}	401.96^{b}	415.60^{bc}	418.82^{bc}	457.71^{d}	4.34
Week-4	432.09^{a}	493.16^{bc}	493.78^{bc}	537.49^{c}	480.67^{b}	478.27^{b}	487.38^{b}	539.16^{c}	5.39
Week-5	336.00^{a}	433.64^{ab}	428.22^{ab}	512.67^{bc}	387.05^{a}	403.27^{a}	420.45^{ab}	535.11^{c}	10.97
Week-6	397.81^{a}	489.20^{b}	508.33^{bc}	585.22^{c}	453.44^{ab}	471.43^{ab}	493.52^{b}	589.98^{c}	10.55
Cumulative Weight Gain	1840.12^{a}	2257.07^{b}	2268.93^{b}	2505.5^{c}	2134.91^{b}	2176.69^{b}	2226.92^{b}	2536.10^{c}	18.14

Means in the same row bearing different superscripts are significantly different ($p<0.05$).

C Control W S *Withania somnifera* Syn Synbiotic

The synergistic effect of supplementation of 0.5% *Withania*+0.05% synbiotic on body weight gain was evident throughout the trial. Broilers supplemented with 0.5% *W. somnifera*+0.05% synbiotic achieved maximum body weight over all other treatments at the end of each week without being affected by the continuous high THI and adverse ambient temperature conditions. The parallel performance of T_8 group with 1.5% level of *Withania* (T_4) indicates the potentiating effect of synbiotic on the pharmacologic effect of *Withania*. The cumulative body weight gain revealed nonsignificant variation among T_2, T_3, T_5, T_6 and T_7 groups with lowest weight gain in T_1 and highest weight gain in T_4 and T_8 treatment groups. The significant ($p<0.05$) anabolic effect of *W. somnifera* (Joshi *et al*., 2015) and synbiotic either alone or their combination was observed in the present study.

A downward trend in body weight gain of all treatments (**Fig. 4.3**) was evident at the end of 5th week which might be due to sudden rise of about 3.28^{0}C mean weekly temperature. However, the depression in body weight gain was significantly less pronounced in broilers supplemented with 1.5% *W. somnifera* or 0.5% *W. somnifera*+0.05% synbiotic based diet, similar to observation recorded in *Withania* supplemented Salmonella infected broilers (Kumari *et al*., 2015). The depression in body weight gain though reverted in subsequent week with reduction in ambient temperature (up to 2^{0}C), but the resilience to regain body weight was found to be remarkably higher in *Withania* supplemented groups, *i.e.,* T_2 to T_4 and T_7 to T_8. Broilers treated with *W. somnifera* root powder @ 1.5% or 0.5% *W. somnifera* with 0.05% synbiotic were found to be least sensitive to environmental changes and especially managed to escaped from adverse effect of climate in 5th week of trial.

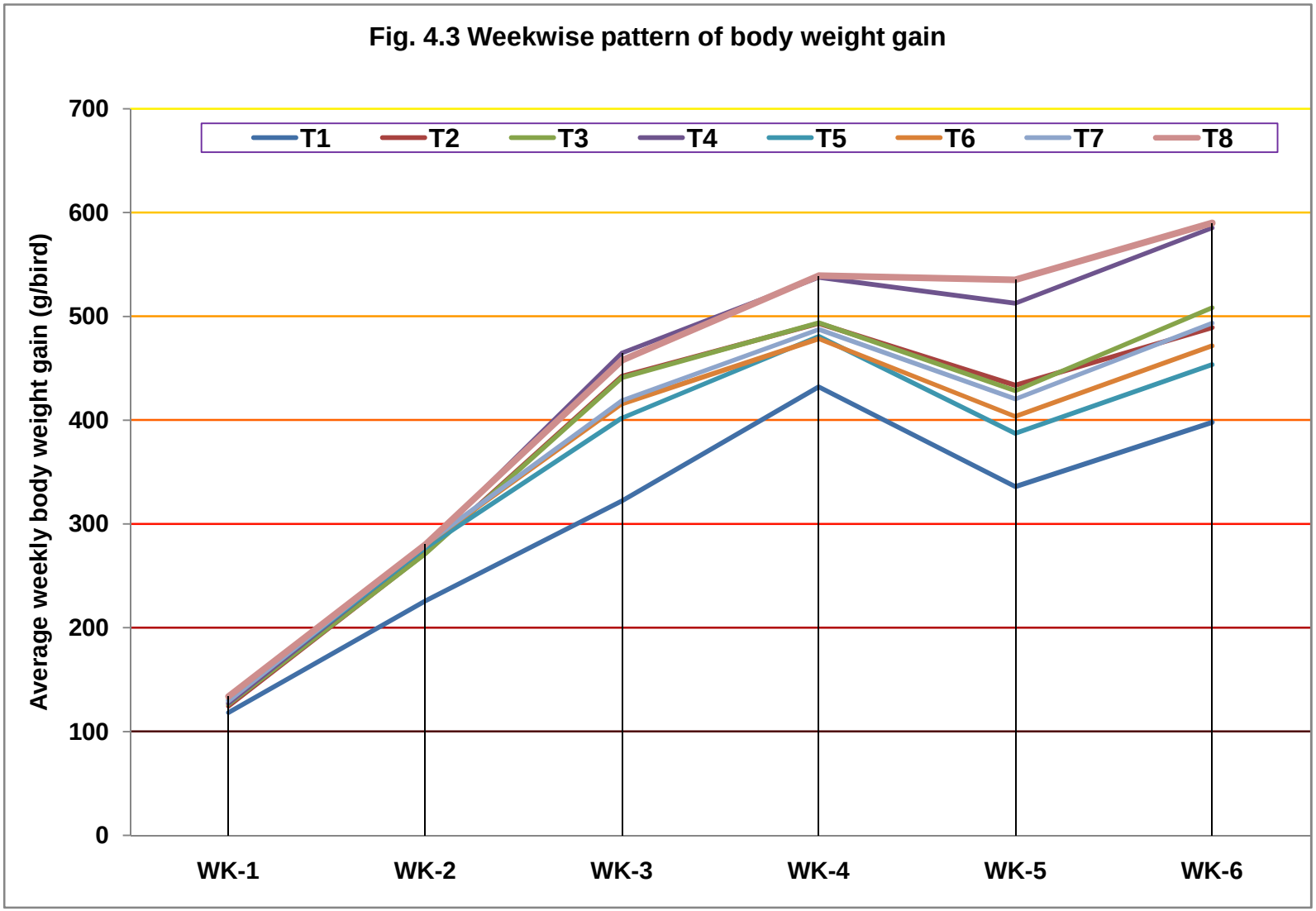
The improvement in growth rate in synbiotic supplemented groups was found to be 16.02% and 18.29% when compared with control in T_5 and T_6 groups, respectively (Bozkurt *et al*., 2009). The observed effects of synbiotic on body weight and body weight gain were in agreement with the results of Abdel-Raheem *et al*. (2011), Ghasemi and Taherpour (2013); and Dizaji *et al*. (2012) but varied in view with the results of Salianeh *et al*. (2011) and Saiyed *et al*. (2015). Similar to the observed overall improvement in weight gain of broilers under heat stress in synbiotic groups, Sohail *et al*. (2013) reported mild to moderate improvement in overall weight gain in heat stressed synbiotic fed groups. A trial on synbiotic formulation (Ahmed *et al*., 2015b) similar to that used in the present study reported similar body weight and weight difference at 4th week of trial between control and 0.025% synbiotic fed broilers.

The results pertaining to significantly higher weight gain in T_2 and T_3 broilers on similar feed intake to that of control group indicate high feed efficiency on *Withania* based diets which is in agreement with the findings of Shisodiya *et al*. (2008) who also observed significantly higher body weight in broilers on low quantity of feed containing *Withania* as feed additive. The growth promoter effect of 0.5% ashwagandha containing diet (T_2) was observed to be higher (2257.07g) than observation made by Ansari *et al*. (2008) and Vasanthakumar *et al*. (2014) who reported 1819g and 2214.78 g body weight in VenCobb 400 broilers for 0.4% and 0.5% *Withania* based diet, respectively. The findings obtained for 1% level of ashwagandha supplementation (2268.93g) are also higher than the weight gain (2126.38 g) recorded by Pandey *et al*. (2013) for similar level of ashwagandha incorporation in diet of broilers. The results are in close agreement with an investigation conducted in VenCobb 400 broilers during summer season by Srivastava *et al*. (2012) who observed weekly body weight gain of 100 g (1), 290 g (2), 340 g (3), 540 g (4), 450 g (5) and 450 g (6) on 2% indigenous herbal formulation containing ashwagandha as main ingredient.

The observed improvement in body weight with age under growth and environmental stress with different level of ashwagandha is in line with findings of Ahmed *et al*. (2015a) who suggested dose dependent effect of ashwagandha on stimulation of thyroid gland directly and/or through the pituitary gland to secret more thyroid hormones. The significant improvement in weight gain with *Withania* supplementation despite continuous high THI (>87.6) in almost all weeks reinforces the findings made by Sujatha *et al*. (2010) in broilers who observed improved weight performances when broilers were reared under high THI (84.74±2.51) above the thermo comfort zone of broilers.

However, in contrast to the present findings, Thange *et al*. (2009) did not observed any effect of various doses of dietary addition of *W. somnifera* on body weights in broilers.

Fig. 4.3 Weekwise pattern of body weight gain



4.3.4 Feed conversion ratio

The economics of broiler rearing depends on the capacity of the broiler to convert feed into body mass. Birds with low FCR could provide higher rate of gain in broiler farming. The addition of phytogenic feed additive *W. somnifera* and/or synbiotic substance in the diet of broiler chicken significantly alters feed conversion rate through modification in metabolism of feed ingredients and vital parameters. The treatment wise FCR under different weeks has been presented in **Table 4.6**.

The weekly FCR of non-supplemented broilers (T_1) was found to be significantly ($p<0.05$) higher in most weeks of feeding trial with cumulative FCR of 1.90. Most treatments (T_1-T_6) in first week revealed non-significant change in FCR (Ansari *et al*., 2008) which is indicative of major role of genetics and maternal effect in earlier stages of broiler life. The impact of either feed additive or their combination and level on FCR become increasingly evident with the advancement in broiler age (**Fig. 4.4**). The overall FCR was found to be significantly lowest ($p<0.05$) in broilers under treatment groups T_3, T_4 and T_8. The synbiotic added groups, T_5 and T_6 performed better until ambient temperature was within acceptable range, *i.e.,* 2nd week. The graded level of ashwagandha supplementation in T_2 to T_4 groups produced an inverse pattern of FCR with increasing level of environmental stress due to continuous rise in ambient temperature from 2nd week onwards. The integrative approach adopted in T_8 treatment was observed to be most fruitful in conversion of feed materials into body masses during the whole trial. The inclusion of 0.025% synbiotic produced intermediate change in FCR.

The differently formulated synbiotic used in the present study was proved to be beneficial in lowering FCR values than reported by Abdel- Raheem *et al.* (2011) and Dizaji *et al*. (2012) in broilers fed synbiotic with different composition. The present study observed similar FCR value in synbiotic added group as reported by Ahmed *et al*. (2015b) in broilers who used similar synbiotic having same composition. Significant reduction in FCR with inclusion of synbiotic in basal feed was in agreement with similar studies conducted in broilers (Awad *et al*., 2008; Bozkurt *et al*., 2009 and Al-Sultan *et al*., 2016). The results are in contrast to the findings of Jung *et al*. (2008) who observed non significant effect of synbiotic on FCR.

Table 4.6 Weekly feed conversion ratio of broilers of broilers under different treatments

Week	Treatment Groups								SEM
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	
	C	0.5% WS	1.0 % WS	1.5% WS	0.025% Syn	0.05% Syn	0.25% WS +0.025 % Syn	0.5% WS +0.05 % Syn	
Week-1	1.41^{c}	1.39^{bc}	1.37^{abc}	1.32^{abc}	1.33^{abc}	1.35^{abc}	1.31^{ab}	1.28^{a}	0.01
Week-2	1.64^{c}	1.61^{bc}	1.59^{abc}	1.55^{abc}	1.51^{a}	1.5^{a}	1.51^{a}	1.53^{ab}	0.01
Week-3	1.68^{d}	1.55^{ab}	1.53^{a}	1.53^{a}	1.63^{c}	1.59^{bc}	1.58^{bc}	1.55^{ab}	0.005
Week-4	1.86^{f}	1.67^{cd}	1.67^{bc}	1.64^{ab}	1.72^{e}	1.70^{cde}	1.70^{de}	1.62^{a}	0.004
Week-5	2.09^{f}	1.92^{cd}	1.88^{bc}	1.83^{a}	2.02^{e}	1.93^{d}	1.89^{cd}	1.84^{ab}	0.005
Week-6	2.30^{g}	1.98^{c}	1.94^{b}	1.90^{a}	2.15^{f}	2.07^{e}	2.07^{d}	1.91^{ab}	0.004
Cumu-lative FCR	1.90^{e}	1.74^{bc}	1.71^{ab}	1.69^{a}	1.79^{d}	1.75^{c}	1.74^{bc}	1.69^{a}	0.003

Means in the same row bearing different superscripts are significantly different ($p<0.05$).

C Control W S *Withania somnifera* Syn Synbiotic

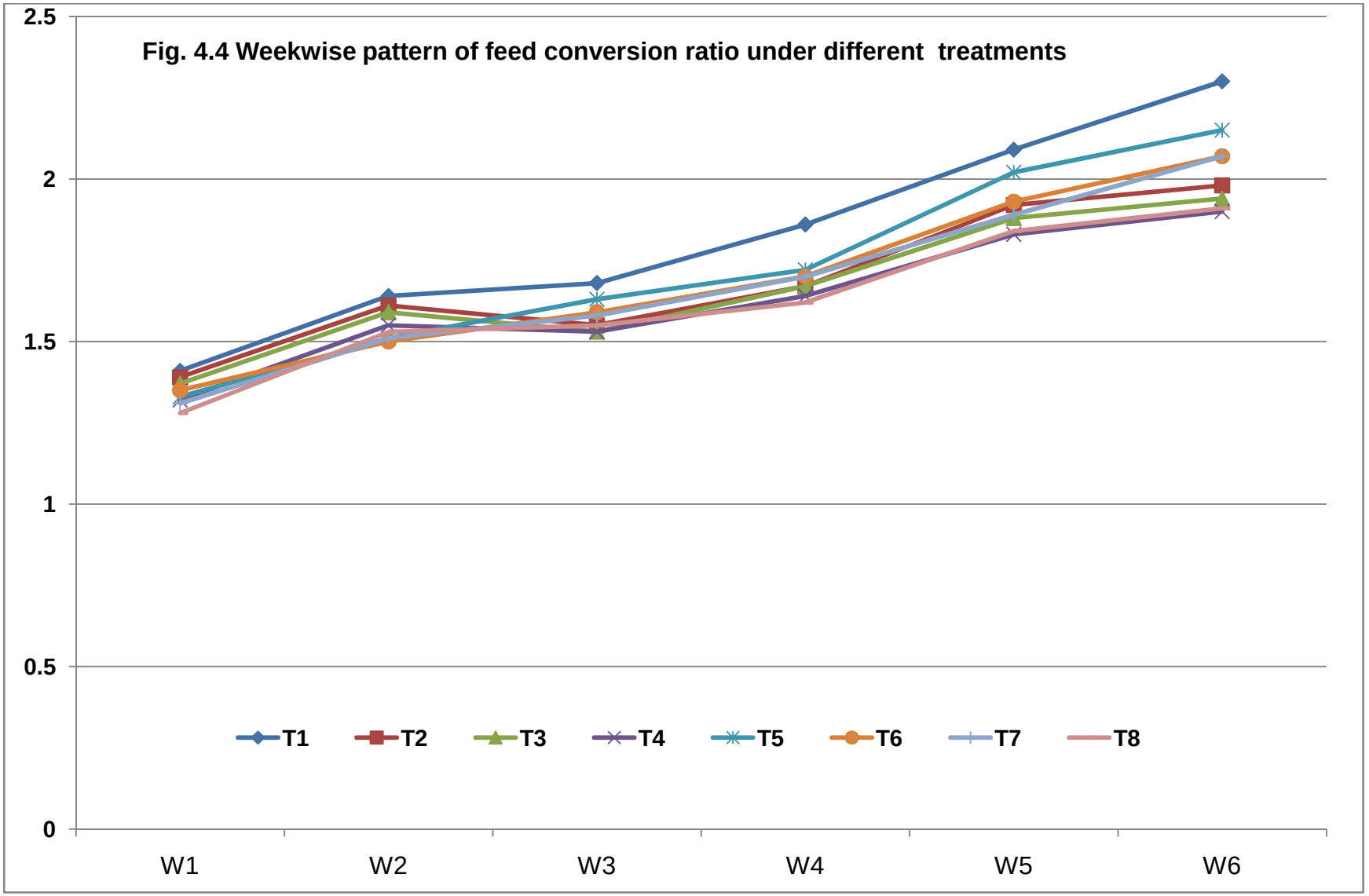


Fig. 4.4 Weekwise pattern of feed conversion ratio under different treatments
2.5
2
1.5
1
0.5
0
W1
W2
W3
W4
W5
W6
T1
T2
T3
T4
T5
T6
T7
T8

The observed beneficial effect of ashwagandha supplementation on FCR in the present study was also demonstrated by various workers (Srivastava *et al*., 2012). The FCR value (1.74) reported by both Kale *et al*. (2015) and Pandey *et al*. (2013) at 0.5% and 1% dietary inclusion of ashwagandha were closely observed in the present study for T_2 (1.74) and T_3 (1.71) treatment groups. The weekly FCR values 0.97 (1), 1.07 (2), 1.25 (3), 1.51 (4), 1.70 (5), 2.03 (6) reported by Ansari *et al*. (2008) at 0.4% level of *Withania* inclusion were not observed in the present study for any treatment during the whole trial.

The study conducted by Vasanthakumar *et al*. (2014) on VenCobb 400 broilers revealed significant difference in FCR at 0.15% ashwagandha root extract supplementation but failed to detect any significant effect of ashwagandha root powder on FCR. In contrast to present findings, Sanjyal and Sapkota (2011); and Joshi *et al*. (2015) reported nonsignificant variation in FCR of broilers raised on ashwagandha containing diets.

4.3.5 Protein efficiency ratio (PER) and performance index (PI)

4.3.5.1 Protein efficiency ratio (PER)

The results on effect of different feed additive and their combination on broiler performance indices such as protein efficiency ratio are presented in **Table 4.7.** Supplementation of any of the feed additive or their combination significantly increased PER in all the treatments during finisher phase and in most of the treatments during starter phase (p□0.05) of trial. Moreover, cumulatively this index was highest ($p<0.05$) in broilers fed 1% and 1.5% ashwagandha root powder than those of control and synbiotic fed groups at the end of 42 day trial. In starter phase, numerically higher but nonsignificant PER variation was recorded in 0.5% *Withania* fed group (T_2) and T_7 treatment group than control group during first and second week of trial which indicates insufficiency of feed additive quantity in initial two weeks. As the level of stress increased with age due to growth and ambient temperature, the comparable decrease in PER of control group and antistress effect of aswagandha become evident on PER. The cumulative PER varied between 2.50 (T_1) to 2.93 (T_3). The performance of synbiotic supplemented groups in terms of PER was higher than control broilers in both starter and finisher period (Ashayerizadeh *et al*., 2011) but lower than 1% and 1.5% ashwagandha treated broilers (p□0.05). Similarly, El-Katcha *et al*. (2014) also reported significant improvement in PER when broiler diets were supplemented with organic growth promoter.

The PER is affected by the composition of intestinal microflora. The efficiency of protein utilization decreases in the presence of harmful gut bacteria due to increased breakdown of proteins (Mikulec *et al*., 1999). In the present study, the beneficial effects of synbiotic products and *Withania* on broiler performance are in agreement with previous studies (Nayebpor *et al*., 2007; Falaki *et al*., 2010). The synergistic effect of probiotic and prebiotic in synbiotic mixture and their multiplicative effect on PER with the addition of ashwagandha could be due to reduction in count of unfavorable bacteria (Kumari and Gupta, 2015) and corresponding increase in beneficial microflora in the gut (Fairchild *et al*., 2001).

The resultant favourable medium in gut with effective increase in absorptive surface area of gut could be responsible for improvement in protein efficiency ratio (Savage and Zakrzewska, 1996; Santin *et al*., 2001). In contrast, Gunal *et al*. (2006), and Willis *et al*. (2007) reported that addition of these additives in the broiler ration had no significant effect on growth performance of broiler chickens.

4.3.5.2 Performance Index (PI)

Effect of dietary addition of feed additive on performance index of broiler chickens are presented in **Table 4.8.** Statistical analysis of the obtained data indicated that synbiotic or *Withania* or their combination in basal diet significantly increased the performance index during most weeks of experimental period. It was observed that inclusion of ashwagandha at 0.5% and synbiotic at 0.05% in broiler chicken ration under T8 group significantly increased PI. The lowest PI value at the end of the trial was observed for nonsupplemented broilers (T_1). The overall PI value for treatment groups T_2 (131.56), T_3 (132.31), T_5 (123.11), T_6 (126.29) and T_7 (130.25) showed only numerical variation ($p>0.05$) over each other. Similarly comparable PI was observed between T_4 and T8 groups. The difference in PI (48.83) between the control (T_1) and T_8 group was quite similar (40.45) to that observed for Marjoram herb in broilers having antioxidant properties (Osman *et al*., 2010).

Inclusion of feed additive significantly improved (p 0.05) PI in the current trial when compared with broiler chicken group fed on the same diet without feed additive supplementation which is in contrast to the findings of El-Katcha *et al*. (2014).

Table 4.7 Weekly protein efficiency ratio of Broilers in different treatment groups

Week	Treatment Groups								SEM
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	
	C	0.5% WS	1.0% WS	1.5% WS	0.025% Syn	0.05% Syn	0.25% WS+ 0.025% Syn	0.5% WS+ 0.05% Syn	
Week-1	3.10^{a}	3.16^{ab}	3.64^{d}	3.42^{bcd}	3.48^{cd}	3.42^{bcd}	3.30^{abc}	3.43^{bcd}	0.032
Week-2	2.67^{a}	2.73^{ab}	3.15^{e}	2.90^{bcd}	3.06^{cde}	3.08^{de}	2.85^{abc}	2.89^{abcd}	0.023
Week-3	2.60^{a}	2.84^{bc}	3.26^{d}	2.95^{c}	2.84^{bc}	2.90^{c}	2.72^{ab}	2.84^{bc}	0.018
Week-4	2.68^{a}	2.98^{bc}	3.03^{bc}	3.09^{c}	2.93^{b}	2.91^{b}	2.94^{b}	3.07^{c}	0.013
Week-5	2.37^{a}	2.60^{bcd}	2.68^{de}	2.76^{e}	2.49^{b}	2.56^{bc}	2.65^{cde}	2.70^{de}	0.013
Week-6	2.16^{a}	2.52^{c}	2.61^{d}	2.66^{d}	2.34^{b}	2.39^{b}	2.48^{c}	2.60^{d}	0.009
Cumula-tive PER	2.50^{a}	2.75^{b}	2.93^{d}	2.88^{cd}	2.73^{b}	2.76^{b}	2.74^{b}	2.82^{c}	0.010

Means in the same row bearing different superscripts are significantly different ($p<0.05$).

C Control W S *Withania somnifera* Syn Synbiotic

Table 4.8 Weekly performance index of broilers in different treatment groups during trial

Week	Treatment Groups								SEM
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	
	C	0.5% WS	1.0% WS	1.5% WS	0.025% Syn	0.05% Syn	0.25% WS+ 0.025% Syn	0.5% WS + 0.05% Syn	
Week-1	8.38^{a}	8.95^{ab}	9.22^{ab}	9.70^{bc}	9.85^{bc}	9.79^{bc}	9.92^{bc}	10.45^{c}	1.13
Week-2	13.81^{a}	16.85^{b}	17.12^{bc}	17.92^{bcd}	18.36^{cd}	18.70^{d}	18.58^{cd}	18.32^{cd}	1.59
Week-3	19.162^{a}	28.61^{cd}	28.92^{cd}	30.46^{d}	24.72^{b}	26.13^{bc}	26.43^{bc}	29.52^{cd}	3.65
Week-4	23.23^{a}	29.45^{b}	29.61^{b}	32.85^{c}	28.02^{b}	28.20^{b}	28.57^{b}	33.29^{c}	3.54
Week-5	16.10^{a}	22.56^{b}	22.76^{b}	28.05^{c}	19.17^{ab}	21.29^{b}	21.73^{b}	29.10^{c}	5.44
Week-6	17.31^{a}	24.67^{bc}	26.21^{c}	30.81^{d}	21.15^{ab}	23.31^{bc}	23.90^{bc}	30.80^{d}	5.15
Cumula-tive PI	100.82^{a}	131.56^{b}	132.32^{b}	148.19^{c}	123.11^{b}	126.29^{b}	130.25^{b}	149.66^{c}	12.18

Means in the same row bearing different superscripts are significantly different ($p<0.05$).

C Control W S *Withania somnifera* Syn Synbiotic

Similar increase in PI was observed by Abdel-Majeed (2013) in Japanese quail. However, negative trend in performance index was reported by Hassan (2014) on supplementation of humic acid in broilers. The resultant improvement in FCR and performance index (Al-Sultan *et al*., 2016) on addition of synbiotics in basal feed alone or with combination of *W. somnifera* could be associated with more efficient nutrient utilization (energy, protein, minerals and vitamins) from feed.

4.3.6 Mortality

Significant reduction in mortality was ensured during the trial through proper handling and management in terms of feeding, watering, spacing, housing and disease prevention. The mortality of broilers under different treatments was recorded and has been presented in **Table 4.9.**

No mortality was observed in any of the treatment groups in initial four weeks of experiment. However, death of six and two broilers occurred in 5^{th} and 6^{th} week, respectively under different treatments due to sudden rise in ambient temperature from 35.12 ^{0}C to 38.4 ^{0}C. Two broilers of T_1 and one each from T_2, T_5, T_6 and T_7 groups were died during 5^{th} week; and one broiler each from T_1 and T_5 was died in 6^{th} week. The overall mortality rate under different treatments was observed to be 6.66% (T_1-Control), 2.22% (T_2), 4.44% (T_5), 2.22% (T_6) and 2.22% (T_7). The trial findings are in agreement with values reported by Joshi *et al*. (2015) who recorded 2.2% mortality in 0.1 and 0.2% *Withania* treated broilers. The broiler mortality rate was found to be reduced up to 1.42% on supplementation of 1% ashwagandha root powder (Pandey *et al*., 2013). The supplementation of ashwagandha root powder considerably decreased mortality (Kumari *et al*., 2015). The antistress and adaptogenic activity of ashwagandha imparted significant protection to broilers under treatment groups T_3, T_4 and T_8.

4.4 Metabolism/Digestibility Trial

4.4.1 Digestibility of proximate principles

The per cent digestibility coefficients of nutrients in the total ration of different treatment groups of experimental broiler chicks are presented in **Table 4.10**. The per cent dry matter digestibility of basal feed fortified with any of the feed additive was observed to be significantly higher (p□0.05) than nonsupplemented basal feed used for broilers in control group (T_1). The three level of ashwagandha affected the digestibility of dry matter in a similar manner with nonsignificant variation among them. Dry matter digestibility in synbiotic fed group (T_5 and T_6) also behaved in the

same manner to that of *W. somnifera*. Significantly highest dry matter digestibility (76.57%) than control was observed in T_8 broilers raised on both type of feed additive. Similar digestibility of dry matter ($p>0.5$) was found among treatment groups T_2, T_3 and T_6. The dry matter digestibility of *Withania* treated feed in T_4 group was nonsignificantly different from T_8 group. The improvement in weight gain of broiler chickens in T_4 and T_8 groups as observed in the present study may be attributed to the fact that the enhanced digestibility of dry matter of basal feed might have increased the availability of nutrients to birds for utilization and overall improvement of body weight. The improvement in body weight gain of broilers in T_5 and T_6 group in the present study could be due to control of pathogenic bacteria and modulation of intestinal morphology and expression of mucin and brush border enzyme.

The organic matter digestibility (OMD) exhibited similar significant variation among treatment groups to that of dry matter with remarkably ($p\square0.05$) lowest and highest values of OMD were observed for T_1 and T_8 group, respectively. OMD was not found to be affected with the level of either *Withania* or synbiotic. Comparable performance in OMD was recorded between T_4 and T_8 group.

The estimation of crude protein digestibility is an important parameter for the assessment of growth promoting effect of feed additive in basal diets. The digestibility of crude protein in ashwagandha supplemented treatment groups was invariably highest than all other groups including T_8 group in which 0.5% ashwagandha was added in diet. The enhanced digestibility of crude protein reflects the anabolic nature of ashwagandha (Singh *et al*., 2011) and supports the hypothesis that in addition to its antistressor effect, this phytoherb could be utilized as an organic source of growth promoter in broilers. The digestibility of crude protein in T_5, T_6 and T_7 groups were statistically similar and significantly higher than nonsupplemented broiler group. Improvement in synbiotic supplemented groups could be due to competitive exclusion of harmful gut pathogens and improved gut epithelial integrity (Yang *et al*., 2009). The lowest digestibility coefficient was observed for broilers under control group. The significance of crude protein variability over the treatments has been reflected in cumulative body weight gain at the end of the trial. The present study observed significant effect of organic growth promoter which is in contrast to the findings of Ang *et* al. (2009).

Table 4.9 Mortality pattern observed in broilers under different treatments during experimental trial

Treatments	Mortality Pattern	
	No. of birds died	Per cent mortality
T_1 (C)	3	6.66
T_2 (0.5% WS)	1	2.22
T_3 (1.0% WS)	0	0
T_4 (1.5% WS)	0	0
T_5 (0.025% Syn)	2	4.44
T_6 (0.05 % Syn)	1	2.22
T_7 (0.25% WS +0.025% Syn)	1	2.22
T_8 (0.5% WS + 0.05% Syn)	0	0

C Control W S *Withania somnifera* Syn Synbiotic

Table 4.10 Per cent digestibility coefficients of nutrients in the total ration of different treatment groups

Treatments	Digestibility coefficients of nutrients					
	DMD	OMD	CPD	EED	NFED	CFD^{NS}
T_1 (C)	73.32^{a}	76.53^{a}	73.19^{a}	79.52^{a}	78.24^{ab}	22.04
T_2 (0.5% WS)	75.29^{bc}	78.54^{bc}	80.53^{c}	83.27^{b}	79.47^{bc}	24.69
T_3 (1.0% WS)	75.26^{bc}	78.54^{bc}	80.15^{c}	83.61^{b}	77.74^{a}	24.67
T_4 (1.5% WS)	76.41^{cd}	79.68^{cd}	80.80^{c}	84.16^{b}	78.58^{abc}	24.93
T_5 (0.025% Syn)	74.92^{b}	78.21^{b}	77.46^{b}	83.06^{b}	78.50^{abc}	24.66
T_6 (0.05 % Syn)	75.30^{bc}	78.76^{bcd}	75.58^{b}	83.24^{b}	77.60^{a}	24.82
T_7 (0.25% WS + 0.025% Syn)	75.01^{b}	78.17^{b}	75.63^{b}	83.21^{b}	78.40^{ab}	24.74
T_8 (0.5% WS + 0.05% Syn)	76.57^{d}	79.84^{d}	82.26^{c}	84.40^{b}	79.88^{c}	25.08
SEM	0.132	0.129	0.246	0.298	0.158	0.324

Means in the same column bearing different superscripts are significantly different ($p<0.05$)

NS Non-Significant C Control W S *Withania somnifera* Syn Synbiotic

The digestibility of ether extract (EED) revealed significant variation between nonsupplemented and supplemented broilers groups. The EED value ranged from 79.52% (T_1) to 84.40% (T_8) with nonsignificant difference was observed among all the supplemented groups. The digestibility coefficient of nitrogen free extract (NFED) was nonsignificantly similar (p□0.05) among T_1, T_3, T_4, T_5, T_6 and T_7 groups. Statistically highest and numerically lowest NFED value was observed in T_8 and T_6 group broilers, respectively. The digestibility of crude fibre bears little relevance in broilers and was found to be nonsignificant (p>0.05) over the treatments. The CFD ranged from 22.04% (T_1) to 25.08% (T_8).

The significantly high digestibility of crude protein and ether extract observed in synbiotic fed broiler group than control could be due to the presence of probiotic culture *Lactobacillus bulgaricus* present in synbiotic (Apata, 2008). The modulation of intestinal morphology (Baurhoo *et al*., 2007) and increase in concentration of amylases in small intestine (Jin *et al*., 2000) through supplementation of synbiotic might have increased the digestibility of nutrients and thus resulted in improved FCR (Baurhoo *et al*., 2007). The results of the present study with respect to dry matter digestibility revealed contrast findings than observed by Thorat *et al*. (2015) in broilers.

4.4.2 Balance of nitrogen, calcium and phosphorus

The retention of nitrogen, calcium and phosphorus (g/head/day) observed during metabolic trial of five days are tabulated in **Table 4.11**. In conformity to the observed body weight in different treatments, the retention of nitrogen in broilers under different treatments was found to be significant. The nitrogen retained in various treated groups ranged between 2.41 (T_1) to 2.68 (T_6) g/head/day. Significantly lowest nitrogen was found to be retained in broilers fed basal diet without any supplementation, *i.e.,* T_1. The supplementation of either one or both feed additive in basal diet of broilers was found to be beneficial in enhancing the quantity of nitrogen to be retained in the body. All supplemented groups except T_5 group, revealed significantly (p□ 0.05) higher nitrogen retention than control group (T_1) (**Fig. 4.5**). The 0.025% synbiotic fed broilers (T_5) retained relatively similar nitrogen content to T_1 group. The nitrogen available for various metabolic processes was found to be similar in all the *Withania* treated groups. Statistically similar nitrogen was retained in T_4 and T_6 group. The 1.5% *Withania* treated broilers (T_4) and 0.05% synbiotic supplemented broiler group (T_6) retained maximum nitrogen over most of the treatments which is manifested in their improved FCR due to efficient utilization of

protein and energy present in the basal diet. The enhanced nitrogen retention observed in synbiotic supplemented T_6 group is in agreement with the findings recorded by Thorat *et al*. (2015).

The major minerals such as calcium and phosphorus are usually considered as growth indicators in broilers production system. Either of the added growth promoters used in the current trial, contributed significantly in enhancing the level of calcium and phosphorus to be retained in the body. Both the calcium and phosphorus retention were found to be significantly highest (p□ 0.05) in T_6 and T_8 groups. The remarkably low calcium and phosphorus retained in nonsupplemented broilers might be responsible for poor weight gain observed in control group. The calcium retention ranged from 0.40 (T_1) to 0.51 (T_6) g/head/day whereas phosphorus over the treatments ranged from 0.34 (T_1) to 0.47 (T_6) g/head/day.

The effect of *Withania* levels on calcium and phosphorus retention was found to be of intermediate nature between control and synbiotic. The synbiotic used in the present study imparted more calcium and phosphorus sparing effect than ashwagandha.

4.5 Haemato-serobiochemical Parameters

4.5.1 Haemoglobin and erythrogram

The haemoglobin and erythrogram are considered as bio-indicator of overall health of flocks and gives comprehensive view about the suitability of particular treatment. The treatment wise values observed for various haematological parameters are tabulated in **Table 4.12**.

4.5.1.1 Blood haemoglobin (Hb)

The mean blood Hb values ranged between 7.51(T_1) to 8.43 (T_4), and 7.90 (T_1) to 9.23 (T_8) at 28th and 42nd day of trial, respectively. Low level of ashwagandha (0.5%) was found sufficient during period of low to moderate stress in initial four week. The combined approach adopted in T_8 or 1.5% ashwagandha root powder was required to significantly enhance the Hb level under higher heat stress conditions. The synbiotic fed to broilers at two different levels failed to demonstrate any remarkable difference in Hb content, in contrast to significantly low Hb value observed by Ahmed *et al*. (2015b) for similar type of synbiotic.

Table 4.11 Balances of nitrogen, calcium and phosphorus (g retained/ bird/ day) in different dietary groups

Treatments	Nitrogen retained (g/head/day)	Calcium retained (g/head/day)	Phosphorus retained (g/head/day)
T_1 (C)	2.41^{a}	0.40^{a}	0.34^{a}
T_2 (0.5% WS)	2.56^{b}	0.44^{b}	0.39^{b}
T_3 (1.0% WS)	2.57^{b}	0.45^{b}	0.40^{b}
T_4 (1.5% WS)	2.62^{bc}	0.47^{b}	0.42^{bc}
T_5 (0.025% Syn)	2.42^{a}	0.45^{b}	0.39^{b}
T_6 (0.05 % Syn)	2.68^{c}	0.51^{c}	0.47^{d}
T_7 (0.25% WS + 0.025% Syn)	2.54^{b}	0.46^{b}	0.39^{b}
T_8 (0.5% WS + 0.05% Syn)	2.57^{b}	0.48^{c}	0.45^{cd}
SEM	0.011	0.004	0.005

Means in the same column bearing different superscripts are significantly different ($p<0.05$)

C Control W S *Withania somnifera* Syn Synbiotic

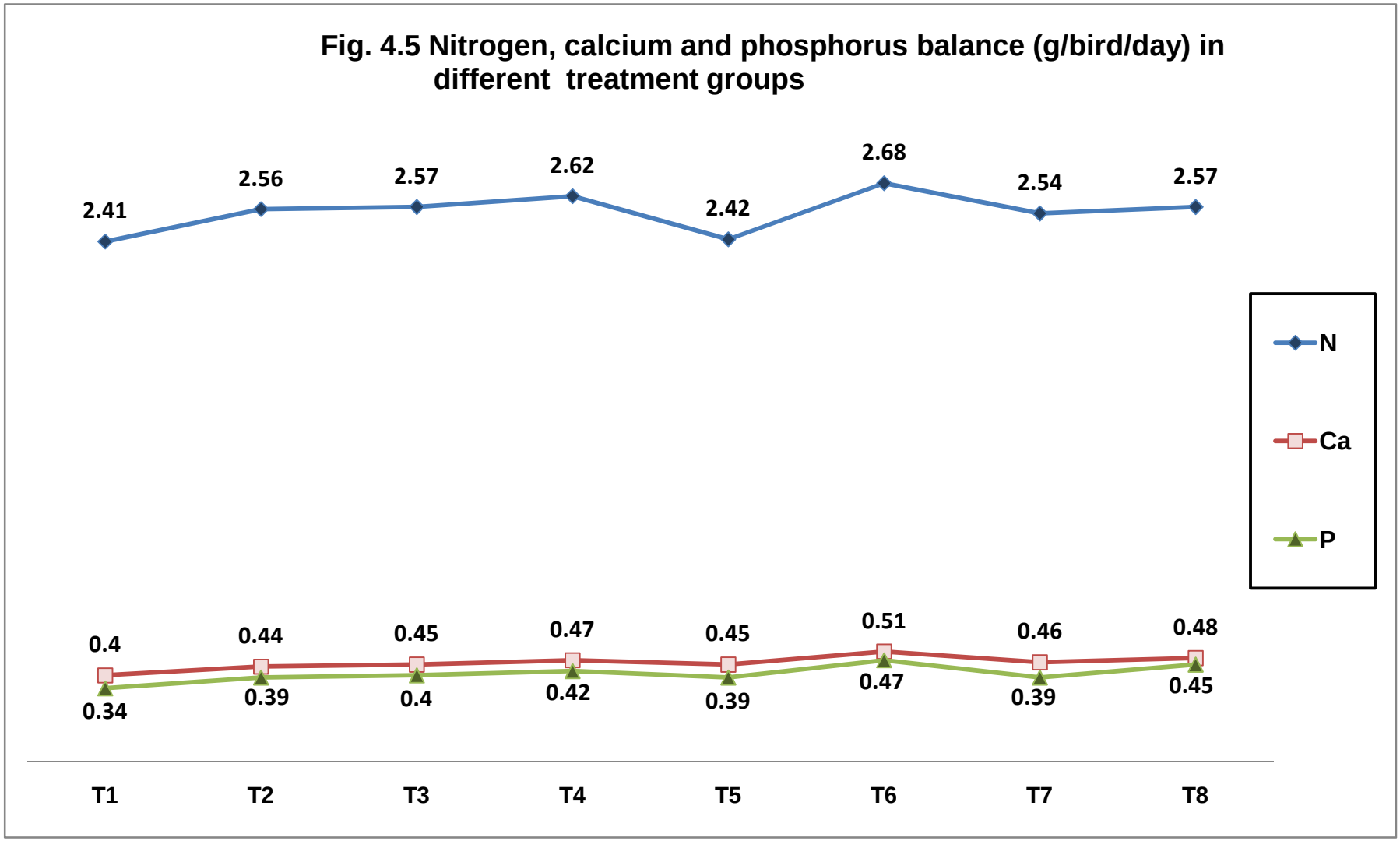
Table 4.12 Mean haemoglobin and erythrogram in different treatment groups of broilers at 28th and 42nd day of experimental trial

Treatments	Haemoglobin (g/dl)		PCV %		TEC*10^6/ul	
	Day of sampling		Day of sampling		Day of sampling	
	28th Day	42nd Day	28th Day	42nd Day	28th Day	42nd Day
T_1 (C)	7.51^{a}	7.90^{a}	22.24^{a}	23.42^{a}	1.53^{a}	1.70^{a}
T_2 (0.5% WS)	8.31^{b}	8.53^{bc}	24.40^{b}	25.11^{bc}	1.87^{b}	1.93^{bc}
T_3 (1.0% WS)	8.20^{b}	8.97^{cd}	24.20^{b}	26.41^{cd}	1.85^{b}	2.02^{cd}
T_4 (1.5% WS)	8.43^{b}	9.13^{d}	25.02^{b}	26.89^{d}	2.01^{b}	2.17^{de}
T_5 (0.025% Syn)	7.67^{a}	8.10^{ab}	22.72^{a}	23.91^{ab}	1.60^{a}	1.79^{ab}
T_6 (0.05 % Syn)	7.60^{a}	8.17^{ab}	22.41^{a}	24.22^{ab}	1.61^{a}	1.82^{abc}
T_7 (0.25% WS +0.025% Syn)	7.80^{a}	8.33^{ab}	23.02^{a}	24.60^{ab}	1.69^{a}	1.91^{bc}
T_8 (0.5% WS + 0.05% Syn)	8.33^{b}	9.23^{d}	24.74^{b}	26.93^{d}	2.01^{b}	2.25^{e}
SEM	0.035	0.062	0.11	0.189	0.019	0.023

Means in the same column bearing different superscripts are significantly different ($p<0.05$)

C Control W S *Withania somnifera* Syn Synbiotic

Fig. 4.5 Nitrogen, calcium and phosphorus balance (g/bird/day) in different treatment groups



The observed nonsignificant effect of synbiotic on Hb values in line with observation recorded by Nyamagonda *et al*. (2009). Significantly higher Hb value on supplementation of synbiotic in broiler was reported by Besky and Al-Sardary (2015). Synbiotic having similar composition though revealed significantly higher value of Hb in guinea fowl (Habibu *et al*., 2016). The variability in reporting of Hb value might be due to differences in species or composition of synbiotic or season of trial used by various workers.

The study is in agreement with findings in broilers (Kumari *et al*., 2015) and mice (Ziauddin *et al*., 1996) in which significant increase in Hb concentration was observed with supplementation of either root powder or root extract of *W. somnifera*. However, contrast findings were reported by Mushtaq *et al*. (2012) in broilers treated with three levels of ashwagandha root extracts. The observed dose dependent increase in Hb value corresponds to the results reported by Bhardwaj *et al*. (2012) in Japanese quails for 0.5%, 1% and 1.5% level of *W. somnifera.*

4.5.1.2 Erythrogram

(a) Packed cell volume (PCV)

The broilers under different treatments generated similar results for PCV to that of Hb values over the period. The PCV values ranged from 22.24% to 25.02% after four weeks of experiment and 23.42% to 26.93% at the end of trial. Ashwagandha significantly enhanced the cellularity and ultimately PCV. However, over the period, the cellularity increased in all the treatments, the per cent increment in cellularity was observed to be highest in T_4 and T_8 groups. The manner of increase in PCV at the end of trial is in agreement with results obtained in Japanese quails (Bhardwaj *et al*., 2012)

(b) Total erythrocyte count (TEC)

The TEC was found to be significantly ($p \square 0.05$) higher in T_2 to T_4 and T_8 groups than rest of the treatments in 28^{th} day sampling. Significantly lowest TEC values were observed for T_1 group during both collections. Highest TEC value was observed for T_8 group at the end of trial. The effect of oxidative stress in the last two weeks was less evident in treatment groups containing variable amount of ashwagandha. Experimental groups such as T_1, T_5 and T_6 failed to increase TEC significantly at the end of trial under heat stress conditions. The absence of dose

effect of ashwagandha on TEC at 4th week age (Bhardwaj *et al*., 2012) corresponds to similar effect in Japanese quails.

The results of the present study are semi contrary to previous study (Ahmed *et al*., 2015b) which stated significant (p 0.05) increase in PCV and nonsignificant differences in TEC in broilers fed synbiotic with similar composition. In agreement to the present findings, Nyamagonda *et al*. (2009); and Besky and Al-Sardary (2015) observed nonsignificant effect of synbiotic on PCV and TEC values in broilers whereas the contrast results were recorded by Habibu *et al*. (2016) in guinea fowl.

The haematinic effect of ashwagandha was visible in terms of enhance TEC, Hb and PCV values on *Withania* included diet in broilers. The haemoproliferative and haemoprotective effect of ashwagandha observed in the present study is in agreement with the findings in broilers (Mushtaq *et al*., 2012; Kumari *et al*., 2015) and mice (Ziauddin *et al*., 1996).

The haemoproliferative effect of *W. somnifera* in broiler chicks might have been due to its positive influence on haemopoiesis through stimulation of stem cell proliferation and increase in bone marrow cellularity (Aphale *et al.,* 1998; Mishra *et al.,* 2000) while the haemoprotective has been ascribed due to its antioxidant activity protecting RBC from oxidative stress and improving erythrocytic enzyme activity (Sujatha *et al.,* 2010). The remarkable role of *W. somnifera* in recovery of blood Hb, TEC and PCV status was also observed in *E. coli* infected guinea pigs (El-Boshy *et al*., 2013)

4.5.2 Leucogram

The leucogram is a reflection of immune status of birds and gives an idea about immunological state and health of the birds. The treatment wise values observed for various haematological parameters are tabulated in **Table 4.13**.

(a) Total leucocytes count (TLC)

The TLC was found to be nonsignificantly different among all the treatments at 28th day of age. Nonsignificant variation among T_2, T_4, T_5, T_6 and T_8 was observed at the end of trial. However, broilers under treatment groups T_1 and T_7 showed significantly enhanced TLC values than T_3 treatment which might be due to mild exposure of broilers to infectious agent under heat stress condition (Kumari *et al*., 2015).

Table 4.13 Blood leucogram in different treatments groups of broilers at 28th and 42nd day of experimental trial

Treatment	TLC *10³/ul		Heterophils*10³/ul		Lymphocytes*10³/ul-		Monocytes*10³/ul		Eosinophil*10³/ul		Basophil*10³/ul	
	Day of sampling		Day of sampling		Day of sampling		Day of sampling		Day of sampling		Day of sampling	
	28th Day^{NS}	42nd Day	28th Day	42nd Day	28th Day	28th Day	28th Day^{NS}	42nd Day^{NS}	28th Day^{NS}	42nd Day^{NS}	28th Day^{NS}	42nd Day^{NS}
T_1 (C)	33.44	55.44^{b}	27.89^{b}	34.22^{b}	61.67^{a}	56.67^{a}	7.67	6.44	1.44	1.33	1.33	1.33
T_2 (0.5% WS)	32.89	51.00^{ab}	24.67^{a}	29.67^{a}	65.44^{bc}	62.00^{b}	7.44	6.11	1.33	1.11	1.11	1.11
T_3 (1% WS)	33.00	46.00^{a}	24.33^{a}	27.89^{a}	66.44^{c}	63.89^{b}	7.00	6.00	1.11	1.11	1.11	1.11
T_4 (1.5% WS)	33.00	52.33^{ab}	24.00^{a}	27.00^{a}	67.00^{c}	65.11^{b}	7.00	5.67	1.00	1.11	1.00	1.11
T_5 (0.025% Syn)	33.00	54.00^{ab}	26.67^{ab}	30.44^{a}	63.56^{ab}	61.44^{b}	7.22	5.67	1.33	1.22	1.22	1.22
T_6 (0.05 % Syn)	37.56	52.00^{ab}	25.44^{ab}	30.00^{a}	65.22^{bc}	62.11^{b}	7.00	5.67	1.22	1.11	1.11	1.11
T_7 (0.25% WS+ 0.025% Syn)	36.00	54.67^{b}	25.11^{a}	29.56^{a}	65.44^{bc}	62.00^{b}	7.00	6.11	1.22	1.22	1.22	1.11
T_8 (0.5% WS+ 0.05% Syn)	31.67	47.33^{ab}	24.22^{a}	27.33^{a}	66.67^{c}	65.00^{b}	6.89	5.33	1.11	1.22	1.11	1.11
SEM	.076	0.90	0.312	0.431	0.316	0.497	0.131	0.12	0.05	0.047	0.043	0.042

Means in the same column bearing different superscripts are significantly different ($p<0.05$)

NS Non significant C Control W S *Withania somnifera* Syn Synbiotic

The TLC values ranged from 31.67 (T_8) to 37.56 (T_6); and 46 (T_3) to 55.44 (T_1) 10^3/µl at 28th day and 42nd day, respectively. The inability to detect variation in TLC in this study disagree with the results of Nyamagonda *et al*. (2009) which observed significant effect of synbiotic on TLC in broilers.

(b) Total heterophil count

The average numbers of heterophils were found to be higher ($p<0.05$) in control group than rest of the treatments during the whole trial except in synbiotic supplemented groups, T_5 and T_6 at 28th day of collection. The heterophils value ranged from 24 (T_8) to 27.89 (T_1); and 27 (T_4) to 34.22 (T_1) 10^3/µl at the end of 4th and 6th week, respectively. Lower value of heterophils in *W. somnifera* treated broilers indicate protective role of ashwagandha on different visceral organs.

(c) Total lymphocyte count

The present study observed lowest lymphocyte values for non-supplemented T_1 group broilers at both interval of estimation. The *Withania* treated T_3, T_4 and T_8 group revealed highest ($p<0.05$) lymphocytes level at 4th week of age whereas 1.5% level of ashwagandha showed numerically highest lymphocyte at 6th week of age. All treatments after the acute heat stress period of 5th and 6th week, exhibited significantly increased lymphocyte numbers than control. Increase in numbers of lymphocyte indicates the immunopotentiative effect of ashwagandha, mediated through varying degree of lymphoproliferative changes in lymphoid organs to improve cell mediated immunity (Gatne *et al*., 2010).

(d) Monocyte, eosinophil and basophil cells

The numbers of monocyte, eosinophil and basophil cells were found to be nonsignificant ($p> 0.05$) among treatments at 4th and 6th week of experiment. The T_8 group revealed numerically lowest number of monocyte whereas highest monocyte count was observed in (T_1) treatment group at both intervals. Eosinophil ranged between 1.00 (T_4) to 1.44 (T_1) and 1.11 (T_2-T_4, T_6) to 1.33 (T_1) 10^3/µl at the end of 4th and 6th week, respectively. Most of the treatments at both intervals revealed basophil value of $1.11X10^3$/µl with highest basophil cells count was observed in T_1 group.

Significant variation in TLC and nonsignificant variation in heterophil and lymphocyte observed in guinea fowl on supplementation of synbiotic (Habibu *et al*., 2016) disagree with present findings. *W. somnifera* significantly increases white

blood cell and erythrocyte counts (Sham *et al*., 2003; Manish *et al*., 2004; Senthilnathan *et al*., 2006).

The obtained results for leucogram are in semi-agreement with the research findings of Mushtaq *et al*. (2012) in broilers which observed variable response of different level of ashwagandha on TLC and nonsignificant variation in monocytes and eosinophils but failed to detect significant difference in heterophils and lymphocytes counts as observed in current investigation. The observed increase in the number of lymphocyte cells (Davis and Kuttan, 2000; Malik *et al*., 2007) is well documented in avian, mammalian and fish species (Mishra *et al*., 2000; Sharma *et al*., 2010) with the treatment of *W. somnifera.* The results obtained in Japanese quails (Bhardwaj *et al*., 2012) on dietary inclusion of 0.5%, 1% and 1.5% level of *W. somnifera*, revealed significant increase in TLC and lymphocytes, similar to that observed in the current study. However, variation reported for heterophils in present study, was not observed in quails.

4.5.3 Blood glucose

The departure of serum glucose value from equilibrium is an indication of stress in monogastric animals. The level and type of stress significantly alters the serum glucose values in broilers. The pharmacological property of ashwagandha and synbiotics plays an important role in glucose homeostasis. The status of blood glucose of representative broiler birds from each replication and treatment at the end of market age (28^{th} day) and after experimental period (42^{nd} day) was studied and the treatment wise results are presented in **Table 4.14**.

Nonsignificant variation in serum glucose values of most of the treatments (T_1, T_3, T_4, T_5, T_7 and T_8) was observed at the end of market age (28^{th} day). The feeding program employed in the treatment T_6 (202.33 mg/dl) significantly lowered serum glucose value than T_2 group (211.89 mg/dl). The lowest serum glucose value was observed for T_6 group at the end of 28^{th} day of trial. The results obtained are in agreement with the findings of Srivastava *et al*. (2012) who also observed nonsignificant variation in serum glucose level between control and *Withania* fed broilers. However, the values observed in present study are slightly higher than reported (183.55 mg/dl) by Srivastava *et al*. (2012) which might be due to moderate stress experienced by broilers in the present study. The results of the present investigation are further supported by the similar findings of Ahmed *et al*. (2015b) in broilers. The failure to detect any significant effect of synbiotic on serum glucose

level in comparison to control corresponds to the observation made by Besky and Al-Sardari (2015) in broilers raised on 0.25 and 0.5% synbiotic preparation.

The serum glucose values observed at the end of 42nd day were found to be statistically different between one and more treatments with highest serum glucose level was observed in broilers under T_1 group. The treatment values for serum glucose ranged between 218.78 (T_3) to 238.44 (T_1) with most treatments except T_5, significantly differed from control broilers (T_1). Though an increase in serum glucose level over the period was observed in all the treatments due to heat stress (Aksit *et al*., 2006) yet the quantitive variation was found to be lowest in *Withania* fed broilers. The quantitive differences between the two collections were found to be highest for broilers under non-supplemented group (T_1).

The therapeutic effect of ashwagandha root powder in induction of hypoglycemia (Andallu and Radhika, 2000) was observed in the present study at 42nd day of trial. Broilers supplemented with *W. somnifera* or synbiotic or both were least affected by heat stress induced hyperglycemia in the last two weeks of trial. The hypoglycemic effect of ashwagandha observed in the present study during thermal stress is in agreement with the findings of Sujatha *et al*. (2010) who also observed significant reduction in serum glucose values in *Withania* fed broilers reared under heat stress conditions. The glucose homeostasis role of *W. somnifera* manifested in the present study corresponds to similar observation recorded in guinea pigs (El-Boshy *et al*., 2013). The glucose lowering effect of ashwagandha could be attributed to its insulin enhancing effect or its antioxidant role in the body (Bhattacharya *et al*., 1997). The reduction in enzymatic activity of gluconeogenic enzyme, hepatic G6P has also been claimed for hypoglycemic effect of ashwagandha (Udayakumar *et al*., 2009).

Similar to ashwagandha, the hypoglycemic effect of synbiotic (Kavitha *et al*., 2016) was observed in broilers at 42nd day of age. The synbiotic exerts hypoglycemia through stimulation of tissue uptake of glucose, alteration of insulin metabolism and inhibition of glucose reabsorption by the kidneys (Roselino *et al*., 2012).

Table 4.14 Mean serum glucose values in different treatment groups of broilers at 28th and 42nd day of experimental trial

Treatments	Mean serum glucose (mg/dl)	
	Day of sampling	
	28th day	**42nd day**
T_1 (C)	209^{ab}	238.44^{b}
T_2 (0.5% WS)	211.89^{b}	222.67^{a}
T_3 (1.0% WS)	208.22^{ab}	218.78^{a}
T_4 (1.5% WS)	209.67^{ab}	219.33^{a}
T_5 (0.025% Syn)	209.11^{ab}	229.44^{ab}
T_6 (0.05 % Syn)	202.33^{a}	221.56^{a}
T_7 (0.25% WS +0.025% Syn)	203.33^{ab}	223.33^{a}
T_8 (0.5% WS + 0.05% Syn)	210.33^{ab}	220.00^{a}
SEM	0.98	1.34

Means in the same column bearing different superscripts are significantly different ($p<0.05$)

C Control W S *Withania somnifera* Syn Synbiotic

In contrast to the present findings, Varma *et al*. (2011) demonstrated the hyperglycemic effect of ashwagandha in cockerels during pesticides induced hypoglycemic stress. The hyperglycemic effect of synbiotic observed by Hassanin *et al*. (2015) in rabbit might be due to species difference.

4.5.4 Serum TSH, triiodothyronine (T3) and thyroxin (T4) hormones

Thyroid hormones are well known to regulate energy metabolism, accelerate basal and oxidative metabolism rate by increasing the respiratory rate, mitochondrial mass and mitochondrial cytochrome contents of the cell (Raeesi *et al*., 2012). The plasma level of thyroid hormones changes with age, fasting, temperature, feeding and pathophysiology (Lin *et al*., 2001). Decreased thyroid hormone level has been claimed to results in failure to provide adequate oxygen delivery to the tissues that may lead to hypoxia and metabolic disturbances (Hassanzadeh, 2009). Thus keeping in view, the significant role of thyroid hormone in growth, the present investigation recorded the serum value of TSH, T3 and T4 hormones at the end of the trial, the values of which are represented in **Table 4.15**.

The mean serum TSH hormone level in broilers at the end of trial significantly differs among treatments with highest value (0.56 ng/ml) observed for broilers under control group. Lowest TSH values were recorded in broilers fed 1.5% ashwagandha treated diet and/or 0.05% synbiotic containing diet. Linear decrease in TSH values was observed with level of *W. somnifera* supplementation (**Fig. 4.6**). The serum TSH concentration of T_5 and T_7 group was statistically similar to nonsupplemented broilers (T_1).

The growth in poultry is dependent upon direct effects of thyroxin (T4) and its active form, triiodothyronine (T3) (Etherton *et al*., 1987). The results of the present study indicated significant increase in serum concentrations of triiodothyronine and thyroxin hormone in broilers under most treatments during the entire experimental period. The triiodothyronine hormone regulates oxygen consumption, particularly in young chickens (Bobek *et al*., 1976) and exerts significant reduction in abdominal body fat of broilers (Wang *et al*., 2007).

Table 4.15 Mean serum TSH, T3 and T4 hormone concentration in broilers under different treatment groups at the end of experimental trial

Treatments	Mean Serum TSH (ng/ml)	Mean Serum T3 (ng/ml)	Mean Serum T4 (ng/ml)
T_1 (C)	0.56^{d}	2.05^{a}	40.33^{ab}
T_2 (0.5% WS)	0.42^{bc}	2.63^{b}	49.33^{d}
T_3 (1.0% WS)	0.33^{ab}	2.63^{b}	43.33^{bc}
T_4 (1.5% WS)	0.28^{a}	2.76^{b}	40.00^{ab}
T_5 (0.025% Syn)	0.47^{cd}	2.43^{ab}	37.67^{a}
T_6 (0.05 % Syn)	0.28^{a}	2.55^{b}	46.67^{cd}
T_7 (0.25% WS +0.025% Syn)	0.49^{cd}	2.60^{b}	41.00^{ab}
T_8 (0.5% WS + 0.05% Syn)	0.39^{bc}	2.83^{b}	48.00^{d}
SEM	0.012	0.058	0.37

Means in the same column bearing different superscripts are significantly different ($p<0.05$)

C Control W S *Withania somnifera* Syn Synbiotic

The concentration of triiodothyronine in serum was significantly higher in all treatments in comparison to control and T_5 treatment group however, the values observed under different treatments were found within normal range (0.5-4.0 ng/ml) as reported by Sturkie (2000). The significantly low serum triiodothyronine hormone in control (T_1) broilers might be one of the possible reasons for poor weight gain observed during trial under heat stress. The three different levels of ashwagandha tried in the present study could not able to produce any significant differences in T3 hormone concentration.

The decrease in serum triiodothyronine level in control broilers (T_1) in response to high ambient temperature agrees with the findings of Mack *et al*. (2013). The plasma level of thyroxin bears more importance in broilers as the affinity and capacity of avian blood plasma for T3 are much weaker than for T4 (Singh *et al*., 1967) The present study observed significant effect of 0.5% *Withania* (T_2), 0.05% synbiotic (T_6) and 0.5% *W. somnifera* + 0.05% synbiotic substances (T_8) on tetra-iodothyronine or thyroxin hormone concentration in comparison to control group. A downward trend in thyroxin hormone concentration was observed with enhanced level of ashwagandha. The findings of the present investigation are in strong agreement with results demonstrated by Sujatha *et al*. (2010) who observed numerically higher triiodothyronine hormone and statistically higher thyroxin hormone concentration in broilers supplemented with herbal formulation containing *W. somnifera* root powder.

The enhanced thyroxin hormone concentration in synbiotic fed broiler groups might be due to thyroxin stimulating effect of probiotic present in the synbiotic mixture (Khan *et al*., 2013). The present findings are in semi agreement with the findings of Panda and Kar (1987, 1999) which reported significant increase in thyroxin hormone but nonsignificant variation in triiodothyronine concentration in cockerels and mice following supplementation of ashwagandha root extract. Inconsistent results on serum thyroid levels in various studies indicate differential regulation of thyroid hormone in response to varied climatic and physiological effect (Elnager *et al*., 2010; Mack *et al*., 2013).

4.5.5 Serum protein profile

The values representing serum total protein, albumin and globulin for different treatments at 28th and 42nd day of trial are represented in **Table 4.16**.

Fig.4.6 Mean serum TSH, T3 and T4 concentration in broilers of different treatment groups

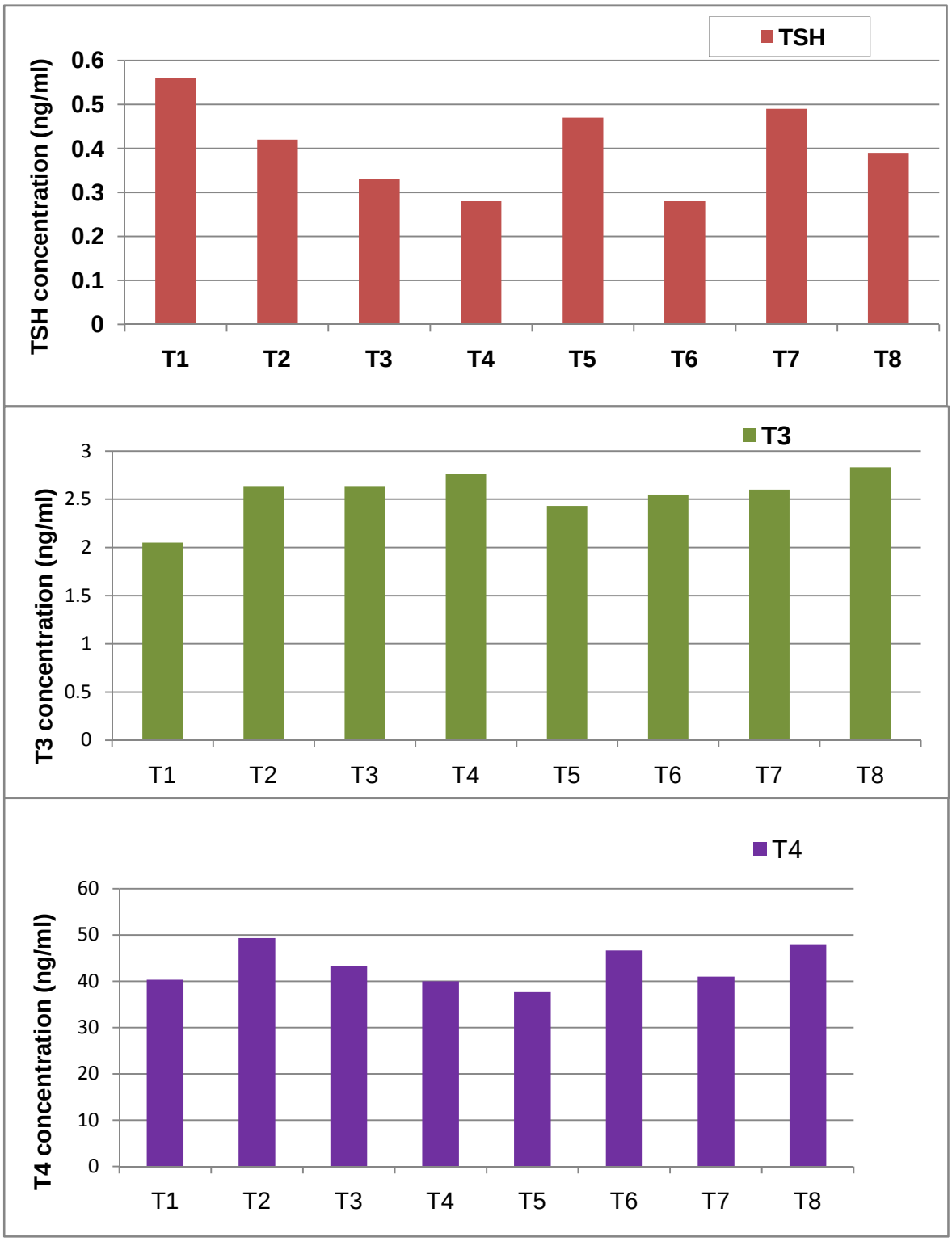


Table 4.16 Mean serum total protein, albumin, globulin (g/dl) and creatinine values (mg/dl) in different treatment groups of broilers at 28th and 42nd day of experimental trial

Treatments	Mean serum total protein		Mean serum albumin		Mean serum globulin		Mean serum creatinine	
	Day of sampling		Day of sampling		Day of sampling		Day of sampling	
	28th day	42nd day	28th day^{NS}	42nd day	28th day	42nd day	28th day^{NS}	42nd day^{NS}
T1 (C)	3.10^{a}	2.59^{a}	1.26	1.12^{a}	1.84^{a}	1.47^{a}	0.32	0.41
T2 (0.5% WS)	3.00^{ab}	3.40^{bc}	1.29	1.33^{b}	2.00^{ab}	2.07^{bc}	0.31	0.39
T3 (1.0% WS)	3.40^{bc}	3.39^{bc}	1.30	1.33^{ab}	2.10^{bc}	2.06^{bc}	0.31	0.38
T4 (1.5% WS)	3.52^{c}	3.58^{bc}	1.33	1.35^{b}	2.19^{c}	2.23^{bc}	0.31	0.37
T5 (0.025% Syn)	3.18^{ab}	3.07^{ab}	1.27	1.24^{ab}	1.91^{a}	1.83^{ab}	0.32	0.39
T6 (0.05 % Syn)	3.24^{ab}	3.20^{b}	1.28	1.30^{ab}	1.97^{ab}	1.90^{b}	0.32	0.39
T7 (0.25% WS + 0.025% Syn)	3.27^{ab}	3.35^{b}	1.28	1.31^{ab}	1.98^{ab}	2.04^{bc}	0.32	0.39
T8 (0.5% WS + 0.05% Syn)	3.53^{c}	3.97^{c}	1.35	1.54^{c}	2.19^{c}	2.42^{c}	0.30	0.37
SEM	0.025	0.068	0.01	0.023	0.019	0.047	0.006	0.004

Means in the same column bearing different superscripts are significantly different ($p<0.05$)

NS Non significant C Control W S *Withania somnifera* Syn Synbiotic

4.5.5.1 Serum total proteins

Administration of higher level of ashwagandha (1.5%) or 0.5% *W. somnifera*+0.05% synbiotic significantly enhanced the serum total protein during initial four weeks. The T_1 group demonstrated nonsignificant variation in serum proteins with T_2, T_5, T_6 and T_7 after 4^{th} week. The serum total protein value ranged between 3.00 (T_2) to 3.53 (T_8) at 28^{th} day. Remarkable decrease in serum protein quantity in non-supplemented T_1 broilers and slight decrease in synbiotic supplemented groups T_5 and T6 broilers was observed after heat stress episode of 5^{th} and 6^{th} week. The dietary inclusion of *W. somnifera* root powder or its combination with synbiotic significantly improved the serum protein status under heat stress conditions. The lowest serum protein at 6th week was observed for broilers under control group whereas highest value was found for T8 group broilers. The graded level of ashwagandha produced nonsignificant variation in total protein content of T_2 to T_4 group at the end of trial. The combination of two growth promoters in T_8 treatment proved to be more beneficial in mimicking the anabolic effect of ashwagandha. The role of synbiotic in serum protein level was not established in the present study after both collections except at 0.05% level in 6^{th} week of age. The findings are in agreement with observation recorded by Panda and Kar (1997) on administration of 20 mg extract of ashwagandha in cockerels. In contrast, Srivastava *et al*. (2012) observed non significant effect of 2% ashwagandha based herbal product on serum protein content of broilers raised during normal climate.

4.5.5.2 Serum albumin

The mean serum albumin value was found to be nonsignificant over the treatments in initial four week of trial with values ranges from 1.26 (T_1) to 1.35 (T_8) g/dl. The 42^{nd} day serum albumin value (g/dl) significantly varied between T_1 (1.12) and other treatments, *viz.,* T_2 (1.33), T_4 (1.35) and T_8 (1.54) whereas the variation observed between T_3, T_5, T_6 and T_7 was found to be non-statistical in nature. The supplementation of synbiotic alone in T_5 and T_6 groups manifested nonsignificant intermediate serum protein values at 4^{th} and 6^{th} week of age. The anabolic role of *Withania* was observed in ashwagandha fed broilers.

4.5.5.3 Serum globulin

The immunomodulatory effect of ashwagandha was revealed through considerable enhancement in serum globulin values in treatment T_3, T_4 and T_8 at 4^{th} week of age and in treatment T_2-T_4, T_7 and T_8 at the end of the trial. The nonsupplemented broilers in T_1 group were observed incapable to increase serum

globulin values during the whole trial. The level of *Withania* produced variable effect in initial four weeks but similar effect was observed with advancement of life. The significant effect of synbiotic on serum globulin was observed only at 42nd day of trial for 0.05% level however, the potentiating effect of synbiotic on *Withania* supplementation was observed throughout in the present study. The difference between the lowest (T_1) and highest (T_8) serum globulin values was widened (0.33 to 0.95) after exposure of broilers to thermal stress during 5th week and 6th week.

4.5.5.4 Serum creatinine

The mean serum creatinine value were observed non significant both at 28th and 42nd day of trial. However, the values were slightly raised at 42nd day in all the treatments.

The study is in agreement with the findings of Ashayerizadeh *et al*. (2009); and Besky and Al-Sardari (2015) in broilers chicken which do not observed any significant effect of synbiotic on total protein, serum albumin and serum globulin at the dose rate of 2.5 g and 5 g/kg of feed. A study in rabbit also revealed nonsignificant effect of synbiotic substances on serum albumin and total protein levels (Hassanin *et al*., 2015). Similar to the present findings in *Withania* fed broilers, Jadhav *et al*. (2014) observed numerically higher levels of total protein, albumin and globulin in birds treated with antioxidant substances during heat stress conditions. The reports of Dhenge *et al.* (2009) in broilers on significant increase in serum total protein and globulin level and nonsignificant increase in albumin level on supplementation of *W. somnifera* corroborate the present findings.

The increased serum total protein and globulin values reported by Sujatha *et al*. (2010) on supplementation of *Withania* based herbal formulation in broilers reared under heat stress condition of 84.74±2.51 THI, was similarly recorded. The serum levels of proteins are considered as reliable indicators of heat stress in broilers (Yalcin *et al*., 2004). The serum protein regulatory role of ashwagandha as demonstrated by Varma *et al*. (2011) in pesticides intoxicated cockerels was observed in the present study. The decrease in total serum protein, albumin and globulin content observed over the period in non-ashwagandha fed broilers, exposed to heat stress in the present study could be attributed to enhanced protein catabolism and gluconeogenesis effect of stress hormone cortisol (Sujatha *et al*., 2010). The effect of *W. somnifera* on serum protein profile became more evident during period of heat stress and is in agreement with study conducted by Arivuchelvan *et al*. (2013),

Kumari *et al*. (2015) which observed effective role of ashwagandha in reversal of changes in serum total protein, albumin and globulin to that of normal (Udayakumar *et al.*, 2009) in drug or disease induced serum hypoproteinaemia, hypoalbuminea and hypoglobulinaemia in broilers.

The observed anabolic effect of *W. somnifera* in the present study might be due to enhanced synthesis of modulator proteins in liver *(*Anbalagan and Sadique, 1981) or indirectly through correction of insulin deficiency by its hypoglycemic effect during period of heat stress (Udayakumar *et al*., 2009) and/or through increase in thyroid hormone concentration which exerts anabolic effect (Panda and Kar, 1997). The present investigation indicates that ashwagandha diverts amino acid pool towards synthesis of globulin protein throughout the trial and during heat stress, more amino acid are diverted for the synthesis of albumin to maintain the pace of growth.

4.5.6 Serum lipid profile

Serum lipid plays an important role in evaluation of animal health and meat quality (Fletcher, 2002). Hyperlipidemia has been claimed as one of the major aetiology behind most of the cardio vascular diseases characterized by the accumulation of cholesterol and low density lipoprotein (LDL) substances. The phytochemical properties of *W. somnifera* and role of synbiotics in reduction of harmful lipogenic substances has been documented in a number of species. The present investigation on the use of ashwagandha or synbiotic either alone or in combination invariably reflects changes in various serum lipid components. The serum lipid profiles of broilers raised under different treatments are represented in **Table 4.17**.

4.5.6.1 Serum triglycerides

The 4th week mean serum glycerides levels were significantly lowered ($p< 0.05$) in T_4 (57.44 mg/dl) and T_8 (57.67 mg/dl) groups than control broilers. The broilers in control group demonstrated maximum serum triglycerides with nonsignificant differences with T_2, T_3, T_5, T_6 and T_7. The effect of synbiotic alone was not evident at 28 days of age. The increase in serum triglycerides level (6th week) in all the treatment was observed with advancement in age under heat stress conditions. Least serum triglycerides value ($p<0.05$) was recorded in group T_8 (82.44 mg/dl) as compared to control (194.22 mg/dl). The inclusion of 0.5%, 1% and 1.5% level of *W. somnifera* in broiler diet showed equivalent concentration ($p>0.05$) of serum triglycerides to that of T_8 treatment. A decreasing trend in total serum

triglycerides in response to increasing level of *W. somnifera* and synbiotics was observed (**Fig. 4.7**). However, total serum triglyceride was increased when the level of *W. somnifera* was decreased to 0.25 % in group T_7.

4.5.6.2 Serum total cholesterol

Addition of 0.5% *W. somnifera*+0.05% synbiotic resulted in minimum total serum cholesterol value (p 0.05) of 113.30 mg/dl and 102.33 mg/dl at 4th and 6th week, respectively. Inclusion of 1.5% ashwagandha also reduced total cholesterol in serum comparable to T_8 group at 6th week of age. The 4th week serum cholesterol values were found to be nonsignificant among treatments T_1 to T_7. The mean serum cholesterol reduced in the last two week under the effect of *Withania* supplementation at all levels.

Low level of synbiotic inclusion (0.025%) in T_5 group and nonsupplemented broilers failed to reduce serum cholesterol and showed an upward trend during heat stress. Comparable performance in serum cholesterol between T_2 and T_3; T_6 and T_7 was observed at 42nd day. The results are analogous to the observation recorded by Sujatha *et al.* (2010) in broilers fed *Withania* containing diet.

4.5.6.3 High density lipoprotein (HDL) cholesterol

Mean HDL cholesterol per broiler for the experimental groups T_1 to T_8 was between 49.33 to 60.78 mg/dl; and 43.33 to 55.33 mg/dl at 4th and 6th week, respectively. The difference in serum HDL was statistically significant and apparently HDL concentration was quite high in T8 treatment as compared to the control (T_1). The serum HDL level was unaffected with the levels of *W. somnifera* or synbiotic. It was drastically reduced in nonsupplemented broilers under T_1 group at both intervals. The findings suggest that 0.5% ashwagandha in combination with 0.05% synbiotic resulted in remarkable increase of serum HDL level.

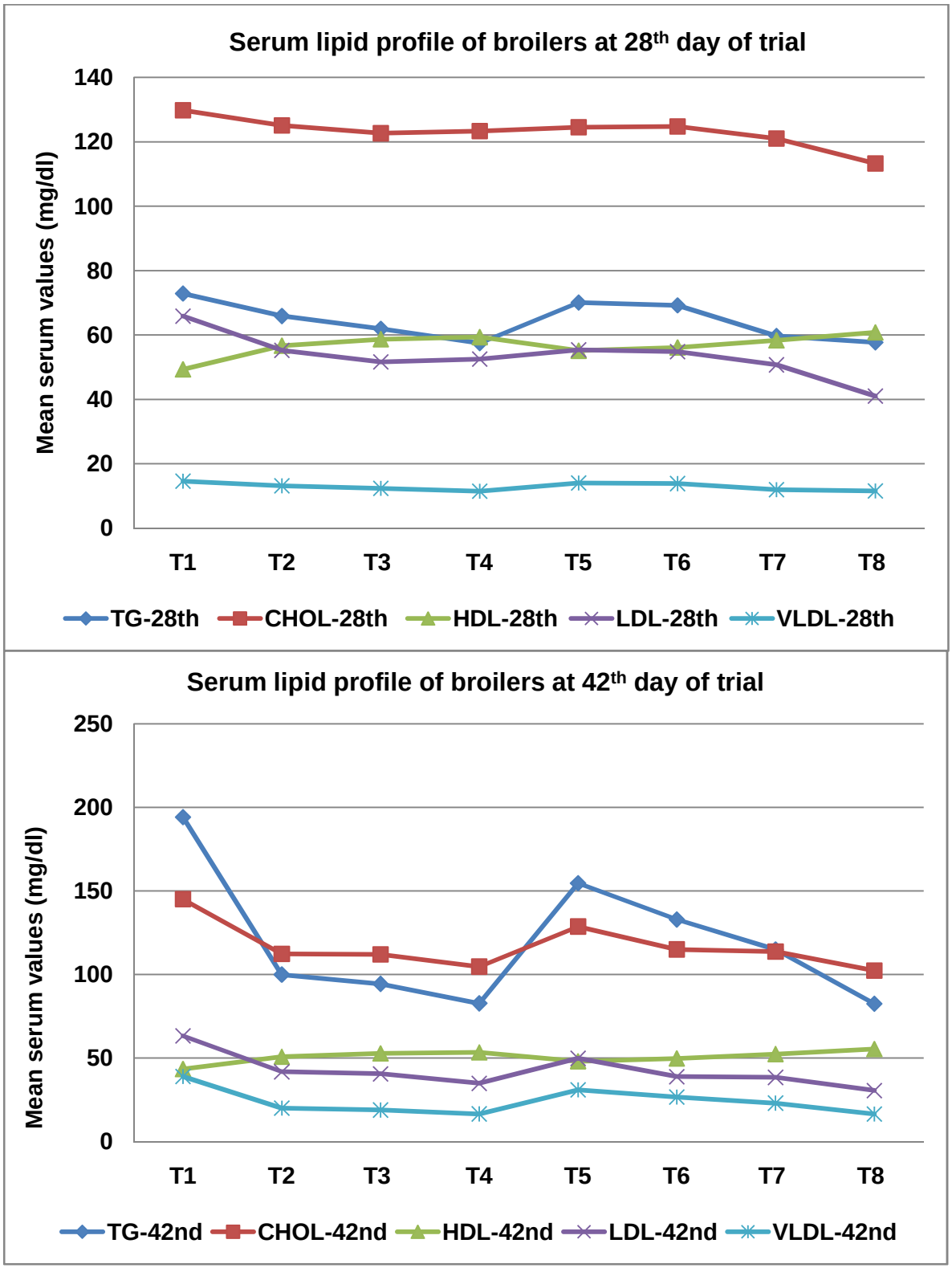
Table 4.17 Serum lipid profile of broilers under different treatment groups at 28th and 42nd day of experimental trial

Treatments	Mean serum triglycerides (mg/dl)		Mean serum cholesterol (mg/dl)		Mean serum HDL (mg/dl)		Mean serum LDL (mg/dl)		Mean serum VLDL (mg/dl)	
	Day of sampling		Day of sampling		Day of sampling		Day of sampling		Day of sampling	
	28th Day	42nd Day	28th Day	42nd Day	28th Day	42nd Day	28th Day	42nd Day	28th Day	42nd Day
T_1 (Control)	72.89^{b}	194.22^{d}	129.78^{b}	145.00^{c}	49.33^{a}	43.33^{a}	65.87^{c}	63.15^{b}	14.58^{b}	38.84^{d}
T_2 (0.5% WS)	65.89^{ab}	99.89^{a}	125.11^{b}	112.33^{ab}	56.67^{bc}	50.67^{bc}	55.27^{b}	41.69^{a}	13.18^{ab}	19.98^{a}
T_3 (1% WS)	61.89^{ab}	94.33^{a}	122.67^{ab}	112.00^{ab}	58.67^{bcd}	52.67^{cd}	51.62^{b}	40.47^{a}	12.38^{ab}	18.87^{a}
T_4 (1.5% WS)	57.44^{a}	82.67^{a}	123.33^{b}	104.67^{a}	59.33^{cd}	53.33^{cd}	52.51^{b}	34.80^{a}	11.49^{a}	16.53^{a}
T_5 (0.025% Syn)	70.11^{ab}	154.67^{c}	124.55^{b}	128.67^{bc}	55.11^{b}	48.00^{b}	55.42^{b}	49.73^{ab}	14.02^{ab}	30.93^{c}
T_6 (0.05% Syn)	69.22^{ab}	132.89^{bc}	124.78^{b}	115.00^{ab}	56.11^{bc}	49.67^{bc}	54.82^{b}	38.75^{a}	13.84^{ab}	26.58^{bc}
T_7 (0.25% WS+ 0.025% Syn)	59.67^{ab}	114.89^{ab}	121.00^{ab}	113.67^{ab}	58.33^{bcd}	52.33^{cd}	50.73^{b}	38.35^{a}	11.93^{ab}	22.98^{ab}
T_8 (0.5% WS+ 0.05% Syn)	57.67^{a}	82.44^{a}	113.30^{a}	102.33^{a}	60.78^{d}	55.33^{d}	41.02^{a}	30.51^{a}	11.53^{a}	16.49^{a}
SEM	1.51	3.674	1.145	2.13	0.456	0.466	1.134	2.529	0.302	0.735

Within a column, means bearing at least one common superscript are statistically (p□ 0.05) similar

C Control W S *Withania somnifera* Syn Synbiotic

Fig. 4.7 Serum lipid profile of broilers under different treatment groups at 28th and 42nd day



4.5.6.4 Low density lipoprotein (HDL) cholesterol

The initial sampling in the present investigation revealed significantly lowest (41.02 mg/dl) and highest (65.87 mg/dl) LDL values in T_8 and T_1 groups, respectively with nonsignificant variation among treatments T_2 to T_7. Later on, serum sampling of T_1 group at the end of trial observed statistically higher serum LDL concentration (63.15 mg/dl) than most treatments excluding T_5 group. Treatments groups T_2 to T_7 reduced serum LDL in a more or less similar manner.

4.5.6.5 Very Low density lipoprotein (HDL) cholesterol

The serum VLDL value was also observed to be significantly highest in untreated control broilers than T_4 and T_8 groups on day 28; and T_2 to T_4 and T_8 on day 42 of the experimental trial. The VLDL values ranged between 11.53 to 14.58 mg/dl and 16.49 to 38.84 mg/dl at the end of 4^{th} and 6^{th} week, respectively.

The effect of synbiotic observed is in agreement with the findings of Abdel-Raheem and Abd-Allah (2011) who observed nonsignificant effect of synbiotic in initial three weeks of trial and significant variation at six weeks of trial in serum cholesterol and triglycerides values. The trend of significant reduction in serum cholesterol and LDL with dietary inclusion of synbiotic at low level and nonsignificant variation in serum triglycerides and HDL reported by Besky and Al-Sardary (2015) in a 42 day trial was not observed in the present study. The present investigation observed significant reduction in all the above parameters (cholesterol, LDL, triglycerides and VLDL) however no reduction in serum cholesterol and LDL was observed at low level (0.025%) of synbiotic inclusion. Similarly, Safalaoh (2006) did not find differences in serum cholesterol when the low level of probiotic was added to the drinking water of broiler chicks. The cholesterol and LDL lowering and HDL increasing effect (Mohamed *et al*., 2014) of synbiotic was replicated in the present study. In contrast, Ashayerizadeh *et al*. (2009, 2011) found that synbiotic addition to the broiler diet had no significant effect in serum triglycerides, HDL, LDL and VLDL level except reduction in serum cholesterol. The cholesterol lowering effect of synbiotic observed at moderate to high level of inclusion was also reported by Ghasemi and Taherpour (2013).

The supplementation of synbiotic in the present study might have reduced activity of acetyl coenzyme A decarboxylase enzyme required for triglyceride synthesis (Santose *et al*., 1995). The prebiotic present in synbiotic exerts

hypocholesterolemic effects through reduction in absorption of lipids by binding to bile acid, increase cholesterol elimination and hepatic synthesis of new bile acid (Zhang *et al*., 2003) whereas the probiotic available in synbiotic inhibits the activity of hydroxymethyl-glutaryl coenzyme A involved in the cholesterol synthesis (Fukashima and Nakano, 1995).

The results obtained by Andallu and Radhika (2000) in a trial on human subject, confirms the significant role of ashwagandha in lowering of serum triglycerides, cholesterol, LDL and VLDL similar to that observed in the present study. The ameliorative role of ashwagandha in cases of pesticide induced hypercholesterolemia in cockerels was proved by Varma *et al*. (2011). The present study observed significant reduction of 27.81%, 57%, 44.89% and 57.44% in serum cholesterol, triglycerides, LDL and VLDL, respectively at 1.5% level of supplementation of *W. somnifera* in basal diet of broilers. A slightly higher level (2%) of ashwagandha root powder feeding for 42 days than used in the present study was found to reduce 30% and 26% cholesterol and triglycerides, respectively in egg of layers (Qureshi *et al*., 2011). The results are parallel to the findings of Visavadiya and Narasimhacharya (2007) which reported significant reduction in cholesterol by 53.01%, triglycerides by 44.85%, LDL by 62.7% and VLDL by 44.8% in plasma on *Withania* supplementation. The cholesterol lowering effect of *Withania somnifera* could be due to elevated excretion of cholesterol and bile acids through fecal sterol excretion (Ebihara and Schneeeman, 1989). Udayakumar *et al*. (2009) determined hypolipidemic activity of *W. somnifera* root extract (200 mg/kg body weight) as feed supplement and observed significant reduction in serum triglycerides of rats. Similarly, Roughani *et al*. (2005) reported that oral administration of *W. somnifera* mixed pelleted food at the dose of 6.25% produced significant reduction in triglycerides, serum cholesterol and LDL level in rats. The above reports are in close conformation to present findings in which *W. somnifera* in dose level of 1.5% caused a gradual and significant reduction in total lipids, cholesterol, triglycerides, LDL and VLDL in 42 day of experimentation.

4.5.7 Serum mineral profile

The serum mineral status of broilers under investigation provides a comprehensive view to observe the effect of different treatments employed in the present study. The mean serum calcium, phosphorus and magnesium level estimated at two different intervals are presented in **Table 4.18**.

Table 4.18 Mean serum calcium, phosphorus and magnesium values in different treatment groups of broilers at 28th and 42nd day of experimental trial

Treatments	Mean serum calcium (mg/dl)		Mean serum phosphorus (mg/dl)		Mean serum magnesium (mg/dl)	
	Day of sampling		Day of sampling		Day of sampling	
	28th Day	42nd Day	28th Day	42nd Day^{NS}	28th Day	42nd Day
T_1 (Control)	8.52^{a}	6.30^{a}	5.84^{a}	5.16	2.06^{a}	1.62^{a}
T_2 (0.5% WS)	9.56^{c}	6.66^{ab}	6.30^{ab}	5.27	2.20^{bc}	1.80^{b}
T_3 (1% WS)	9.33^{c}	6.96^{ab}	6.33^{ab}	5.27	2.20^{bc}	1.83^{b}
T_4 (1.5% WS)	9.61^{c}	7.33^{bc}	6.38^{ab}	5.37	2.25^{bc}	1.84^{b}
T_5 (0.025% Syn)	8.49^{a}	7.32^{bc}	5.89^{a}	5.33	2.14^{ab}	1.82^{b}
T_6 (0.05 % Syn)	9.24^{bc}	7.37^{bc}	6.59^{b}	5.62	2.30^{c}	1.85^{b}
T_7 (0.25% WS+ 0.025% Syn)	8.64^{ab}	721^{bc}	6.28^{ab}	5.30	2.22^{bc}	1.84^{b}
T_8 (0.5% WS+ 0.05% Syn)	9.57^{c}	7.76^{c}	6.56^{b}	5.71	2.25^{bc}	1.87^{b}
SEM	0.08	0.088	0.063	0.091	0.016	0.017

Means in the same column bearing different superscripts are significantly different ($p<0.05$)

NS Non significant C Control W S *Withania somnifera* Syn Synbiotic

The serum calcium level is considered as an important indicator of growth status of the body. The serum calcium level of control (T_1), T_5 and T_7 treatment groups were nonsignificantly different ($p>0.05$) at 4th week of trial whereas at 6th week, the serum calcium of T_1 group nonsignificantly varied with values of T_2 and T_3 treatments. The *Withania* at every level up to market age imparted significant and similar effect on calcium absorption or its retention in the body but after the period of high heat stress, the calcium sparing effect of 0.5% and 1% level of *Withania* were reduced. Exceptionally, the combined treatment group 'T_8' significantly enhanced the serum calcium status during the whole trial period. The low serum calcium values during the whole trial in control group might affected the body weight gain in broilers under control group. The higher level of synbiotic (0.05%) in basal diet produced moderate to high level of calcium boosting effect in serum.

The serum phosphorus level significantly increased with 0.05% synbiotic or 0.5% *W.somnifera*+0.05% synbiotic inclusion in the diet with respect to control at 4th week of age. The broilers in T_1 group nonsignificantly differed with T_2 to T_5 and T_7 group. The phosphorus level was numerically higher in all dietary supplemented groups at 42nd day of collection however no significant variation was observed between any of the treatments.

The serum level of magnesium significantly affects the activity of various enzymes and functioning of oxidative phosphorylation. The four week serum magnesium level was significantly higher in all the supplemented groups except T_5 group with highest value was observed in 0.05% synbiotic fed broilers (T_6). Broilers fed ashwagandha with three different levels did not show any variation in serum magnesium concentrations at 28th day of trial.

The serum sampling at 6th week revealed significant variation of all supplemented treatments with non supplemented T_1 broilers. The magnesium values in blood ranged from 1.62 (T_1) to 1.87mg/dl (T_8). Very scant literature is available regarding the effect of ashwagandha on serum minerals. Similar calcium sparing effect of ashwagandha was observed in pesticides intoxicated cockerels (Varma *et al*., 2011). The enhanced serum calcium and phosphorus level in synbiotic supplemented broilers in present study was not observed by Abdel-Raheem and Abd-Allah (2011). The probiotic used by Khan *et al*. (2013) also failed to observe significant effect on serum calcium and magnesium levels. The well documented role of magnesium in attenuation of free radicals (Garcia *et al*. 1998) was evident in 6th week during the period of highest heat stress. Elevated serum magnesium

concentration in supplemented groups at both intervals indicates improved antioxidant capacity due to key role of magnesium in activity of hepatic catalase (Guo *et al*., 2003).

4.5.8 Serum enzyme profile

Low AST and ALT levels are an indicator of better health in animals. According to Peric *et al*. (2009), the determination of AST and ALT is an indicator of oxidative damage in liver tissue and their elevated levels are usually associated with liver diseases. Mean serum concentration of hepatic enzymes, AST and ALT of broilers as influenced by dietary inclusion of *W. somnifera* and synbiotic are presented in **Table 4.19**.

4.5.8.1 Serum AST and ALT values

Mild to moderate climatic stress experienced by broilers under different treatments did not alter the AST and ALT values and thus nonsignificant variation among treatments was observed at the end of 4th week of trial. The value of AST and ALT (IU/L) at market age ranged between 169.67 (T_8) to 184 (T_1); and 13.33 (T_1) to 15.78 (T_1), respectively. The unchanged AST and ALT values observed in the present study give an indication that liver and kidney function were not disturbed during period of moderate climatic stress. Significant deviation in AST and ALT values among treatments was observed in the last two weeks of trial with the increase in weekly mean ambient temperature. Analysis of serum enzyme activity revealed that T_2, T_3, T_4 and T_8; and T_2, T_3, T_4, T_6, T_7 and T_8 recorded significantly ($p<0.05$) lowered AST and ALT enzyme activity, respectively than control group (T_1) at the end of trial.

The amelioration action was noted for AST (IU/l) and ALT (IU/l) wherever *W. somnifera* supplementation was carried out which significantly improved and optimized their serum levels as compared to control group. Similarly, concentrations of blood enzymes (AST and ALT) were further lowered ($p<0.05$) with synbiotic supplementation in broiler birds. The different level of either *Withania* or synbiotic resulted in statistically similar decrease in AST and ALT values. The significant decrease in the levels of biochemical marker enzymes like AST and ALT in treated animals might be due to decreased leakage of the enzymes in liver cells. Ashwagandha feeding significantly prevented the elevation of serum AST and ALT in stress induced hepatic damage, which reflected the hepatoprotective role of ashwagandha in broilers.

Table 4.19 Mean serum AST and ALT values in different treatment groups of broilers at 28th and 42nd day of experimental trial

Treatments	Mean Serum AST (IU/L)		Mean Serum ALT(IU/L)	
	Day of sampling		Day of sampling	
	28th DayNS	42nd Day	28th DayNS	42nd Day
T1 (C)	184.00	263.44d	15.78	32.00c
T2 (0.5% WS)	177.00	151.33a	14.78	14.67a
T3 (1.0% WS)	177.55	171.67ab	14.78	18.33ab
T4 (1.5% WS)	169.78	186.67abc	14.44	14.56a
T5 (0.025% Syn)	177.11	239.67d	15.00	20.33b
T6 (0.05 % Syn)	172.44	233.67cd	14.89	16.00ab
T7 (0.25% WS +0.025% Syn)	172.22	221.67bcd	13.44	16.00ab
T8 (0.5% WS+ 0.05% Syn)	169.67	172.33ab	13.33	13.33a
SEM	1.99	5.76	0.426	0.61

Means in the same column bearing different superscripts are significantly different ($p<0.05$)

NS Non significant C Control W S *Withania somnifera* Syn Synbiotic

Abdel-Raheem and Abd-Allah (2011) in a trial on broiler chicken, observed nonsignificant effect of synbiotic on serum AST and ALT values at 21 days and 42 days of experiment whereas Ahmed *et al*. (2015b) detected significant increase in level of serum ALT in broilers fed synbiotic based diet. However, the current study observed findings similar to Abdel-Raheem and Abd-Allah (2011) for AST and ALT at 4^{th} week and AST at 6^{th} week but in contrast (Ahmed *et al*., 2015b) detected significant reduction of ALT on synbiotic supplementation at 42^{nd} days of trial. The differential findings might be due to compositional difference of synbiotic employed in the present study. Studies in broilers have detected no effect of probiotics on serum AST and ALT levels (Khan *et al*., 2013). Thus the observed effect of synbiotic on serum ALT and AST could be ascribed to prebiotic mannon oligosaccharides (MOS) present in synbiotic used in the current study (Yalcinkaya *et al*., 2008).

The hepatoprotective effect of ashwagandha seems to be due to its antioxidant effect during disease, thermal and intoxication stress. The present study observed stress ameliorative effect on heat induced serum elevation of AST and ALT hepatic enzymes through supplementation of ashwagandha herb (Jadhav *et al*., 2014). Similar study in lead intoxicated mice demonstrated the significant effect of *W. somnifera* on reduction of serum AST and ALT level (Sharma *et al*., 2012). The current finding of 0.5% level as optimum dose for hepatoprotective effect is in agreement with study of Kumari *et al*. (2015) in broilers who also observed suitability of 0.5% ashwagandha in basal diet for significant reduction of serum AST and ALT in *Salmonella* infected broilers. The serum AST (55%) and ALT (60%) reduction effect of *Withania somnifera* in alloxan induced diabetic rats (Udayakumar *et al*., 2009) was observed in the current investigation in heat stressed broilers. El-Boshy *et al*. (2013) also reported that oral administration of *W. somnifera* extract in *E. coli* infected guinea pigs returns the liver transaminase enzymes to their normal level. In contrast, Varma *et al*. (2011) did not observe any effect of ashwagandha root extract on pesticide intoxicated cockerels. The study conducted in broilers on effect of 2% herbal drug containing ashwagandha as main ingredient also failed to detect any significant effect of ashwagandha on serum AST and ALT levels (Srivastava *et al*., 2012).

4.6 Immunological Parameters

The effect of different level of supplementation of ashwagandha and synbiotic on immune status of broilers against IBD and RD virus was estimated through ELISA and HI titre, respectively and the results are presented in **Table 4.20**. The immune status of the birds as assessed for IBD virus was found to be significantly better in ashwagandha treated broilers groups, *viz.,* T_2 (714.44), T_3 (831.67), T_4 (961.66), T_8 (897) as compared to T_1 (431) at 4th week of age. Dose dependent effect (p□ 0.05) of ashwagandha on serum IBD titre was observed at 4th week whereas such effect at 6th week of age was observed only for T_3 and T_4 when the environmental stress was most. Immunomimetic effect of ashwagandha increased with the level of *W. somnifera* supplementation. The two level of synbiotic (T_5 and T_6) produced no significant change in serum IBD titre as compared to control during both collections. However, inclusion of synbiotic at 0.05% level in T_8 group significantly potentiated the effect of 0.5% *Withania* and ultimately improved the antibody status in T_8 group in equivalence to T_4 group at 28th and 42nd days. The ELISA titre (IBD) was observed to be significantly lowest in nonsupplemented broilers in T_1 group during the whole trial. The IBD titre ranged from 431 (T_1) to 961.67 (T_4); and 364.89 (T_1) to 773.67 (T_4) at 4th and 6th week, respectively. An overall reduction in antibody titre was observed in all the treatment groups in 6th week in comparison to 4th week titre due to heat stress. Mushtaq *et al*. (2011) also recorded significant increase in IBD titre of broilers fed with three different levels of *Withania* root extract however the dose dependent effect was not reported.

The mean serum RD titre at 28 days for control, *Withania*, synbiotic and their combined group was observed to 3.76 (T_1); 4.13 (T_2), 4.19 (T_3), 4.20 (T_4); 3.93 (T_5), 3.95 (T_6); 3.97 (T_7), 4.21 (T_8), respectively. The observations recorded for RD titre at 42 days were found in range of 2.62 (T_1) to 3.08 (T_4 and T_8). Similar to IBD titres, lowest RD titres was found for T_1 groups during the whole experiment. A non variable response in serum titre value of RD was observed for different doses of ashwagandha (T_2-T_4) at 28th and 42nd day of trial except for T_2 treatment in 6th week. The 0.05% synbiotic imparted significant intermediate effect on RD titre in 28th day but subsequently failed to increase RD titre during heat stress in 6th weeks. The low level of both feed additive used in T_7 treatment could not contributed significantly in raising immune status against RD at 42nd day. Treatment groups T_3, T_4 and T_8 exerted similar effect on RD antibody titre during the trial.

Table 4.20 Mean serum IBD and ND titre values (log_{10}) in broilers under different treatment groups at 28th and 42nd day of experimental trial

Treatments	Mean Serum IBD Titre		Mean Serum RD Titre	
	Day of sampling		Day of sampling	
	28th Day	42nd Day	28th Day	42nd Day
T_1 (C)	431^{a}	364.89^{a}	3.76^{a}	2.62^{a}
T_2 (0.5% WS)	714.44^{b}	541.11^{cd}	4.13^{c}	2.86^{b}
T_3 (1.0% WS)	831.67^{c}	595.66^{d}	4.19^{c}	3.06^{c}
T_4 (1.5% WS)	961.67^{d}	773.67^{e}	4.20^{c}	3.08^{c}
T_5 (0.025% Syn)	464.11^{a}	391.00^{ab}	3.93^{ab}	2.70^{ab}
T_6 (0.05 % Syn)	500.78^{a}	458.33^{abc}	3.95^{b}	2.73^{ab}
T_7 (0.25% WS+0.025% Syn)	504.89^{a}	491.67^{bcd}	3.97^{b}	2.82^{ab}
T_8 (0.5% WS+0.05% Syn)	897.00^{cd}	733.33^{e}	4.21^{c}	3.08^{c}
SEM	11.03	14.41	0.020	0.023

Means in the same column bearing different superscripts are significantly different ($p<0.05$)

C Control W S *Withania somnifera* Syn Synbiotic

1% *Withania* root powder was proved to be sufficient to increase the level of RD titre value both during moderate and high heat stress period (Vasanthakumar *et al*., 2014). Similar significant effect of 1% ashwagandha on RD titre in broilers was reported by Akotkar *et al*. (2007). The findings of this study are in contrast with those reported in broilers (Mushtaq *et al*., 2011) in which no significant effect of *W. somnifera* extract was observed on RD titre value. The absence of synbiotic effect on serum RD titre during the whole trial except for T_6 treatment at 28^{th} day is in contrast with the results obtained by Al-Sultan *et al*. (2016) which could be due to difference in level of synbiotic or the adverse environment conditions observed in the present study.

The effect of synbiotic on antibody titre was poorly studied during different trial in broilers as most of the trials were restricted for performance. The immunostimulant effect of *W. somnifera* is well documented in various animal species. The observed immunomodulatory effect of ashwagandha could be due to increase in number of immunoglobulin's producing cells, *i.e.,* lymphocytes, due to the presence of active principle glycowithanolides (Jadhav *et al*., 2014) in the roots of the ashwagandha. The findings of this study are also augmented by the earlier researcher (Agarwal *et al*., 1999; Sham *et al.,* 2003; Manoharan *et al*., 2004). Ashwagandha supplementation in broilers was found to improve both humoral and cell mediated immune response (Kumari *et al*., 2011). The skin thickness and antibody titre against RD were enhanced in broilers supplemented with 1% ashwagandha root powder (Akotkar *et al*., 2007). The response of broilers observed at each level was found to be absent in quail in which minimum level of 1.5% ashwagandha was required to produce significant change in antibody titre (Bhardwaj *et al*., 2012). The routine administration of ashwagandha in broilers was found to enhance antibody response against gumboro disease (Okonkwo *et al*., 2015).

The heat stress induced reduction in antibody titre in response to tropical environmental conditions was found to affect cell-mediated and humoral immunity in chickens (Niu *et al*., 2009). The antistressor effect of ashwagandha observed in the present study is in accordance with the findings of the Arivuchelvan *et al*. (2013) wherein significant recovery in RD titre was reported on supplementation of 2% ashwagandha in enrofloxacin mediated reduction in RD titre value.

4.7 Carcass Evaluation

4.7.1 Carcass yield and meat yield

The dressing%, eviscerated weight and meat yield from broilers under different treatments are summarized in **Table 4.21**. The dressing% of broilers significantly (p□ 0.5) differed between control (T_1) and treatments groups (T_2-T_8). Significantly lower dressing% was observed in broilers reared on basal diet without any feed additive (T_1). The treatment groups T_2-T_8 revealed statistically similar (p> 0.5) dressing values. The dressing% over the treatments ranged from 67.51 (T_1) to 70.81 (T_8).

The eviscerated weight over the treatments also revealed similar pattern to dressing% with significant difference between control (T_1) and treatment groups (T_2-T_8). All the supplemented, *i.e.,* T_2 toT_8, manifested nearly similar eviscerated weight with nonsignificant variation over other treatments. The eviscerated weight ranged from 63.11% (T_1) to 65.89% (T_3, T_4 and T_8).

The meat yield as per cent of live weight from breast muscle part showed remarkably higher (p□ 0.05) yield in broilers fed basal diet containing ashwagandha alone or combined with synbiotic. The differently prepared ashwagandha treated diets varied nonsignificantly with each other. The beast meat obtained from synbiotic fed broilers differed nonsignificantly from control (T_1) broilers. Highest breast yield (18.39%) (p□ 0.05) was obtained in T_8 treatment in which 0.5% *W. somnifera* was combined with 0.05% synbiotic substances. Lowest breast meat (15.41%) was recovered in T_1 group broilers fed on basal diet. The per cent leg muscle in all the treatments except T_1 and T_5 treatments remained statistically similar. The nonsupplemented T_1 group devoid of any supplementation revealed lowest leg meat content (13.02%) whereas the T_8 group significantly (p□ 0.05) exhibited maximum quantum of leg muscle. The variable level of ashwagandha employed in the present study could not able to create differences in the leg muscle content.

The 0.05% concentration of synbiotic in broiler feed demonstrated intermediate increase (p□0.05) in carcass yield and leg muscle however its inclusion in broiler rations seem to confer no extra additional benefits on the breast muscle yield (Ashayerizadeh *et al*., 2009). This finding on breast meat is in contrast to the observation of Abdel-Raheem and Abd-Allah (2011) that might be due to heat stress experienced by the broilers in the present study.

Significant improvement in per cent dressing weight, breast and leg muscle yield in variedly treated ashwagandha fed broilers in current study is in concurrence with the findings of Rindhe *et al*. (2012). The enhanced proportion of breast and leg muscle in ashwagandha treated groups indirectly indicates the anabolic nature and stress ameliorative nature of ashwagandha on dressing%, breast and leg meat during hot environment (Gu *et al*., 2008). Thus, the reported reduction in carcass weight and breast weight at 34ºC (Aksit *et al*., 2006) was not observed in *Withania* fed broilers in present investigation.

The present study observed safe and eco friendly role of phytoherb *W. somnifera* in the production of lean meat with overall quality (Guo *et al.* 2004). Similarly, Samarth *et al*. (2002) and Pedulwar *et al*. (2007) concluded that dietary supplementation of ashwagandha root powder increased the dressing percentage and meat yield of broiler birds. Use of 1% *W. somnifera* in current study increased the dressing per cent similar to that reported by Pandey *et al*. (2013). The improvement (3.30%) in dressing% of *Withania* treated broilers (T_8) than control was observed to be higher than reported (1.92%) by Sanjyal and Sapkota (2011) however the observed leg muscle yield (T_8:14.96%) was lower than reported (22.20%). The positive results in dressing weight, breast and leg muscle content indicates the nutritive effect of *W. somnifera* in broilers (Javed *et al*., 2009). In contrast, Vasanthakumar *et al*. (2014) observed the nonsignificant effect of *W. somnifera* root powder on dressing percentage.

4.7.2 Giblet and offals yield

The weight of different giblet and offals part as per cent of live weight is tabulated in **Table 4.22**. The weight of the liver in broilers under different treatments revealed nonsignificant variation with supplementation of either ashwagandha or synbiotic.

Table 4.21 Dressing weight, eviscerated weight and muscle weight (% of live weight) of broilers under different treatments

Parameters	Treatment Groups								SEM
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	
	C	0.5% WS	1.0% WS	1.5% WS	0.025% Syn	0.05% Syn	0.25% WS+ 0.025 Syn	0.5% WS+ 0.05% Syn	
Dressing Wt	67.51[a]	69.79[b]	70.22[b]	70.42[b]	69.49[b]	70.00[b]	70.03[b]	70.81[b]	0.171
Eviscerated Wt	63.11[a]	65.22[b]	65.89[b]	65.89[b]	64.89[b]	65.44[b]	65.44[b]	65.89[b]	0.186
Breast Muscle	15.41[a]	17.71[b]	17.43[b]	18.14[b]	16.86[ab]	17.07[ab]	17.65[b]	18.39[b]	0.208
Leg muscle	13.02[a]	14.52[b]	14.49[b]	14.76[b]	14.06[ab]	14.56[b]	14.61[b]	14.96[b]	0.139

Means in the same row bearing different superscripts are significantly different ($p<0.05$)

C Control W S *Withania somnifera* Syn Synbiotic

Table 4.22 Giblet and offals yield (% of live weight) from broilers under different treatments

Organs	Treatment Groups (%)								SEM
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	
	C	0.5% WS	1% WS	1.5% WS	0.025% Syn	0.05% Syn	0.25%WS + 0.025% Syn	0.5%WS + 0.05% Syn	
Giblet Yield									
Giblet	4.43^{a}	4.59^{ab}	4.54^{ab}	4.56^{ab}	4.57^{ab}	4.63^{ab}	4.53^{ab}	4.83^{b}	0.042
Liver^{NS}	2.11	2.06	2.01	2.01	2.01	2.01	2.11	2.17	0.033
Heart	0.42^{a}	0.43^{a}	0.43^{ab}	0.46^{ab}	0.43^{ab}	0.45^{ab}	0.43^{ab}	0.48^{b}	0.006
Gizzard	1.89^{a}	2.10^{b}	2.11^{b}	2.09^{b}	2.13^{b}	2.17^{b}	2.00^{ab}	2.19^{b}	0.023
Offals Yield									
Blood loss	2.52^{a}	2.89^{bc}	2.89^{bc}	2.91^{bc}	2.62a	2.65a	2.74ab	3.03c	0.027
Head^{NS}	2.17	2.25	2.29	2.21	2.26	2.28	2.30	2.29	0.015
Feath	13.82^{b}	12.75^{a}	12.61^{a}	13.21^{ab}	13.61^{b}	13.32^{ab}	13.57^{b}	13.12^{ab}	0.087
Shank	3.37^{a}	3.38^{a}	4.14^{b}	3.97^{b}	3.40^{a}	3.46^{a}	3.84^{b}	3.96^{b}	0.036
Lung	0.38^{ab}	0.38^{a}	0.37^{a}	0.43^{b}	0.39^{ab}	0.41^{ab}	0.38^{a}	0.43^{b}	0.005
Spleen	0.12^{a}	0.13^{b}	0.13^{b}	0.15^{c}	0.13^{ab}	0.13^{b}	0.14^{bc}	0.14^{bc}	0.002
Bursa	0.14^{a}	0.18^{b}	0.19^{bc}	0.22^{d}	0.20^{bcd}	0.21^{cd}	0.22^{cd}	0.23^{d}	0.003
Crop	0.55^{a}	0.56^{ab}	0.56^{ab}	0.62^{cd}	0.59^{bc}	0.65^{d}	0.62^{cd}	0.63^{cd}	0.005
Proventriculus^{NS}	0.33	0.33	0.33	0.36	0.36	0.37	0.35	0.34	0.005
Gall bladder	0.11^{ab}	0.13^{b}	0.11^{ab}	0.11^{ab}	0.12^{ab}	0.10^{a}	0.10^{a}	0.10^{a}	0.003
Intestine	4.28^{a}	4.32^{ab}	4.56^{abc}	4.42^{ab}	4.78^{c}	4.62^{bc}	4.78^{c}	4.81^{c}	0.035
Caeca^{NS}	0.95	0.94	0.92	0.95	1.04	1.06	0.98	1.05	0.016

Means in the same row bearing different superscripts are significantly different ($p<0.05$)
NS Non-significant C Control W S *Withania somnifera* Syn Synbiotic

Numerically highest liver weight was observed in broilers of T_8 group. Similar effect of 1% ashwagandha root powder or 0.15% ashwagandha root extract on liver weight was reported during summer season in broilers (Vasanthakumar *et al*., 2014). The other giblets such as heart revealed significant difference between control group and T_8 group with value of 0.42% and 0.48% for T_1 and T_8 groups, respectively. The other treatments, *viz.,* T_2-T_7, nonsignificantly differed with each other and control. No effect of single application of either growth promoter, i.e., *Withania* or synbiotic, was observed on heart weight. The increased weight of the gizzard reflects the increase in digestive or metabolic capacity of birds. The proportion of gizzard in response to treatments was significantly higher in T_2-T_6 and T_8 (p□ 0.05) in comparison to T_1 and T_7 group. The highest (2.19%) and lowest (1.89%) gizzard content were observed in T_8 and T_1 group, respectively. In terms of overall giblet weight, significant variation was only observed between nonsupplemented (T_1) and 0.5% *W. somnifera*+0.05% synbiotic treated broilers. Sanjyal and Sapkota (2011) reported slightly higher heart (0.53%) and liver (2.30%); and lower gizzard (2.06%) weight in ashwagandha fed broilers than observed for 0.5% *W.somnifera*+0.05% synbiotic fed broilers. The significant increment in total giblet yield in T_8 treatment corresponds to the findings of Rindhe *et al*. (2013) in *Withania* fed broilers. The observation recorded for synbiotic supplemented group for giblet, liver and heart weight are in agreement with results obtained by Saiyed e*t al*. (2015).

In meat industry, the bleeding loss during slaughter is associated with the quality of meat. Significantly higher blood loss in ashwagandha fed groups (T_2 toT_4) and T_8 group than control and other groups was observed after slaughter. Minimum blood loss (2.52%) occurred in control group whereas the broilers under T_8 treatment efficiently lost more blood. The proportion of head as per cent of total live weight in various treatments was found to be nonsignificant and ranged from 2.17% (T_1) to 2.29% (T_8). The feather weight was significantly higher in T_1, T_5 and T_7 group than T_2 and T_3 group. Treatment groups T_4, T_6 and T_8 differed nonsignificantly with other treatments with respect to feather weight. The reduction in feather weight with supplementation of phytoherb *Withania* is in agreement with observation recorded by Ahmed *et al*. (2015a). The shank weight being representative of strength of broiler to support higher body weight was observed to be significantly higher in T_3, T_4, T_7 and T_8 treatments than rest of the treatments. The shank weight of broilers under different treated groups ranged from 3.37% (T_1) to 4.14% (T_3).

The lung is one of the major vital organs that has significant role in perfusion and oxygen supply to body during period of active growth. Numerically higher lungs weight was observed in T_4 and T_8 groups. The broilers under T_4 and T_8 groups revealed significantly higher lung mass in comparison to T_2, T_3 and T_7 groups. The spleen and bursa, the two immunologically active organs of birds, were found to be significantly different in various treatments. The spleen weight was significantly higher in T_2-T_4 and T_6-T_8 groups than control. The 0.5% and 1% level of *Withania* in basal diet; and T_7 and T_8 treatments resulted in similar weight gain in spleen than control. The other immune organ, bursa also demonstrated the significant effect of either *Withania* or synbiotic or their combination on weight. All supplemented broiler groups revealed significantly higher bursa weight than nonsupplemented control broilers with maximum effect (p 0.05) observed for T_8 group. The significantly lowest bursa weight (0.14%) was observed in control group.

The crop is the prime organ of feed storage in birds and indirectly represents the feed intake capacity of broilers. The empty weight of crop in the present study ranged from 0.55% (T_1) to 0.65 (T_6). The control group revealed significantly lowest crop weight than most treatments except T_2 and T_3. The storage capacity in terms of crop weight was statistically similar in T_4, T_7 and T_8 treatment groups. The weight of proventriculus remains differed nonsignificantly in all the treatments with minimum and maximum weight of 0.33% and 0.37%, respectively. The gall bladder weight also nonsignificantly varied over the treatments except treatment group T_2 which significantly deviated from T_6, T_7 and T_8 groups. The weight of intestine indirectly reflects absorptive capacity of the intestine. The weight of intestine in treated groups, T_5 to T_8 was found to be significantly (p 0.5) higher than control broilers. The higher relative weight of intestine in supplemented groups may be associated with improvement in intestinal micro architecture (Burkholder *et al*., 2008; Awad *et al*., 2008). The *Withania* based diet in treatment T_2 to T_4 failed to demonstrate any difference with control group which indicate poor role or no role of ashwagandha in development of intestinal parts. In contrast to the study of Sohail *et al*. (2013), the variedly prepared dietary formulation failed to become source of variation for difference in caecal weight of broilers.

The effect of dietary synbiotic was statistically observed only for gizzard, bursa, crop and intestinal weight in the present study. Significant beneficial effect (p 0.05) of an experimental blend of *Withania* and synbiotic was observed for heart and gizzard (giblets); lung, spleen, bursa, crop, intestine and shank weight (offals). The

investigated MOS in combination with probiotic significantly affected the dressing per cent and intestine weight of broilers in contrast to the findings of Bozkurt *et al*. (2009). The results reported for effect of MOS containing synbiotic (Abdel-Raheem and Abd-Allah, 2011) were replicated only for head weight and could not be observed for gizzard, spleen, proventriculus and bursa in the present study. The possible reason for variation in result could be due to increasing trend (33℃ to 36.7℃) of environmental temperature observed in the present trial in contrast to reverse trend (32℃ to 25℃) observed by Abdel-Raheem and Abd-All ah (2011). The differently prepared synbiotic used by Dizaji *et al*. (2012) did not produced any significant difference ($p > 0.05$) between experimental groups for proventriculus, gizzard, liver and bursa. The increased weights of immune organs (bursa and spleen) on supplementation of either *Withania* or synbiotic in heat stressed birds (Sohail *et al*., 2013) have resulted in enhancement of antibody titre.

4.7.3 Shank length, intestine length and caecal length

The length of three important part of carcass, *i.e.,* shank, intestine and caeca, are recorded treatment wise and represented in **Table 4.23**.

The shank is considered as an important organ of heat dissipation in broilers. Due to the thick insulation coat of feathers on most of the body surface, broilers efficiently increase their sensible heat loss from featherless parts such as shank and feet through increase in blood flow when exposed to heat stress (Cangar *et al*., 2008). The shank length of nonsupplemented broilers in control group (T_1) was observed to be significantly highest. The lowest shank length was obtained in broilers under T_8 group. Nonsignificant variation in shank length was observed among treatments T_2, T_3, T_4 and T_8. The effect of synbiotic supplementation on reduction of shank length was also observed in the present study. The two level of synbiotic or three level of ashwagandha produced similar decrease in shank length. The increased shank length in nonsupplemented broilers (T_1) indicates an effort of the broilers to increase their surface area to enhance heat loss from body during heat stressed condition. The least shank length observed in *Withania* supplemented broilers supports its antistressor effect (Kumari *et al*., 2015).

Table 4.23 Length of shank, intestine and caecum in different treatments during experimental trial

Treatments	Shank length (cm)	Intestine length (cm)	Caecum length (cm)
T_1 (Control)	9.75^{d}	178.44^{a}	16.70^{a}
T_2 (0.5% WS)	8.67^{abc}	206.78^{b}	19.91^{b}
T_3 (1.0% WS)	8.33^{abc}	207.11^{b}	19.94^{b}
T_4 (1.5% WS)	8.17^{ab}	205.44^{b}	20.55^{bc}
T_5 (0.025% Syn)	8.83^{c}	204.22^{b}	19.73^{b}
T_6 (0.05 % Syn)	8.71^{bc}	208.55^{b}	19.99^{b}
T_7 (0.25% WS+ 0.025% Syn)	8.83^{c}	206^{b}	20.49^{bc}
T_8 (0.5% WS+ 0.05% Syn)	8.08^{a}	216.67^{b}	21.72^{c}
SEM	0.069	1.525	0.20

Means in the same column bearing different superscripts are significantly different ($p<0.05$)

C Control W S *Withania somnifera* Syn Synbiotic

The intestinal length of broilers as revealed in the current trial was observed to be highly significant between nonsupplemented control group (T_1) and any of the supplemented groups. The different supplemented groups, *viz.,* T_2 to T_8 differed nonsignificantly with each other. The length (cm) of intestine under different treatments ranged from 178.44 (T_1) to 216.67 (T_8). In contrast to present findings, nonsignificantly higher intestinal length indicative of better gut health, was reported (Vasanthakumar *et al*., 2014) in broilers fed 1% ashwagandha root powder or 0.15% ashwagandha root extract. However, the values observed for 1% *Withania* (207.11cm) was quite similar (213.50cm) to values observed by Vasanthakumar *et al*. (2014). Similarly, the synbiotic used by Saiyed *et al*. (2015) in broiler ration was found to produce only numerical difference among treatments with highest length was observed in synbiotic supplemented groups.

The caecum is the prime organ of microbial digestion in birds and plays an important role in mineral and vitamins metabolism. Significant variation in caecal length (p□0.05) was observed between control and supplemented groups. Lowest caecal length was estimated in broilers under T_1 group. The caecal length in different treatments was found to vary between 16.70cm (T_1) to 21.72cm (T_8). The ashwagandha treated broiler groups (T_2-T_4) did not differ among themselves on ground of level of incorporation of ashwagandha. In correspondence to ashwagandha treated groups, the two level of synbiotic also increased the caecal length in a similar fashion.

4.8 Evaluation of Gut Health

The results obtained on pH of intestinal contents (duodenum and caecum) and total coliforms counts in broilers of various treatments during the present investigation have been statistically analyzed and presented in **Table 4.24**.

4.8.1 Intestinal pH

The regulation of digestive system of broiler chicken depends on the pH of different parts of intestine (Rahmani *et al*., 2005). The health of the chicken and kind of nutrients consumed affect the pH level of digestive system of broilers. The pH in particular part of the gut also affects the growth of the microbes (Mabelele *et al*., 2013). The duodenal pH as recorded in the present study revealed significantly lower pH value for T_8 treatment followed by ashwagandha treated broiler groups (T_2-T_4).

The duodenal pH of T_5 to T_7 group was statistically similar whereas highest pH in duodenum was observed in broilers of control group. Nir *et al*. (1993) and Engberg *et al*. (2002) reported a negative relationship between intestinal pH and gizzard pH and suggested that increase in intestinal pH results in lower gizzard pH.

The pH of the caecal content exerts significant effect on the microbial digestion of the ingesta through modulation of the caecal environment. The volatile fatty acids produced during anaerobic microbial digestion maintain the caecal pH. Any disturbance in the caecal microbial population through intrinsic or extrinsic factor significantly alters the caecal pH. The low pH produced in the caecum by beneficial microorganism was found to be partly responsible for the suppression of harmful coliforms in the caecum (Denev, 2006). The present study demonstrated the beneficial effect of addition of either synbiotic or ashwagandha or both on the caecal pH. The caecal pH in control group was observed to be significantly highest with numerical value of 7.27. In *Withania* supplemented groups, it ranged from 7.12 (T_2) to 6.66 (T_4). The T_5 and T_6 groups reflected pH of 6.95 and 6.66, respectively. The groups T_7 and T_8 that fed combined feed additives exhibited pH of 6.77 and 6.32, respectively. Significant reduction in pH of the caecal content was observed with the addition of both feed additive in broiler ration which suggest their synergistic effect on the caecal health.

The effective role of synbiotic in maintenance of caecal pH could be due to its probiotic component (Kumprecht *et al*., 1994; Rada *et al*., 1995) which might favored the growth of beneficial bacteria in the caeca. A study conducted in broiler chickens also revealed the significant effect of probiotic in enhancement of numbers of beneficial lactobacilli and depression in the number of coliforms and *Salmonella* spp. through decrease in pH of caecal content (Denev, 2006). The supportive effect of ashwagandha on caecal pH could be indirectly through its antibacterial effect on coliforms bacteria (Kumari and Gupta, 2015). The *in vitro* antibacterial activity of *W. somnifera* against Gram-negative bacteria, particularly *Salmonella typhi* and *E. coli* has been observed by many other workers (Sundaram *et al*., 2011; Velu and Baskaran, 2012).

4.8.2 Total coliforms count

Caecum is considered as an area of high microbial activity in the intestine of broilers.

Table 4.24 Caecal pH and total coliforms count in caecal content of broilers in different treatment groups during experimental trial

Treatments	Intestinal pH		Total coliforms count (Log_{10} CFU/g)
	Duodenal pH	Caecal pH	
T_1 (Control)	5.99^{c}	7.27^{c}	5.66^{d}
T_2 (0.5% WS)	5.82^{b}	7.12^{bc}	5.06^{c}
T_3 (1.0% WS)	5.81^{b}	6.95^{bc}	5.04^{c}
T_4 (1.5% WS)	5.78^{b}	6.66^{ab}	4.90^{b}
T_5 (0.025% Syn)	5.71^{ab}	6.95^{bc}	5.06^{c}
T_6 (0.05 % Syn)	5.69^{ab}	6.66^{ab}	4.85^{ab}
T_7 (0.25% WS+0.025% Syn)	5.69^{ab}	6.77^{abc}	5.01^{c}
T_8 (0.5% WS+0.05% Syn)	5.57^{a}	6.32^{a}	4.83^{a}
SEM	0.020	0.066	0.008

Means in the same column bearing different superscripts are significantly different ($p<0.05$)

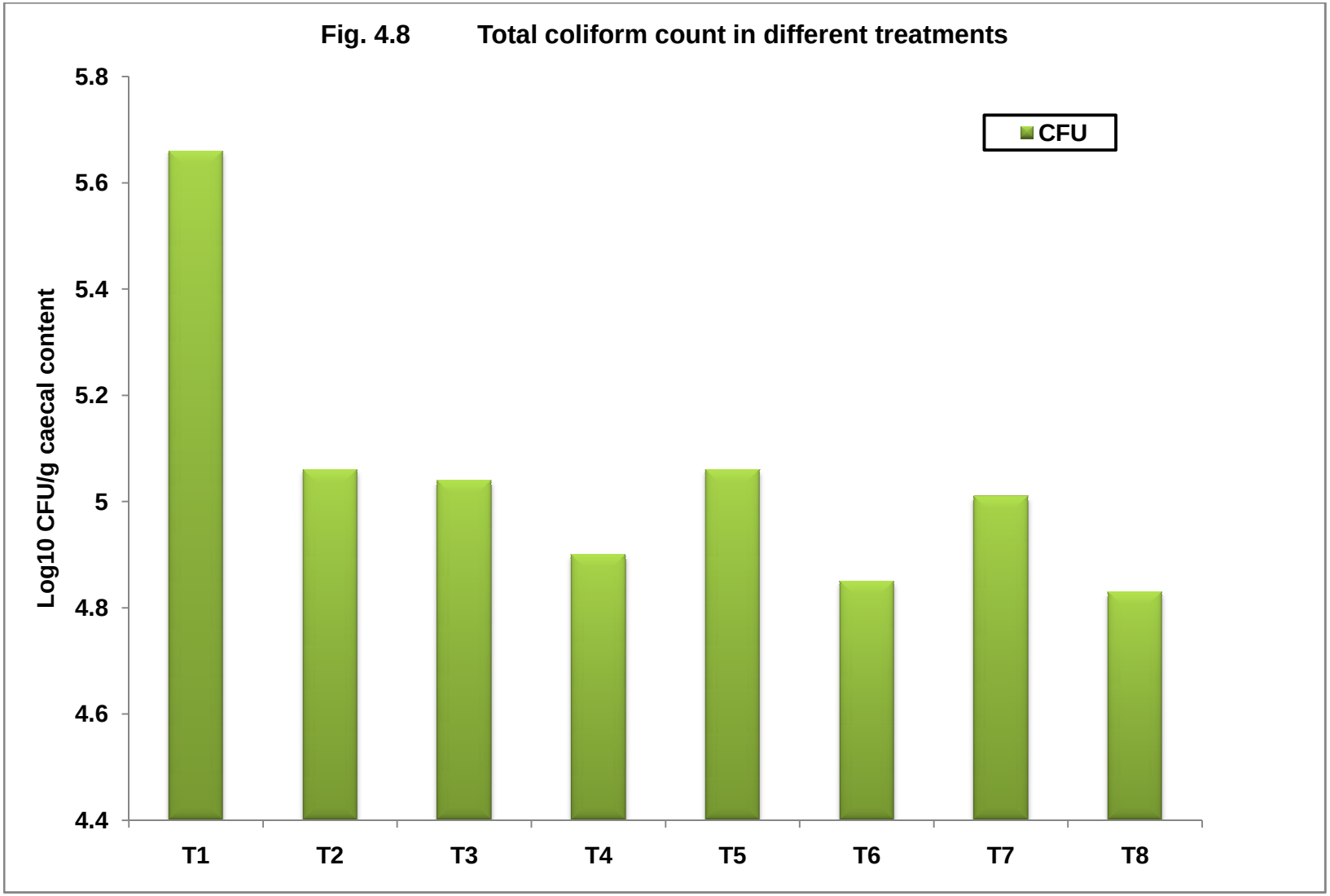
C Control W S *Withania somnifera* Syn Synbiotic

The caecal microflora of the alimentary tract has a significant effect on the health and performance of poultry and exerts significant protection against the establishment of microbial pathogens belongs to coliforms group (Barrow, 1992). It is well established that manipulation of gastric microflora in poultry has significant effect on the growth rate and efficiency of feed utilization. Modulation of caecal bacteria towards a “healthy community” by feeding probiotics, prebiotics and phytoherb is an alternative approach to improve gastrointestinal health by favoring beneficial microflora and suppressing pathogenic bacteria (Apajalathi *et al*., 2004).

The present study has shown that the inclusion of synbiotic and phytoherb ashwagandha in basal diet significantly decreased the number of coliforms in the cecal content of broiler chicks compared to the control ($p<0.05$). The total coliforms count estimated was found to be significantly highest (5.66 $\log_{10}$ cfu/g caecal content) in nonsupplemented broilers in T_1 group (**Fig. 4.8)**. The inclusion of synbiotic in basal diet of T_6 treatment group or its combination with 0.5% *W. somnifera* in T_8 treatment resulted in significant reduction of coliforms count. Mild to moderate decrease ($p \square 0.05$) in coliforms counts were observed with varying level of ashwagandha fed groups (T_2-T_4). The combined group, T_7 with low level of *Withania* and synbiotic demonstrated mild but significant decrease in coliforms counts. The total coliforms counts in different treatments ranged from 5.66 (T_1) to 4.83 (T_8) $\log_{10}$ cfu/g caecal content.

A number of studies (Pail *et al*., 1990; Bonomi *et al*., 1995) have reported beneficial effect of addition of probiotics to the diet through increase in number of lactic acid bacteria and by decrease in the number of coliforms, particularly *E. coli*. Many other investigators have studied the potentials of probiotics, which exert *in vitro* inhibitory effects toward enteric microorganisms (Edens, 2003) and *in vivo* growth competition with *E. coli* in chickens (Watkins *et al*., 1982). The present study is in conformity with the findings of Denev (2006). The significant effect of *W. somnifera* in reduction of coliforms bacteria could be due to its direct inhibitory effect on coliforms bacterial growth, specifically *E. coli* (Kumari and Gupta, 2015). An *in vitro* assessment of antibacterial activity of ashwagandha revealed significant antimicrobial effect on many harmful and pathogenic bacteria (Singh and Kumar, 2012). Thus the lowest value of cfu observed for coliforms bacteria in T_8 treatment group could be attributed to direct antibacterial effect of ashwagandha on coliforms bacteria and indirect effect of synbiotic through lowering of caecal pH.

Fig. 4.8 Total coliform count in different treatments



4.9 Meat Quality Evaluation

Poultry meat is an excellent source of high quality protein, vitamins and minerals to balance the human diet. The breast muscle is very developed in broiler lines and constitute about 22-25% of the whole carcass weight (Wang *et al*., 2009). Thus the qualitative assessment of the broiler breast meat was carried out in the present study to observe the anabolic and hypolipidemic effect of *W. somnifera* and the nutrient sparing effect of synbiotics on broiler breast meat composition.

4.9.1 Proximate composition of broiler meat

The proximate composition of broiler breast meat revealed significant (p□ 0.05) difference in mean crude protein (CP) content (**Table 4.25**). The mean CP (dry matter basis) was highest in the broiler group supplemented with 1.5% *Withania* however comparable performance was observed in T_8 group supplemented with 0.5% Withania+0.05% synbiotic. The CP content in different treatments ranged from 86.84% to 90.10% with lowest value observed in non-supplemented boiler group (T_1). Mean ether extract of breast meat also exhibited significant ($p<0.05$) differences among different treatments. A downward trend in ether extract value was observed with increase level of *Withania* supplementation. The combined feeding approach through *W. somnifera* and synbiotic supplementation at 0.5% and 0.05% level, respectively showed the lowest ether extract value. The results indicate the health promoting synergistic effect of herb *Withania* and synbiotic substances in reduction of fatty substances. The highest and significant ether extract value was observed in the control group (T_1). The ash content of the breast muscle was found to be highest in 0.05% synbiotic supplemented group (T_6) whereas the treatment groups T_5, T_7 and T_8 showed comparable ash content. Comparable ash content was observed in control and *Withania* supplemented groups (T_1- T_4).

The present study observed gradual enrichment of protein content in breast meat of broilers which supports the anabolic effect of ashwagandha as claimed in traditional Indian medicine (Panda and Kar, 1997). The study also revealed the synergistic effect of synbiotic on *Withania* supplementation in improving the protein content of muscle which could be due to enhanced level of absorption of nutrients in the gut. The broilers in the nonsupplemented group (T_1) failed to respond towards high ambient temperatures and showed lowest CP content in breast meat through decreased protein synthesis (Lin *et al*., 2006).

Table 4.25 Proximate composition (% DM) of broiler breast meat under different treatments

Treatments	Crude protein	Ether extract	Ash
T_1 (C)	86.84^{a}	7.50^{d}	4.75^{a}
T_2 (0.5% WS)	87.83^{bc}	6.58^{c}	4.67^{a}
T_3 (1% WS)	88.43^{cd}	5.91^{c}	4.65^{a}
T_4 (1.5% WS)	90.10^{e}	4.33^{a}	4.68^{a}
T_5 (0.025% Syn)	87.66^{abc}	6.39^{c}	4.99^{ab}
T_6 (0.05 % Syn)	87.41^{ab}	6.37^{c}	5.31^{b}
T_7 (0.25% WS+ 0.025%Syn)	88.77^{d}	5.19^{b}	5.05^{ab}
T_8 (0.5% WS+ 0.05% Syn)	89.82^{e}	4.20^{a}	5.10^{ab}
SEM	0.297	0.236	0.141

Means in the same column bearing different superscripts are significantly different ($p<0.05$)

C Control W S *Withania somnifera* Syn Synbiotic

The comparative reduction in breast meat protein in control group following exposure to continuous heat stress above 34℃ is in line with the findings of (Akit *et al*., 2005) who also observed 8% reduction in breast meat protein when the broilers were exposed to 34℃ after three weeks of age. Studies have shown the association of heat stress with depression in meat chemical composition with low CP and higher ether extract content observed in the breast meat of broilers (Gonzalez and Leeson, 2005). The higher ash values observed in the synbiotic supplemented groups might have occurred due to contributory effect of synbiotic. Significant anabolic impact of 1% *W. somnifera* supplementation on VenCobb broilers was also observed by (Vasantkumar *et al*., 2014).

4.9.2 pH and water holding capacity of broiler meat

The pH and water holding capacity of broiler breast meat was estimated 24h post slaughter and the results for different treatments are presented in **Table 4.26.** Highest and significant pH ($p \leq 0.05$) was observed in broilers devoid of any supplementation. The T_5 broiler group supplemented with lower level of synbiotic also revealed statistically similar result to that of control broilers with numerically lower value of pH. The broilers group, T_5 to T_7 also revealed nonsignificant variation among themselves. All the three levels of ashwagandha (T_2 to T_4) and combined treated group T_8 imparted similar effect on pH that ranged between 5.84 (T_4) to 5.86 (T_6). The higher ultimate pH in broilers of control group (T_1) could be the resultant effect of heat stress leading to depletion of glycogen reserve. Gu *et al*. (2015) also reported the pH value of breast and thigh meat was 6.2 and 6.4, respectively in stressful environment and suggested that heat stress could contribute to the development of pale, soft, exudative (PSE) meat.

The antistressor effect of ashwagandha seemed to maintain the energy storage of the muscle. The observed pH value of breast meat in ashwagandha fed broilers (T_2-T_4) and T_8 groups nearly matches with the pH value of fresh broiler meat (5.96) as reported by Castellini *et al*. (2002). Nearly similar value of pH (5.96±0.03) was also determined by Qiao *et al*. (2002) for broiler breast meat. The pH affects the interfilamental space in muscle and ultimately the water holding capacity. Sarcoplasmic proteins responsible for water holding capacity (WHC) are affected by post mortem fall in pH.

Table 4.26 pH and water holding capacity of broiler breast meat (24 hr post slaughter) under different treatments

Treatments	pH	WHC
T_1 (C)	6.01^{c}	59.06^{a}
T_2 (0.5% WS)	5.86^{a}	60.31^{ab}
T_3 (1% WS)	5.85^{a}	62.09^{bc}
T_4 (1.5% WS)	5.84^{a}	63.37^{bc}
T_5 (0.025% Syn)	5.96^{bc}	61.67^{abc}
T_6 (0.05 % Syn)	5.93^{b}	63.06^{bc}
T_7 (0.25% WS+ 0.025%Syn)	5.93^{b}	62.42^{bc}
T_8 (0.5% WS+ 0.05% Syn)	5.86^{a}	63.75^{c}
SEM	0.007	0.340

Means in the same column bearing different superscripts are significantly different ($p<0.05$)

C Control W S *Withania somnifera* Syn Synbiotic

The water holding capacity is the ability of muscle to retain its own water and reflects the amount of water held in the inter-filamental space and indirectly reflects the quality and structural framework of muscle required to hold the water. Significantly higher water holding capacity of broiler breast meat was observed in T_8 treatment followed by T_4, T_6, T_7 and T_3.

The meat of control broilers were found to retain 4.69% less water (p□0.05) than T8 groups which could affects the taste, flavour and juiciness of muscle. The tenderness, juiciness, firmness, and appearance of meat have been reported (Anadon, 2002) to improve as the content of water in the muscle increases. Nonsignificant variation in water holding was observed in T_1, T_2 and T_5 treatment groups. The present study is in contrast with findings of Ogunwole *et al*. (2013) who observed non-significant difference in water holding capacity and pH of meat of broilers fed graded levels of ascorbic acid. Low WHC (Honikel *et al.,* 1996) results in serious economic losses through reduction in quantum of salable products and loss of export to customers who demands high quality end products. Meat with low WHC often tends to produce inferior meat products.

4.9.3 Sensory characteristics of broiler meat

The sensory evaluation of broiler breast meat was carried out through nine panel based Hedonic scale method for different attributes of meat quality such as appearance, colour, odour, juiciness, texture, tenderness, flavor and overall palatability (**Table 4.27**). The attributes like odour, colour and tenderness appeared to be nonsignificant among the different treatment groups. The overall acceptability, colour and juiciness were observed significantly highest for breast meat of T_4 and T_8 treatments than rest of the treatments (**Fig 4.9**). The palatability of breast meat from T_1 (control) and T_5 treatment was observed comparable with each other. The colour of the meat from *Withania* fed broilers (T_2-T_4) and T_8 group was significantly better than other treatments (Ogunwole *et al*., 2013). Significantly better appearance was observed in meat of T_8 broilers than other meat group. Chouliara *et al*. (2007) remarked that color parameter of fresh chicken breast meat did not vary by adding grape seed and bearberry extracts with statistically similar acceptability in all the treatments.

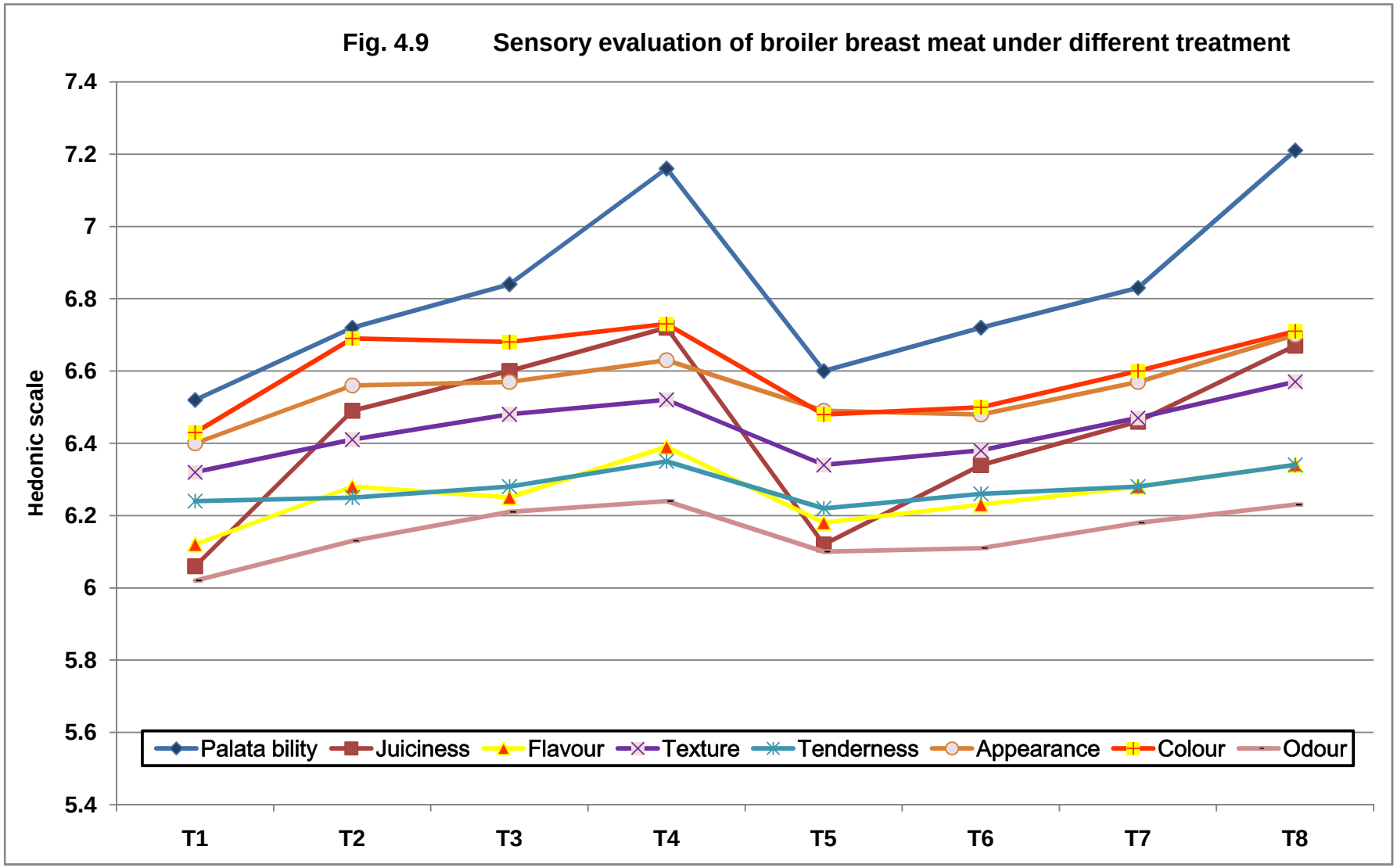
Table 4.27 Sensory evaluation of broiler breast meat under different treatments

Treat-ments	Appea-rance	Colour	Odour^{NS}	Juici-ness	Texture	Tender ness^{NS}	Flavour^{NS}	Over all Palata-bility
T_1 (C)	6.40^{a}	6.43^{a}	6.02	6.06^{a}	6.32^{a}	6.24	6.12	6.52^{a}
T_2 (0.5% WS)	6.56^{bc}	6.69^{c}	6.13	6.49^{ab}	6.41^{abc}	6.25	6.28	6.72^{bc}
T_3 (1.0% WS)	6.57^{bc}	6.68^{c}	6.21	6.6^{bc}	6.48^{cd}	6.28	6.25	6.84^{c}
T_4 (1.5% WS)	6.63^{bc}	6.73^{c}	6.24	6.72^{d}	6.52^{cd}	6.35	6.39	7.16^{d}
T_5 (0.025% Syn)	6.49^{ab}	6.48^{ab}	6.10	6.12^{a}	6.34^{a}	6.22	6.18	6.60^{ab}
T_6 (0.05 % Syn)	6.48^{ab}	6.50^{ab}	6.11	6.34^{b}	6.38^{ab}	6.26	6.23	6.72^{bc}
T_7 (0.25% WS + 0.025% Syn)	6.57^{bc}	6.60^{bc}	6.18	6.46^{bc}	6.47^{bcd}	6.28	6.28	6.83^{c}
T_8 (0.5% WS + 0.05% Syn)	6.70^{c}	6.71^{c}	6.23	6.67^{d}	6.57^{d}	6.34	6.34	7.21^{d}
SEM	0.017	0.015	0.032	0.019	0.014	0.025	0.033	0.019

Means in the same column bearing different superscripts are significantly different ($p<0.05$)

NS Non-Significant C ControlW S *Withania somnifera* Syn Synbiotic

Fig. 4.9 Sensory evaluation of broiler breast meat under different treatment



4.10 Economics of Single and Combined Use of *W. somnifera* and Synbiotic Feed Additive in Ration of Broiler Chicks

The cost benefit analysis for inclusion of *W. somnifera* and synbiotic in ration of broiler chicks under different treatments was carried out to evaluate the economic feasibility of their inclusion in broiler diets. The total input cost and other expenditure incurred on feed, chick and miscellaneous expenses on per bird basis under different treatments has been worked out at the end of experimental period (**Table 4.28** and **4.29)**. The total input cost varied with the amount of feed consumed by broilers under each treatment and the type and amount of feed additive used.

The average feed cost per broiler on account of average feed intake was observed to significantly lower in control due to no input cost of feed additive and low ultimate body weight at the end of the trial. The feed cost per broiler in synbiotic supplemented T_5 and T_6 groups was found to be statistically comparable with control group. Similarly the feed cost for 0.5% and 1% *W. somnifera* were found to be nonsignificantly similar but significantly higher than control broilers. Significantly higher feed cost was recorded in broilers under T_4 group than all other group which could be due to higher input cost on account of ashwagandha. The feed cost per broiler in T_7 group was found to be similar ($p \square 0.05$) to that of broilers in T_2, T_5 and T_6 groups whereas the feed cost incurred to raise broilers of T_8 group was observed to be similar to T_3 group.

On the basis of average body weight gained by broilers in each treatment, the total income varied significantly among treatments (**Table 4.30**). The average income achieved per bird was significantly lowest (Rs.150.62) in broilers not supplemented with any feed additive whereas the highest selling income of Rs.206.31 and Rs.203.87 was recovered from broilers in T_8 and T_4 group, respectively. Statistically similar amount per bird was fetched from broilers reared under T_2, T_3, T_5, T_6 and T_7 groups. The profit per bird was significantly highest (Rs.47.05) in T_8 group with a profit of about 29.54%. The profit % of broilers in control group was found to be restricted to 12.14% with Rs.16.34 only. The 1.5% ashwagandha supplemented T_4 broilers delivered intermediate return (Rs. 31.14) with profit % of 18.02. The profit % per bird in T_2, T_3, T_4, T_5, T_6 and T_7 groups were observed to be 22.90, 19.37, 18.02, 22.89, 26.34 and 25.02, respectively.

Table 4.28 Total cost of dietary rations fed to broilers under different treatments

Treatment	Replicate	Broiler control feed			*W. somnifera* root powder			Synbiotic powder			Total feed cost-(Rs.) 5+8+11
		Feed intake(kg)	Cost (Rs/ kg)	Feed cost (Rs.)	WS intake (kg)	Cost (Rs./ kg)	Cost of WS (Rs.)	Syn. Intake (kg)	Cost (Rs/ kg)	Cost of Syn (Rs.)	
1	2	3	4	5	6	7	8	9	10	11	12
T_1 (Control)	1	3.646	25.00	91.16	-	-	-	-	-	-	91.16
	2	3.471	25.00	86.76	-	-	-	-	-	-	86.76
	3	3.837	25.00	95.91	-	-	-	-	-	-	95.91
	Mean total feed cost										**91.28^{a}**
T_2 (0.5%WS)	1	4.045	25.00	101.13	0.020	375.00	7.585	-	-	-	108.71
	2	3.909	25.00	97.72	0.019	375.00	7.329	-	-	-	105.05
	3	3.946	25.00	98.65	0.019	375.00	7.398	-	-	-	106.04
	Mean total feed cost										**106.61^{cd}**
T_3 (1.0%WS)	1	3.686	25.00	92.14	0.036	375.00	13.82	-	-	-	105.96
	2	3.813	25.00	95.32	0.038	375.00	14.29	-	-	-	109.61
	3	4.173	25.00	104.33	0.041	375.00	15.64	-	-	-	119.98
	Mean total feed cost										**111.85^{de}**
T_4 (1.5%WS)	1	4.193	25.00	104.82	0.062	375.00	23.58	-	-	-	128.41
	2	4.324	25.00	108.10	0.064	375.00	24.32	-	-	-	132.43
	3	4.190	25.00	104.74	0.062	375.00	23.56	-	-	-	128.31
	Mean total feed cost										**129.72^{f}**
T_5 (0.025% SYN)	1	4.150	25.00	103.76	-	-	-	0.001	350.00	0.363	104.12
	2	3.752	25.00	93.79	-	-	-	0.001	350.00	0.328	94.12
	3	3.918	25.00	97.94	-	-	-	0.001	350.00	0.342	98.28
	Mean total feed cost										**95.84^{abc}**
T_6 (0.05 %SYN)	1	4.076	25.00	101.90	-	-	-	0.002	350.00	0.713	102.61
	2	3.751	25.00	93.78	-	-	-	0.002	350.00	0.656	94.43
	3	3.768	25.00	94.18	-	-		0.002	350.00	0.659	94.84
	Mean total feed cost										**97.30^{ab}**
T_7 (0.25% WS + 0.025%	1	4.183	25.00	104.57	0.010	375.00	3.921	0.001	350.00	0.366	108.86
	2	3.981	25.00	99.52	0.009	375.00	3.732	0.001	350.00	0.348	103.60
	3	3.636	25.00	90.89	0.009	375.00	3.408	0.001	350.00	0.318	94.61
	Mean total feed cost										**102.36^{bc}**
T_8 (0.5% WS+ 0.05% SYN)	1	4.218	25.00	105.44	0.021	375.00	7.908	0.002	350.00	0.738	114.08
	2	4.215	25.00	105.37	0.021	375.00	7.903	0.002	350.00	0.737	114.01
	3	4.461	25.00	111.51	0.022	375.00	8.363	0.002	350.00	0.780	120.66
	Mean total feed cost										**116.25^{e}**

Table 4.29. The total input cost (Rs./bird) and total income (Rs./bird) under different treatment groups during experimental trial

Treatment	Replicate	Total feed cost- (Rs.)	Chick cost (Rs./ chick)	Miscellaneous exp. (Rs./ bird)	Total input cost (Rs./bird) 3+4+5	Income		
						Total body wt (kg)	Selling price (Rs./kg)	Bird sold (Rs./bird) 7*8
1	2	3	4	5	6	7	8	9
T_1 C	1	91.16	28.00	15.00	134.16	1.920	80.00	153.65
	2	86.76	28.00	15.00	129.77	1.781	80.00	142.51
	3	95.91	28.00	15.00	138.91	1.946	80.00	155.69
	Mean input cost (Rs./bird)				**134.28^{a}**	**Mean income (Rs./bird)**		**150.62^{a}**
T_2 0.5% WS	1	108.71	28.00	15.00	151.72	2.374	80.00	189.92
	2	105.05	28.00	15.00	148.06	2.280	80.00	182.42
	3	106.04	28.00	15.00	149.05	2.241	80.00	179.31
	Mean input cost (Rs./bird)				**149.61^{cd}**	**Mean income (Rs./bird)**		**183.89^{b}**
T_3 1.0% WS	1	105.96	28.00	15.00	148.96	2.196	80.00	175.73
	2	109.61	28.00	15.00	152.62	2.271	80.00	181.70
	3	119.98	28.00	15.00	162.98	2.467	80.00	197.36
	Mean input cost (Rs./bird)				**154.85^{de}**	**Mean income (Rs./bird)**		**184.93^{b}**
T_4 1.5% WS	1	128.41	28.00	15.00	171.41	2.523	80.00	201.89
	2	132.43	28.00	15.00	175.43	2.611	80.00	208.88
	3	128.31	28.00	15.00	171.31	2.5103	80.00	200.82
	Mean input cost (Rs./bird)				**172.72^{f}**	**Mean income (Rs./bird)**		**203.87^{c}**
T_5 0.025% Syn	1	104.12	28.00	15.00	147.12	2.2799	80.00	182.34
	2	94.12	28.00	15.00	137.12	2.116	80.00	169.30
	3	98.28	28.00	15.00	141.29	2.141	80.00	171.33
	Mean input cost (Rs./bird)				**141.84^{abc}**	**Mean income (Rs./bird)**		**174.33^{b}**
T_6 0.05% Syn	1	102.61	28.00	188.66	145.61	2.358	80.00	188.66
	2	94.43	28.00	167.80	137.44	2.097	80.00	167.80
	3	94.84	28.00	175.55	137.85	2.194	80.00	175.55
	Mean input cost (Rs./bird)				**140.30^{ab}**	**Mean income (Rs./bird)**		**177.34^{b}**
T_7 0.25%WS+ 0.025% Syn	1	108.86	28.00	15.00	151.86	2.346	80.00	187.71
	2	103.60	28.00	15.00	146.60	2.316	80.00	185.30
	3	94.61	28.00	15.00	137.62	2.151	80.00	172.10
	Mean input cost (Rs./bird)				**145.36^{bc}**	**Mean income (Rs./bird)**		**181.71^{b}**
T_8 0.5%WS+ 0.05% Syn	1	114.08	28.00	15.00	157.09	2.550	80.00	204.00
	2	114.01	28.00	15.00	157.01	2.527	80.00	202.16
	3	120.66	28.00	15.00	163.66	2.659	80.00	212.76
	Mean input cost (Rs./bird)				**159.25^{e}**	**Mean income (Rs./bird)**		**206.31^{c}**
SEM					1.01			1.59

Means in the same column bearing different superscripts are significantly different ($p<0.05$)

C Control W.S. *Withania somnifera* Syn Synbiotic

Table 4.30 The profit- Rs/bird, Rs./kg live weight and %/bird realized under different feed supplement groups during experiment

Treatment	Replicates	Total input cost (Rs./bird)	Income from bird sold (Rs./bird)	Profit (Rs./bird)	Profit (Rs./kg live weight)	Profit (%/bird)
T_1 C	1	134.16	153.65	19.49	10.15	14.53
	2	129.77	142.51	12.74	7.15	9.82
	3	138.91	155.69	16.77	8.62	12.08
	Mean	**134.28^{a}**	**150.62^{a}**	**16.34^{a}**	**8.64^{a}**	**12.14^{a}**
T_2 0.5% WS	1	151.72	189.92	38.20	16.09	25.18
	2	148.06	182.42	34.36	15.07	23.21
	3	149.05	179.31	30.26	13.50	20.31
	Mean	**149.61^{cd}**	**183.89^{b}**	**34.28^{bc}**	**14.89^{cd}**	**22.90^{cd}**
T_3 1.0% WS	1	148.96	175.73	26.76	12.19	17.97
	2	152.62	181.70	29.08	12.81	19.06
	3	162.98	197.36	34.37	13.94	21.09
	Mean	**154.85^{de}**	**184.93^{b}**	**30.08^{b}**	**12.98^{bc}**	**19.37^{bc}**
T_4 1.5% WS	1	171.41	201.89	30.47	12.08	17.78
	2	175.43	208.88	33.44	12.81	19.07
	3	171.31	200.82	29.51	11.76	17.23
	Mean	**172.72^{f}**	**203.87^{c}**	**31.14^{bc}**	**12.21^{b}**	**18.02^{b}**
T_5 0.025% SYN	1	147.12	182.34	35.22	15.45	23.94
	2	137.12	169.30	32.18	15.21	23.47
	3	141.29	171.33	30.04	14.03	21.26
	Mean	**141.84^{abc}**	**174.33^{b}**	**32.48^{bc}**	**14.89^{cd}**	**22.89^{cd}**
T_6 0.05% SYN	1	145.61	188.66	43.04	18.25	29.56
	2	137.44	167.80	30.36	14.48	22.09
	3	137.85	175.55	37.71	17.18	27.36
	Mean	**140.30^{ab}**	**177.34^{b}**	**37.04^{c}**	**16.64^{de}**	**26.34^{de}**
T_7 0.25% WS+0.025 % SYN	1	151.86	187.71	35.85	15.28	23.61
	2	146.60	185.30	38.70	16.71	26.40
	3	137.62	172.10	34.48	16.03	25.06
	Mean	**145.36^{bc}**	**181.71^{b}**	**36.35^{bc}**	**16.00^{d}**	**25.02^{d}**
T_8 0.5%WS+ 0.05% SYN	1	157.09	204.00	46.91	18.40	29.86
	2	157.01	202.16	45.14	17.87	28.75
	3	163.66	212.76	49.10	18.46	30.00
	Mean	**159.25^{e}**	**206.31^{c}**	**47.05^{d}**	**18.24^{e}**	**29.54^{e}**
SEM		1.01	1.59	0.073	0.228	0.422

Means in the same column bearing different superscripts are significantly different ($p<0.05$)

C Control W.S. *Withania somnifera* Syn Synbiotic

The profit estimated on basis of per kg live weight was remarkably highest in T_8 group (Rs. 18.24). The profit/kg live weight in T_2 and T_5 group was observed to be Rs. 14.89. The broilers in T_6 and T_7 group returned Rs.16.64 and Rs.16/kg live weight, respectively. Similarly, broilers under T_3 and T_4 groups generated Rs.12.98 and Rs. 12.21/kg live weight, respectively. Thus broilers in all the supplemented groups earned significantly more profit than broilers in control group. The broilers reared through combined approach in T_8 group gave maximum economic return in terms of profit % per bird and profit/kg live weight with no mortality of broilers.

Incorporation of synbiotic yields more return than control and the present study is in accordance with Saiyed *et al*. (2015) though they observed higher values of profit as compared to present findings. The profit obtained corresponds to the results of Patel *et al*. (2015) in broilers. The economic evaluation observed for 0.5% aswagandha fed broiler (T_3) (Rs.34.28) in the present study revealed higher profit per bird than that observed by Ansari *et al*. (2008) (Rs.21.44) and Shisodiya *et al*. (2008) (Rs.26.77) for 0.4% and 0.5% *W. somnifera* supplementation in broiler diets, respectively. Similar to the results of Pandey *et al*. (2013) for use of 1% *Withania* containing herb in broilers, the present study observed nearly double net profit per bird than control group. The results of present study are supported by Narahari (1995) and Prajapati (1997) who reported extra profit/bird by using medicinal plants as growth promoter in broilers. The findings of higher net profit per bird in broiler supplemented with ashwagandha as noticed by Pedulwar (2004) and Shisodiya *et al*. (2008) was also observed in the present study. In contrast, Sanjyal and Sapkota (2011); and Kale *et al*. (2015) reported less income per bird in *Withania* treated broilers than control group which could be due to smaller number of bird that might have imposed higher cost of maintenance.

5. SUMMARYAND CONCLUSION

The role of broiler industry in nutritional security of human society through supplementation of low cost quality meat has sharply increased in the last few decades. Broiler meat has scientifically been proved on different quality criterion and is devoid of social and religious prejudice. The exponential increase in demand of broiler meat over the years has put an immense pressure for faster and economical growth performances which has led to the wide spread use of antibiotic growth promoter in the broiler production. In addition, high ambient temperatures along with erratic climatic pattern in tropical countries like India are also inherently associated with higher level of oxidative stress and thus affect the sustainable production from the broiler industry.

The economic efficiency of feed with higher ultimate net returns could be ensured in broiler production through nutrient sparing effect of feed additive in broiler ration. It is also well recognized that the quality, chemical composition and sensorial characteristics of produced meat are highly affected by active principles present in the chicken feed. The ban on antibiotics growth promoter substances in many countries of the world has diverted the concern of the world community towards finding of a sustainable source of organic growth promoter.

Natural Growth Promoters such as herbs, probiotics, prebiotics and synbiotics etc. have been identified as an effective and safe alternative to antibiotic growth promoters to promote the natural, traditional and alternate health system. Many indigenous herbs have also traditionally been recognized and acclaimed by native people for their multiple health benefits. *W. somnifera* or ashwagandha is one such herb which is literally claimed as *Indian Ginseng* in Indian literature due to its health restorative, anabolic, antioxidant, adaptogenic and hypolipidemic effect. The active constituents present in the form of alkaloids and other substances like withanolides in the root of ashwagandha imparts significant health benefit to host. The broad spectrum antibacterial and immunomodulatory behavior of ashwagandha improves the disease resistance status of the flocks and thus decreases the mortality. Likewise, synbiotics substances are known for their significant nutrient sparing effect in the gut of broilers and affect the growth and performance of broilers.

Thus the present investigation was designed to explore the individual as well as combined effect of supplementation of ashwagandha and synbiotic substances on the growth, haemato-serobiochemical, carcass and meat quality parameters of the broilers. 360 numbers of day old VenCobb broilers were randomly distributed in eight treatments with three replicates each to test the efficacy of supplementation of three levels of ashwagandha (T_2:0.5%, T_3:1%, T_4:1.5%), two levels of synbiotic (T_5:0.025%, T_6:0.05%) and two levels of combined feed additive (T_7:0.25% *W.somnifera*+0.025%synbiotics;T_8: 0.5% *W.somnifera*+0.05%synbiotics) in addition to nonsupplemented basal feed (T_1) on the growth and performances.

The growth performance of broilers under different treatments was assessed through change in weekly feed intake, body weight and body weight gain, FCR, PER, PI and mortality. In overall, the performance of control broilers (T_1) was found to be statistically lower in comparison to all other treatment groups in all the weeks. The higher level of alkaloids (3.4%) present in root powder of ashwagandha imparted significant effect on the growth performance of broilers. Low quantity of ashwagandha was found to be sufficient to raise feed intake in broilers during period of low environmental stress in earlier weeks whereas higher level of ashwagandha (1.5%) was required to maintain the feed intake during period of high heat stress.

The average weekly body weight was found to be higher in supplemented groups than control for overall experimental period. All the three levels of ashwagandha (T_2-T_4) affected the body weight in a similar fashion during initial three weeks of experiment however 1.5% level was observed appropriate to produce significant difference in body growth after 3rd week onwards during consistently higher temperature. T_5 and T_6 treatments also failed to demonstrate any statistical difference in body weight and body weight gain between them during whole trial. The growth promoting effect of synbiotic was most pronounced during initial stages of broiler life. The T_7 group demonstrated equivalent performance with all other treatments except T_4 and T_8. Maximum body weight in each week was observed in broilers supplemented with 0.5% *W. somnifera*+0.05% synbiotic without being affected by the continuous high THI and adverse climatic conditions. The weekly FCR of non-supplemented broilers (T_1) was found to be significantly ($p<0.05$) higher in most weeks of feeding trial with cumulative FCR of 1.90. The overall FCR was found significantly lowest ($p<0.05$) in broilers under treatment groups T_3, T_4 and T_8. The synbiotic added groups, T_5 and T_6 performed better until ambient temperature was within acceptable range, *i.e.,* 2nd week. The integrative approach adopted in T_8

treatment was observed to be most fruitful in conversion of feed materials into body masses during the whole trial period. Cumulatively PER index was highest ($p<0.05$) in broilers fed 1% and 1.5% ashwagandha root powder. The cumulative PER for 1-42 days varied between 2.50 (T_1) to 2.93 (T_3). Statistical analysis indicated significant increase in performance index of broiler fed synbiotic or *Withania* or their combination during most weeks of trial. The overall mortality rate under different treatments was observed to be 6.66% (T_1-Control), 2.22% (T_2), 4.44% (T_5), 2.22% (T_6) and 2.22% (T_7).

The dry matter digestibility was significantly higher (p 0.05) in supplemented groups than control group with highest value (76.57%) was observed in T_8 broilers. Remarkably (p 0.05) lowest and highest OMD values were observed in T_1 and T_8 group. Invariably highest digestibility of crude protein observed in ashwagandha supplemented groups reflected the anabolic nature of ashwagandha. The lowest CP digestibility coefficient was observed in control group. EED value ranged from 79.52% (T_1) to 84.40% (T_8) with nonsignificant difference among all the supplemented groups. The digestibility coefficient of NFE was nonsignificant ($p>0.05$) among T_1, T_3, T_4, T_5, T_6 and T_7 groups with statistically highest and numerically lowest NFED value was observed in T_8 and T_6 group broilers, respectively. The CFD ranged from 22.04% (T_1) to 25.08% (T_8).

The nitrogen retained in various treated groups ranged between 2.41 (T_1) to 2.68 (T_6) g/head/day. Significantly lowest nitrogen was found to be retained in T_1. All supplemented groups except T_5 group, revealed significantly (p 0.05) higher nitrogen retention than control group (T_1). The calcium retention ranged from 0.40 (T_1) to 0.51 (T_6) g/head/day whereas phosphorus over the treatments ranged from 0.34 (T_1) to 0.47 (T_6) g/head/day. Both the calcium and phosphorus retention were found to be significantly highest (p 0.05) in T_6 and T_8 groups.

The mean blood Hb values ranged between 7.51(T_1) to 8.43 (T_4), and 7.90 (T_1) to 9.23 (T_8) at 28^{th} and 42^{nd} day of trial, respectively. The synbiotic fed to broilers at two different levels failed to demonstrate any remarkable difference in Hb content. The PCV values ranged from 22.24% to 25.02% after 28 days and 23.42% to 26.93% at the end of trial. The TEC was found to be significantly (p 0.05) higher in T_2, T_3, T_4 and T_8 groups than rest of the treatments in 28^{th} day sampling. T_1, T_5 and T_6 failed to increase TEC significantly at the end of trial.

Nonsignificant variation in TLC was observed among all the treatments at 28th day of age whereas similar effect was observed among T_2, T_4, T_5, T_6 and T_8 at the end of trial. The TLC values ranged from 31.67 (T_8) to 37.56 (T_6); and 46 (T_3) to 55.44 (T_1) 10^3/µl at 28th day and 42nd day, respectively. Heterophils counts was higher ($p<0.05$) in control group than rest of the treatments and ranged from 24 (T_8) to 27.89 (T_1); and 27 (T_4) to 34.22 (T_1) 10^3/µl at the end of 4th and 6th week, respectively. Lowest lymphocyte value was observed for non-supplemented T_1 group during both intervals. T_3, T_4 and T_8 group revealed highest ($p<0.05$) lymphocytes level at 4th week whereas T_4 group revealed numerically highest lymphocyte at 6th week of age. The numbers of monocyte, eosinophil and basophil cells were nonsignificant among treatments at 4th and 6th week of experiment.

Nonsignificant variation in serum glucose values of most of the treatments was observed at the end of 28th day with lowest serum glucose was observed for T_6 group. The therapeutic effect of ashwagandha root powder in induction of hypoglycemia was observed at 42nd day. The treatment values for serum glucose ranged between 218.78 (T_3) to 238.44 (T_1) with most treatments except T5, significantly differed from control broilers (T_1). The hypoglycemic effect of synbiotic was observed in broilers at 42nd day. Highest serum TSH value (0.56ng/ml) was observed in T_1 broilers at the end of trial whereas lowest TSH values were recorded in broilers fed 1.5% ashwagandha treated diet and/or 0.05% synbiotic containing diet. The concentration of triiodothyronine hormone was significantly higher in all treatments in comparison to control and T_5 treatment group. Significant effect of 0.5% *Withania* (T_2), 0.05% synbiotic (T_6) and 0.5% *W. somnifera* + 0.05% synbiotic substances (T_8) on tetra-iodothyronine or thyroxin hormone was observed.

The effect of *W. somnifera* on serum protein profile became more evident during period of heat stress. 1.5% or 0.5% *W. somnifera*+0.05% synbiotic significantly enhanced the serum total protein during initial four weeks with values ranged between 3.00 (T_2) to 3.53 (T_8). The lowest serum protein at 6th week was observed for broilers under control group whereas highest value was found in T_8 group. Mean serum albumin value was found nonsignificant over the treatments in initial four week with values ranged from 1.26 (T_1) to 1.35 (T_8) g/dl. The 42nd day serum albumin value (g/dl) significantly varied between T_1 (1.12) and other treatments, *viz.,* T_2 (1.33), T_4 (1.35) and T_8 (1.54). Considerable enhancement in serum globulin values was observed in treatment T_3, T_4 and T_8 at 4th week of age and in treatment T_2-T_4, T_7 and T_8 at the end of the trial

Significantly lowered serum triglycerides ($p<0.05$) in T_4 (57.44 mg/dl) and T_8 (57.67 mg/dl) groups than control broilers was observed after 28th day. A decreasing trend in total serum triglycerides in response to increasing level of *W. somnifera* and synbiotics was observed. Minimum serum cholesterol value ($p \square 0.05$) of 113.30mg/dl and 102.33mg/dl was observed in T_8 after 4th and 6th week, respectively. The mean serum cholesterol reduced in the last two week under the effect of *Withania* supplementation either alone or in combination with synbiotic. The difference in serum HDL was statistically significant and HDL concentration was quite high in T_8 treatment as compared to the control (T_1). HDL cholesterol ranged between 49.33 to 60.78 mg/dl; and 43.33 to 55.33 mg/dl at 4th and 6th week, respectively. Significantly lowest (41.02 mg/dl) and highest 65.87%) LDL values in T_8 and T_1 groups, respectively with nonsignificant variation among treatments T_2 to T_7. Statistically higher serum LDL concentration (63.15 mg/dl) than most treatments was observed in T_1 after 42nd day. The VLDL values ranged between 11.53 to 14.58mg/dl and 16.49 to 38.84mg/dl at the end of 4th and 6th week, respectively.

The *W. somnifera* imparted significant effect on calcium absorption or its retention in the body but after the period of high heat stress, the calcium sparing effect of 0.5% and 1% level of *Withania* was reduced. T_8 group significantly enhanced the serum calcium status during the whole trial period. Significant increase in serum phosphorus level was observed in 0.05% synbiotic or 0.5% *W.somnifera*+0.05% synbiotic included group with respect to control at 28th week. No significant variation was observed in phosphorus level at 42nd day. Serum magnesium level was significantly higher in all the supplemented groups except T_5 group after 4th week. The serum magnesium at 6th week revealed significant variation in all supplemented groups than non supplemented T_1 broilers.

Serum AST and ALT values were observed nonsignificant at the end of 4th week during mild to moderate stress and the value of AST and ALT ranged from 169.67 (T_8) to 184 (T_1); and 13.33 (T_8) to 15.78 (T_1), respectively. At the end of trial, the AST value for T_2, T_3, T_4 and T_8; and ALT value for T_2, T_3, T_4, T_6, T_7 and T_8 was observed lowered ($P<0.05$) than control group.

The IBD antibody titre was found significantly better in ashwagandha treated broilers groups, *viz.,* T_2 (714.44), T_3 (831.67), T_4 (961.66), T_8 (897) with nonsignificant effect of synbiotic (T_5 and T_6) as compared to T_1 (431) at 4th week of age. The synbiotic inclusion in T_8 group improved the antibody status of T8 group in equivalence to T_4 group at 28th and 42nd days. The IBD titre ranged from 431 (T_1) to

961.67 (T_4); and 364.89 (T_1) to 773.67 (T_4) at 4th and 6th week, respectively. An overall reduction in antibody titre was observed in all the treatment groups in 6th week in comparison to 4th week. The mean serum RD titre at 28 days was observed as 3.76 (T_1); 4.13 (T_2), 4.19 (T_3), 4.20 (T_4); 3.93 (T_5), 3.95 (T_6); 3.97 (T_7) and 4.21 (T_8). The RD titre at 42 days was found in range of 2.62 (T_1) to 3.08 (T_4 and T_8).

The dressing percentage over the treatments ranged from 67.51 (T_1) to 70.81 (T_8) with T_2-T_8 revealed statistically similar ($p>0.5$) values. The eviscerated weight ranged from 63.11% (T_1) to 65.89% (T_3, T_4 and T_8) with T_2 toT_8 manifested nearly similar ($p>0.05$) eviscerated weight. The per cent yield of breast muscle was significantly highest (18.39%) in T_8 group and lowest (15.41%) in T_1 group broilers. Similarly, T_1 group devoid of any supplementation revealed lowest leg meat content (13.02%) whereas the T_8 group significantly ($p\square 0.05$) exhibited maximum quantum of leg muscle (14.96%).

The overall giblet weight revealed significant variation between nonsupplemented (T_1) and T_8 group broilers. The liver weight under different treatments revealed nonsignificant variation with numerically highest liver weight T_8 group broilers. The heart revealed significant difference between control and T8 group with value of 0.42% and 0.48% for T_1 and T_8 groups, respectively. The other treatments, *viz.,* T_2-T_7, nonsignificantly differed with each other and control. The proportion of gizzard in response to treatments was significantly higher ($p\square 0.05$) in T_2-T_6 and T_8 in comparison to T_1 and T_7 group.

Significantly higher blood loss in ashwagandha fed groups (T_2 toT_4) and T_8 group than control and other groups was observed after slaughter. The proportion of head as per cent of total live weight in various treatments was found to be nonsignificant and ranged from 2.17% (T_1) to 2.29% (T_8). The feather weight was significantly higher in T_1, T_5 and T_7 group than T_2 and T_3 group. The shank weight was observed significantly higher in T_3, T_4, T_7 and T_8 treatments than rest of the treatments. The broilers under T_4 and T_8 groups revealed significantly higher lung mass in comparison to T_2, T_3 andT_7 groups. The spleen weight was significantly higher in T_2-T_4 and T_6-T_8 groups than control. All supplemented broiler group revealed significantly higher bursa weight than nonsupplemented control broilers with maximum effect for T_8 group and lowest in control. The crop weight ranged from 0.55% (T_1) to 0.65 (T_6) with lowest crop weight in control group than most treatments except T_2 and T_3. The proventriculus weight differed nonsignificantly in all the treatments with minimum and maximum weight of 0.33% and 0.37%, respectively.

The gall bladder weight also nonsignificantly varied over the treatments except T_2 which significantly deviated from T_6, T_7 and T_8 groups. The intestine weight in T_5 to T_8 groups was found to be significantly (p□ 0.5) higher than control broilers.

Nonsignificant variation in shank length was observed among treatments T_2, T_3, T_4 and T_8 with highest and lowest values in T_1 and T_8, respectively. The intestinal length was observed highly significant between T_1 group and any of the supplemented groups. The caecal length in different treatments varied between 16.70cm (T_1) to 21.72cm (T_8) with lowest caecal length in T_1 group.

Significantly lower duodenal pH value for T_8 treatment followed by T_2-T_4 was observed in broilers whereas highest pH was observed in control group. Significant reduction in pH of the caecal content was observed in *Withania* supplemented groups, *i.e.,* 7.12 (T_2) to 6.66 (T_4) with highest pH (7.27) in T_1 group and lowest (6.32) in T_8 group. The total coliforms count estimated was significantly highest (5.66 $\log_{10}$ cfu/g caecal content) in nonsupplemented broilers (T_1). The total coliforms counts in different treatments ranged from 5.66 (T_1) to 4.83 (T_8) $\log_{10}$ cfu/g caecal content.

Significant (P□0.05) difference in mean crude protein (CP) content of breast muscle was observed which ranged from 86.84% to 90.10%. The lowest CP value was observed in T_1 group and highest in T_4 broiler group. Mean ether extract of breast meat revealed downward trend with higher level of ashwagandha supplementation. Lowest and highest ether extract value was observed in T_8 and T_1 group, respectively. The ash content of the breast muscle was found to be highest in T_6 whereas the treatment groups T_5, T_7 and T_8 showed comparable ash content.

Highest and significant pH of breast meat (p□0.05) was observed in T_1 group. All the three levels of ashwagandha (T_2 to T_4) and combined group T_8 imparted similar effect on pH that ranged between 5.84 (T_4) to 5.86 (T_6). Significantly higher water holding capacity of broiler breast meat was observed in T_8 treatment followed by T_4, T_6, T_7 and T_3. The meat of control broilers was found to retain 4.69% less water (p□0.05) than T_8 groups.

The attributes like odour, colour and tenderness appeared to be nonsignificant among the different treatment groups. The overall acceptability, colour and juiciness were observed significantly highest for breast meat of T_4 and T_8 treatments than other treatments. The palatability of breast meat from T_1 and T_5 treatment was comparable with each other. The meat colour of *Withania* fed broilers

(T_2-T_4) and T_8 group was significantly better than other treatments. Significantly better meat appearance was observed in T_8 broilers than other group.

The average feed cost per broiler on account of average feed intake was observed to significantly lower in control with statistically comparable value in T_5 and T_6 groups. The feed cost per broiler in T_7 group was found to be similar (p□0.05) to that of broilers in T_2, T_5 and T_6 groups whereas the feed cost incurred to raise broilers of T_8 group was observed to be similar to T_3 group. The average income achieved per bird was significantly lowest (Rs.150.62) in T_1 group broilers whereas highest selling income was recovered from broilers of T_8 and T_4 groups. Statistically similar amount per bird was fetched from broilers reared under T_2, T_3, T_5, T_6 and T_7 groups. The profit per bird was significantly highest (Rs.47.05) in T_8 group with a profit of about 29.54%. The profit % of broilers in control group was found to be restricted to 12.14% with Rs.16.34 only. The profit per kg live weight was remarkably highest in T_8 group (Rs.18.24). The profit/kg live weight in T_2 and T_5 group was observed to be Rs.14.89. The broilers in T_6 and T_7 group returned Rs.16.64 and Rs.16/kg live weight, respectively. Similarly, broilers under T_3 and T_4 groups generated Rs.12.98 and Rs.12.21/kg live weight, respectively.

Conclusion

The present feeding trial on ashwagandha and synbiotic substance either alone or in combination indicates their potential to use as feed additive in the ration of broiler chicks for better production performance, overall health and improvement in carcass characteristics. The present study concluded that low level (0.5%) of ashwagandha is optimum for growth of broilers in initial stages of life whereas higher level (1.5%) is most successful in negating the effect of environmental stress. The growth promoting effect of synbiotic are observed to be most pronounced during initial stages of life. The combined feeding approach was observed as most successful in promoting the growth during whole trial. Keeping in view the observed beneficial effects of ashwagandha and its combination with synbiotic, further studies are envisaged to explore the growth promoting potential of combination of ashwagandha herb and synbiotic on large number of broilers birds. In addition, ashwagandha could be explored on large platform for its environmental resilient effect in broilers and the production of low lipid containing designer meat.

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Effect of Single or Combined Dietary Supplementation of *Withania somnifera* and Synbiotic Mixture (Prebiotic and Probiotic) on Performance and Carcass Characteristics of Broilers

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ABSTRACT

The present study was conducted in broilers chickens with an objective to explore the inclusion of herbal feed additive *Withania somnifera* root powder and natural growth promoter synbiotic either alone or in combination on the growth performance, haematobiochemical parameters and carcass characteristics. A 42 day feeding trial was conducted under standard feeding and managemental conditions with broiler starter (0-21 days) and finisher (21-42 days) ration on 360, day old Vencobb broiler chicks randomly divided into 8 treatment groups (T_1-T_8) with three replicates of 15 chicks each. The T_1 group was kept as control whereas T_2, T_3 and T_4 were supplemented with 0.5%, 1% and 1.5% *Withania* root powder; T5 and T6 were supplemented with 0.025% and 0.050% synbiotic and T_7 and T_8 were fed on diet containing 0.25% *Withania*+0.025% synbiotic and 0.50% *Withania*+0.05% synbiotic, respectively. Three birds per replicate under each treatment were randomly selected and shifted to metabolic cages for five day digestion/metabolic trial (37^{th}- 42^{nd} day) to assess the digestibility of different dietary principles and nitrogen, calcium and phosphorus retention. Blood (3 ml) was collected from wing vein of each of the three randomly selected birds from each replication at 28^{th} and 42^{nd} day of experiment for the estimation of different haemato-serobiochemical parameters. Three broilers from each replicate were sacrificed at the end of trial to estimate the different carcass and meat quality parameters. Intestinal contents were collected for estimation of gut health. Samples of feed, voided excreta and meat were analysed for proximate principles as per standard method of AOAC. The total feed intake was significantly higher in T_4 and T_8 group than control (T_1). All supplemented groups revealed significantly higher body weight gain than control with highest weight gain in T_4 and T_8 treatments. The overall FCR was found significantly lowest ($p<0.05$) in broilers under treatment groups T_3, T_4 and T_8. Cumulatively PER index was highest ($p<0.05$) in broilers fed 1% and 1.5% ashwagandha root powder. The PI value was significantly higher in all the supplemented groups than control with highest value for T_8. No mortality was observed at intermediate and higher level of ashwagandha and in combined group, T_8. Remarkably (p 0.05) lowest and highest values of OMD were observed for T_1 and T_8 group, respectively. The digestibility of crude protein in ashwagandha supplemented treatment groups was invariably highest than all other groups including T_8 group. The EED value ranged from 79.52% (T_1) to 84.40% (T_8) with nonsignificant difference was observed among all the supplemented groups. The

digestibility coefficient of nitrogen free extract (NFED) was nonsignificantly similar (p□0.05) among T_1, T_3, T_4, T_5, T_6 and T_7 groups. The nitrogen retained in various treated groups ranged between 2.41 (T_1) to 2.68 (T_6) g/head/day. Both the calcium and phosphorus retention were found to be significantly highest (p□0.05) in T_6 and T_8 groups.

Significant effect of *Withania* alone or in combination with synbiotic was observed on haemoglobin, PCV and TEC. Significantly higher ($p<0.05$) heterophils counts and lower lymphocyte count was observed in control group broilers (T_1). Hypoglycemic effect of synbiotic and ashwagandha was observed in broilers during stress conditions. Significantly positive effect of ashwagandha supplementation on serum thyroxin hormone level was observed. Considerable enhancement in serum protein, albumin, globulin values was found in ashwagandha supplemented groups. Considerably lower serum triglycerides, cholesterol, LDL, VLDL and higher level of HDL was recorded in supplemented groups. Remarkable effect of synbiotic on blood mineral levels, *viz.,* calcium, phosphorus and magnesium was observed. Significantly higher AST and ALT values were observed in broilers under T_1 treatment. Improvement in antibody titre against IBD and RD was recorded in ashwagandha fed broilers. All supplemented groups revealed higher ($p<0.05$) dressing% than T_1 group. The percentage meat yield of breast and leg muscle was significantly higher in T_8 group. The overall giblet weight revealed significant variation between nonsupplemented (T_1) and T_8 group broilers. All supplemented broiler group revealed significantly higher bursa weight than nonsupplemented control broilers with maximum effect for T_8 group and lowest in control. The intestine weight in synbiotic supplemented groups (T_5 to T_8) was found to be significantly (p□0.5) higher than control broilers. Nonsignificant variation in shank length was observed among treatments T_2, T_3, T_4 and T_8. The intestinal length was observed highly significant between T_1 group and any of the supplemented groups. Significantly lower duodenal pH value for T_8 treatment followed by T_2-T_4 was observed. Significant reduction in pH of the caecal content was observed in *Withania* supplemented groups. The total coliforms count estimated was significantly highest (5.66 $\log_{10}$ cfu/g caecal content) in nonsupplemented broilers (T_1). Significantly highest breast meat crude protein content was observed in T_4 group. Mean ether extract of breast meat revealed downward trend with higher level of ashwagandha supplementation. Lowest and highest ether extract value was observed in T_8 and T_1 group, respectively. The ash content of the breast muscle was found to be highest in synbiotic fed group T_6. Supplementation of ashwagandha and synbiotic improved the meat quality in terms of meat pH and water holding capacity. The overall acceptability, colour and juiciness was observed significantly highest for breast meat of T_4 and T_8 treatments than other treatments. The profit per kg live weight was remarkably highest in T_8 group (Rs.18.24). Therefore, the present study revealed the anabolic, hypolipidemic, hypoglycemic and hepatoprotective effect of ashwagandha and growth promotion effect of synbiotic which acts synergistically in enhancing the performance of broilers.

ब्रायलर चूजों में विथानिया सोमनीफेरा और सिन्बायोटिक मिश्रण (प्रोबायोटिक और प्रीबायोटिक) को खाद्य संकाली के रूप में अकेले और संयोजन में खिलाने पर उपयोजन क्षमता और कारकस अभिलक्षणों पर प्रभाव

पशु पोषण विभाग
पशु चिकित्सा एवं पशु विज्ञान महाविद्यालय
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अनुक्षेपण

वर्तमान शोध में ब्रायलर चूजों में वृद्धि, रक्त रासायनों एवं कारकस अभिलक्षणों पर हर्बल खाद्य संकाली अश्वगंधा जड़ और प्राकृतिक वृद्धिकारक पदार्थ सिन्बायोटिक का एकल एवम् संयोजी रूप से अध्ययन किया गया। 360–एक दिवस की आयु के वेनकोब ब्रायलर चूजों में मानक आहार एवम् प्रबन्ध व्यवस्था में 0–21 दिन (ब्रायलर स्टार्टर) तथा 21–42 दिन तक (ब्रायलर फिनीशर) का एक 42 दिवसीय खाद्य परिक्षण किया गया जिसमें चूजों को यादृच्छिक रूप से आठ परिक्षण समूह में 15–15 चूजों के तीन प्रतिकृति समूहों में विभक्त किया गया। टी–1 समूह को नियंत्रण में रख कर टी–2, टी–3 एवम् टी–4 को क्रमशः 0.5%, 1.0% और 1.5% विथानिया जड़ पाउड़र खिलाया गया जबकि टी–5 एवम् टी–6 समूह को 0.025% एवम् 0.05% सिन्बायोटिक तथा टी–7 एवम् टी–8 को क्रमशः 0.25% विथानिया+0.025% सिन्बायोटिक; और 0.5% विथानिया+0.05% सिन्बायोटिक खिलाया गया। विभिन्न पोषक पदार्थों के पाचन परिक्षण और नत्रजन, कैल्शियम व फॉस्फोरस के शरीर में संतुलन ज्ञात करने हेतु 37वें दिवस पर पांच दिवस का प्रति प्रतिकृति समूह तीन ब्रायलर्स का उपापचयी परिक्षण किया गया। आहार परीक्षण के 28वें व 42वें दिन पर प्रत्येक प्रतिकृति समूह से यादृच्छिक रूप से तीन ब्रायलर से विभिन्न जैव–रासायनिक परिक्षणों हेतु 3 एम.एल. रक्त लिया गया। खाद्य परिक्षण के अन्त में प्रत्येक प्रतिकृति समूह से तीन ब्रायलर्स को विभिन्न कारकस व मांस संबंधी गुणों के लिये चयन किया गया। आहार नाल के स्वास्थय परिक्षण हेतु आहार नाल पदार्थों का परिक्षण किया गया। आहार, अपशिष्ठ एवम् मांस पदार्थों का पोषक तत्वों की गणना हेतु मानक तरीके से परिक्षण किया गया। टी–4 एवम् टी–8 समूह में टी–1 के बजाय आहार खपत अधिक पायी गयी, साथ ही सभी उपचारित समूहों में नियंत्रण समूह के बजाय ज्यादा शारीरिक भार में वृद्धि पायी गयी। टी–3, टी–4 एवम् टी–8 समूहों में खाद्य रूपान्तरण अनुपात आवश्यक रूप से कम पाया गया। 1.0 व 1.5% अश्वगन्धा उपचारित समूह में प्रोटीन दक्षता अधिक प्राप्त हुयी जबकि प्रदर्शन के संदर्भ में सभी उपचारित समूहों का प्रदर्शन नियंत्रण समूह से बेहतर पाया गया। मध्यम एवं उच्च उपचारित अश्वगन्धा समूह में एवम् टी–8 में मृत्यु दर शून्य पायी गयी। कार्बनिक पदार्थ पाचन

अधिकतम एवम् न्यूनतम क्रमशः टी–8 व टी–1 में पाया गया। क्रूड़ प्रोटीन का पाचन अश्वगन्धा व टी–8 समूह में सर्वाधिक पाया गया। वसा पाचन विभिन्न समूहों के मध्य 79. 52% से 84.40% के बीच पाया गया। नत्रजन, कैल्शियम एवम् फॉस्फोरस का संतुलन उपचारित समूहों में विशेष रूप से सकारात्मक पाया गया।

अश्वगन्धा व उसके सिन्बायोटिक के संयोजन का हीमोग्लोबिन, पी.सी.वी., टी.ई. सी., टी.एल.सी. व डी.एल.सी. पर प्रभाव देखा गया। अश्वगन्धा व सिन्बोयोटिक, तनावयुक्त स्थितियों में रक्त शर्करा को कम करने में प्रभावी पाया गया। सीरम प्रोटीन प्रोफाईल एवम् लिपिड प्रोफाईल में आहार उपचार की भूमिका स्पष्ट दिखाई दी। जैव खनिज जैसे कैल्शियम, फास्फोरस व मैग्नीशियम तथा यकृत स्वास्थ्य के लिए महत्वपूर्ण ए.एस.टी. व ए.एल.टी. एन्जाईम की सान्द्रता मे उपचारित समूहों में विशिष्ठ अन्तर पाया गया। अश्वगन्धा उपचारित समूह में जैव प्रतिरोधी पदार्थो की मात्रा अधिक पायी गई। ड्रैसिंग परसेन्ट व मांस प्राप्ति में उपचारित समूहों में नियंत्रण समूह की तुलना में भिन्नता पायी गयी। रोग प्रतिरोधी बर्सा का भार टी–8 ग्रुप में अधिक पाया गया। सिन्बायोटिक उपचारित समूहों में आहार नाल का भार अधिक पाया गया जबकि आंत की लम्बाई सबसे कम टी–1 समूह में देखी गई। अश्वगन्धा उपचार का सीकल पी.एच. एवम् कुल कोलीफार्म संख्या में विशेष प्रभाव देखा गया। अधर भाग के सफेद मांसीय हिस्से में अश्वगन्धा के उपचार से प्रोटीन की मात्रा अधिक तथा वसा की मात्रा कम होना पाया गया। मांस की पी.एच. एवम् जल धारण क्षमता में अश्वगन्धा व सिन्बायोटिक द्वारा सुधार पाया गया। सकल ग्राह्यता, रंग व रस के आधार पर टी–4 व टी–8 उपचारित समूह का मांस सर्वश्रेष्ठ पाया गया। प्रतिकिलोग्राम जीवित भार पर सर्वाधिक लाभ टी–8 समूह से प्राप्त हुआ। अतः वर्तमान शोध से ब्रायलर चूजों में अश्वगन्धा व सिन्बायोटिक पदार्थो का वृद्धिकारक वसा एवम् रक्त शर्करा नियंत्रक तथा यकृत रक्षक के रूप में प्रभाव देखा गया।

7. APPENDICES

APPENDIX- I

Mean sum of squares (MSS) and F-ratios of weekly feed intake

Weeks	Source of Variation	d. f.	Feed Intake	
			MSS	F-ratio
1	Treatments	7	42.303	1.756
	Error	16	24.091	
2	Treatments	7	1359.62	14.248
	Error	16	95.424	
3	Treatments	7	8335.28	5.256
	Error	16	1585.92	
4	Treatments	7	2284.29	0.711
	Error	16	3213.69	
5	Treatments	7	24730.23	2.973
	Error	16	8316.89	
6	Treatments	7	16927.822	1.743
	Error	16	9710.87	
Cumulative	Treatments	7	133997.59	4.364
	Error	16	30707.44	

APPENDIX- II

Mean sum of squares (MSS) and F-ratios of weekly body weight and body weight gain

Weeks	Source of Variation	d. f.	Body Weight		Body Weight gain	
			MSS	F-ratio	MSS	F-ratio
0	Treatments	7	0.203	0.246	-	-
	Replication	2	2.45	2.97	-	-
	Error	350	0.825		-	
1	Treatments	7	1145.65	5.48	1131.72	5.43
	Replication	2	213.52	1.02	177.10	0.85
	Error	350	209.05		208.53	
2	Treatments	7	23737.42	17.27	15301.85	11.24
	Replication	2	1299.9	0.95	550.44	0.40
	Error	350	1374.38		1361.9	
3	Treatments	7	191807.57	33.97	91568.71	13.49
	Replication	2	26807.21	4.75	16792.28	2.47
	Error	350	5646.51		6787.70	
4	Treatments	7	424492.65	26.25	52847.16	5.04
	Replication	2	46881.11	2.9	4345.05	0.41
	Error	350	16168.69		10474.14	
5	Treatments	7	1073227.04	38.88	185355.32	4.36
	Replication	2	86440.68	3.13	6804.49	0.16
	Error	344	27600.30		42555.77	
6	Treatments	7	2074976.85	17.92	181219.05	4.63
	Replication	2	129546.75	1.12	5693.12	0.14
	Error	342	115755.71		39181.97	
Cumu-lative	Treatments	7	2074976.85	17.92	2074084.75	17.916
	Replication	2	129546.75	1.12	128576.20	1.111
	Error	342	115755.71		115769.56	

APPENDIX- III

Mean sum of squares (MSS) and F-ratios of weekly feed conversion ratio

Weeks	Source of Variation	d. f.	Feed Conversion Ratio	
			MSS	F-ratio
1	Treatments	7	0.006	2.295
	Error	16	0.003	
2	Treatments	7	0.008	3.594
	Error	16	0.002	
3	Treatments	7	0.009	14.669
	Error	16	0.001	
4	Treatments	7	0.016	47.796
	Error	16	0.001	
5	Treatments	7	0.025	34.809
	Error	16	0.001	
6	Treatments	7	0.055	108.68
	Error	16	0.001	
Cumulative	Treatments	7	0.014	48.407
	Error	16	0.001	

APPENDIX- IV

Mean sum of squares (MSS) and F-ratios of weekly protein efficiency ratio and performance index

Weeks	Source of Variation	d. f.	Protein Efficiency Ratio		Performance Index	
			MSS	F-ratio	MSS	F-ratio
1	Treatments	7	0.093	3.805	1.268	4.128
	Error	16	0.024		0.307	
2	Treatments	7	0.086	6.588	7.861	12.894
	Error	16	0.013		0.609	
3	Treatments	7	0.113	13.788	39.348	12.322
	Error	16	0.008		3.193	
4	Treatments	7	0.051	12.876	29.407	9.798
	Error	16	0.004		3.001	
5	Treatments	7	0.048	12.380	55.024	7.751
	Error	16	0.004		7.098	
6	Treatments	7	0.081	40.497	62.795	9.844
	Error	16	0.002		6.379	
Cumulative	Treatments	7	0.049	19.133	701.72	19.695
	Error	16	0.003		35.629	

APENDIX- V

Mean sum of squares and F-ratio of digestibility of proximate principles

Particulars	Source of variation	d.f.	Mean sum of squares	F-ratios
DM digestibility	Treatments	7	2.99	7.12
	Error	16	0.42	
OM digestibility	Treatments	7	3.14	7.81
	Error	16	0.40	
CP digestibility	Treatments	7	30.67	21.10
	Error	16	1.45	
EE digestibility	Treatments	7	6.80	3.20
	Error	16	2.13	
CF digestibility	Treatments	7	2.91	1.16
	Error	16	2.51	
NFE digestibility	Treatments	7	1.85	3.10
	Error	16	0.60	

APENDIX- VI

Mean sum of square and F-ratios of nitrogen, calcium and phosphorus

Source of variation	d.f.	Nitrogen retention (g/bird/day)		Calcium retention (g/bird/day)		Phosphorus retention (g/bird/day)	
Treatments	7	0.025	8.13	0.003	5.64	0.005	8.78
Error	16	0.003		0.001		0.001	

APENDIX- VII

Mean sum of squares and F-ratios of hematological parameters

Particulars	Source of variation	d.f.	Mean sum of squares		F-ratios	
			Days of collection		Days of collection	
			28^{th}	42^{nd}	28^{th}	42^{nd}
Haemoglobin	Treatments	7	1.264	2.314	14.431	8.425
	Replicates	2	1.113	5.075	12.712	18.479
	Error	62	0.088	0.275		
PCV	Treatments	7	11.170	17.317	12.6	6.712
	Replicates	2	13.108	43.88	14.78	17.009
	Error	62	0.887	2.58		
TEC	Treatments	7	0.318	0.325	12.406	8.212
	Replicates	2	0.265	1.208	10.33	30.547
	Error	62	0.026	0.04		
TLC	Treatments	7	33.903	103.585	0.812	1.763
	Replicates	2	132.097	229.35	3.166	3.903
	Error	62	41.728	58.767		
Heterophils	Treatments	7	16.474	47.585	2.351	3.56
	Replicates	2	2.542	74.056	0.363	5.54
	Error	62	7.008	13.367		
Lymphocytes	Treatments	7	28.601	64.794	3.981	3.638
	Replicates	2	0.514	61.347	0.072	3.445
	Error	62	7.184	17.810		
Monocytes	Treatments	7	0.665	1.125	0.538	1.083
	Replicates	2	2.056	10.792	1.665	10.387
	Error	62	1.235	1.039		
Eosinophils	Treatments	7	0.190	0.062	1.066	0.38
	Replicates	2	0.014	0.097	0.078	0.60
	Error	62	0.179	0.162		
Basophils	Treatments	7	0.093	0.062	0.696	0.456
	Replicates	2	0.181	0.264	1.348	1.957
	Error	62	0.134	0.135		

APENDIX- VIII

Mean sum of squares and F-ratios of blood glucose

Particular	Source of variation	d.f.	Mean sum of squares		F-ratios	
			Days of collection		Days of collection	
			28^{th}	42^{nd}	28^{th}	42^{nd}
Glucose	Treatments	7	102.24	400.15	1.49	3.09
	Replicates	2	0.181	0.514	0.003	0.004
	Error	62	68.53	129.53		

APENDIX- IX

Mean sum of squares and F-ratios of serum T3, T4 and TSH parameters

Particulars	Source of variation	d.f.	Mean sum of squares	F-ratios
T3	Treatments	7	0.515	2.154
	Replicates	2	3.577	14.973
	Error	62	0.239	
T4	Treatments	7	162.696	9.033
	Replicates	2	217.625	12.082
	Error	62	18.012	
TSH	Treatments	7	0.091	9.137
	Replicates	2	0.036	3.633
	Error	62	0.010	

APENDIX- X
Mean sum of squares and F-ratios of sero-biochemical parameters

Particulars	source of variation	d.f.	Mean sum of squares		F-ratios	
			Days of collection		Days of collection	
			28th	42nd	28th	42nd
Total Protein	Treatments	7	0.217	1.427	4.872	4.3
	Replicates	2	0.040	0.017	0.889	0.052
	Error	62	0.044	0.332		
Albumin	Treatments	7	0.008	0.125	1.08	3.16
	Replicates	2	0.001	0.004	0.051	0.099
	Error	62	0.007	0.039		
Globulin	Treatments	7	0.144	0.725	5.649	4.519
	Replicates	2	0.035	0.036	1.38	0.226
	Error	62	0.026	0.160		
Creatinine	Treatments	7	0.001	0.001	0.121	0.871
	Replicates	2	0.001	0.001	0.174	0.579
	Error	62	0.003	0.001		
Triglyceride	Treatments	7	324.71	13845.08	1.98	14.249
	Replicates	2	134.76	30.042	0.82	0.031
	Error	62	164.13	971.62		
Cholesterol	Treatments	7	197.49	1758.21	2.09	5.387
	Replicates	2	41.72	25.79	0.44	0.079
	Error	62	94.43	326.39		
HDL	Treatments	7	112.08	125.43	7.498	8.02
	Replicates	2	15.29	4.167	1.023	0.27
	Error	62	14.95	15.64		
VLDL	Treatments	7	12.99	553.80	1.978	14.25
	Replicates	2	5.391	1.202	0.821	0.031
	Error	62	6.565	38.86		
LDL	Treatments	7	423.366	922.09	4.575	2.002
	Replicates	2	158.352	10.80	1.711	0.023
	Error	62	92.537	460.52		
Calcium	Treatments	7	2.15	1.89	4.79	3.43
	Replicates	2	0.30	0.095	0.67	0.173
	Error	62	0.45	0.553		
Phosphorus	Treatments	7	0.676	0.325	2.36	0.548
	Replicates	2	0.053	0.009	0.185	0.016
	Error	62	0.287	0.593		
Magnesium	Treatments	7	0.051	0.056	2.725	2.714
	Replicates	2	0.008	0.007	0.450	0.357
	Error	62	0.019	0.021		
ALT	Treatments	7	5.97	326.73	0.457	12.23
	Replicates	2	11.06	1.556	0.434	0.058
	Error	62	13.06	26.73		
AST	Treatments	7	213.36	14286.22	0.745	5.984
	Replicates	2	182.76	46.22	0.637	0.019
	Error	62	285.88	2387.32		
IBD	Treatments	7	411105.10	201649.30	46.93	13.478
	Replicates	2	46781.51	55155.29	5.34	3.686
	Error	62	8760.20	14961.50		
ND	Treatments	7	0.264	0.309	10.319	7.852
	Replicates	2	0.001	0.028	0.026	0.70
	Error	62	0.026	0.039		

APENDIX- XI

Mean sum of squares and F-ratios of carcass parameters (per cent of live weight)

Particulars	Source of variation	d.f.	Mean sum of squares	F-ratios
Eviscerated weight	Treatments	7	7.714	3.013
	Replicates	2	1.847	0.721
	Error	62	2.560	
Dressed weight	Treatments	7	9.026	4.309
	Replicates	2	1.725	0.824
	Error	62	2.094	
Giblet weight	Treatments	7	0.120	0.940
	Replicates	2	0.218	1.706
	Error	62	0.128	
Breast weight	Treatments	7	7.706	2.464
	Replicates	2	3.183	1.018
	Error	62	3.127	
Leg weight	Treatments	7	3.258	2.350
	Replicates	2	0.410	0.296
	Error	62	1.387	

APENDIX- XII

Mean sum of squares and F-ratios of carcass organs weight (per cent of live weight)

Particulars	Source of variation	d.f.	Mean sum of squares	F-ratios
Liver	Treatments	7	0.034	0.446
	Replicates	2	0.300	3.944
	Error	62	0.076	
Heart	Treatments	7	0.004	1.583
	Replicates	2	0.005	2.232
	Error	62	0.002	
Gizzard	Treatments	7	0.086	2.192
	Replicates	2	0.058	1.480
	Error	62	0.039	
Blood	Treatments	7	0.274	5.348
	Replicates	2	0.087	1.699
	Error	62	0.051	
Head	Treatments	7	0.018	1.10
	Replicates	2	0.03	1.86
	Error	62	0.016	
Feather	Treatments	7	1.59	2.956
	Replicates	2	3.386	6.285
	Error	62	0.539	
Shank	Treatments	7	0.913	10.058
	Replicates	2	0.375	4.132
	Error	62	0.091	
Lung	Treatments	7	0.005	2.491
	Replicates	2	0.002	0.894
	Error	62	0.002	
Spleen	Treatments	7	0.001	4.096
	Replicates	2	0.001	2.047
	Error	62	0.001	
Bursa	Treatments	7	0.007	11.808
	Replicates	2	0.003	5.100
	Error	62	0.001	
Crop	Treatments	7	0.013	8.082
	Replicates	2	0.002	1.303
	Error	62	0.002	
Proventriculus	Treatments	7	0.002	1.097
	Replicates	2	0.003	1.580
	Error	62	0.002	
Gall bladder	Treatments	7	0.001	2.293
	Replicates	2	0.001	1.476
	Error	62	0.001	
Intestinal	Treatments	7	0.412	4.604
	Replicates	2	0.360	4.018
	Error	62	0.090	
Caeca	Treatments	7	0.028	1.500
	Replicates	2	0.051	2.752
	Error	62	0.019	

APENDIX- XIII

Mean sum of squares and F-ratios of organ length and gut health parameters

Particulars	Source of variation	d.f.	Mean sum of squares	F-ratios
Shank length	Treatments	7	2.508	7.37
	Replicates	2	1.704	5.008
	Error	62	0.34	
Intestinal length	Treatments	7	1102.66	6.581
	Replicates	2	246.22	1.47
	Error	62	167.552	
Caeca length	Treatments	7	18.478	6.447
	Replicates	2	9.433	3.291
	Error	62	2.866	
Duodenal pH	Treatments	7	0.142	4.750
	Replicates	2	0.100	3.336
	Error	62	0.030	
Caecal pH	Treatments	7	0.80	2.55
	Replicates	2	1.197	3.81
	Error	62	0.314	
Total coliform count	Treatments	7	0.626	138.94
	Replicates	2	0.001	0.058
	Error	62	0.005	

APENDIX- XIV

Mean sum of squares and F-ratios of broiler breast meat quality

Particulars	Source of variation	d.f.	Mean sum of squares	F-ratios
Crude protein	Treatments	7	21.236	26.665
	Replicates	2	0.067	0.084
	Error	62	0.796	
Ether extract	Treatments	7	11.942	23.269
	Replicates	2	0.080	0.155
	Error	62	0.513	
Ash	Treatments	7	0.489	2.784
	Replicates	2	0.005	0.030
	Error	62	0.176	
Breast meat pH	Treatments	7	0.036	9.028
	Replicates	2	0.008	1.961
	Error	62	0.004	
Breast meat WHC	Treatments	7	22.98	2.756
	Replicates	2	64.858	7.778
	Error	62	8.338	

APENDIX- XV

Mean sum of squares and F-ratio of sensory evaluation of broiler breast meat

Particulars	Source of variation	d.f.	Mean sum of squares	F-ratios
Appearance	Treatments	7	0.076	3.717
	Error	64	0.020	
Colour	Treatments	7	0.125	7.991
	Error	64	0.016	
Odour	Treatments	7	0.053	0.693
	Error	64	0.076	
Juciness	Treatments	7	0.529	20.014
	Error	64	0.026	
Texture	Treatments	7	0.068	4.514
	Error	64	0.015	
Tenderness	Treatments	7	0.020	0.440
	Error	64	0.046	
Flavour	Treatments	7	0.064	0.821
	Error	64	0.078	
Over all palatability	Treatments	7	0.538	21.792
	Error	64	0.025	

APENDIX-XVI

Mean sum of squares and F-ratios of economics

Particulars	Source of variation	d.f.	Mean sum of squares	F-ratios
Total Feed Input cost	Treatments	7	451.882	18.465
	Error	16	24.473	
Total Input cost	Treatments	7	451.882	18.465
	Error	16	24.473	
Income/bird	Treatments	7	917.34	15.07
	Error	16	60.87	
Profit/bird	Treatments	7	221.34	17.27
	Error	16	12.82	
Profit/kg live weight	Treatments	7	26.89	21.49
	Error	16	1.25	
%profit/bird	Treatments	7	88.40	20.72
	Error	16	4.27	

Plate 1: ***Withania somnifera*** **(Plant, Dry Roots and Root Powder)**

a. Housing

b. Brooding

c. Wing Banding

d. Vaccination

Plate 2: General management during the experimental trial

Plate 4: Feeding of experimental chicks during experimental trial

Plate 3: Watering of broiler chicks under experimental trial

Plate 5: Weighing of broiler chicks during trial period

Plate 6: Broiler chicks for metabolic trial in metabolic cages

Plate 7: Collection of blood sample from broiler chicks

Plate 8: Weighing of carcass (Eviscerated weight)

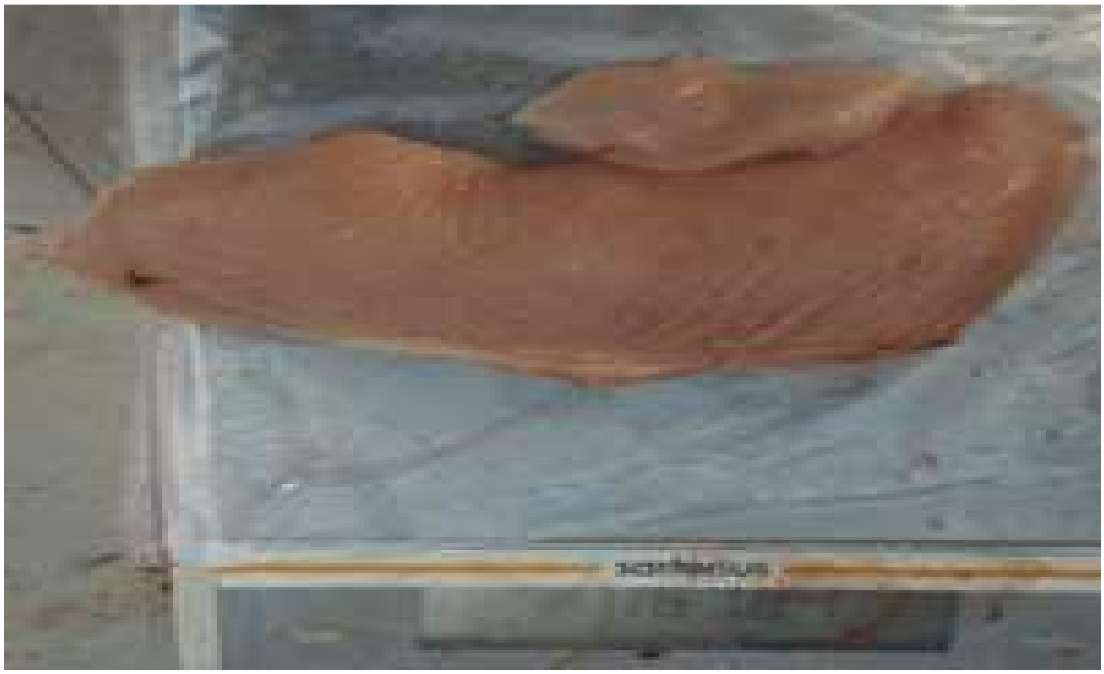

Plate 9: Weighing of breast muscle

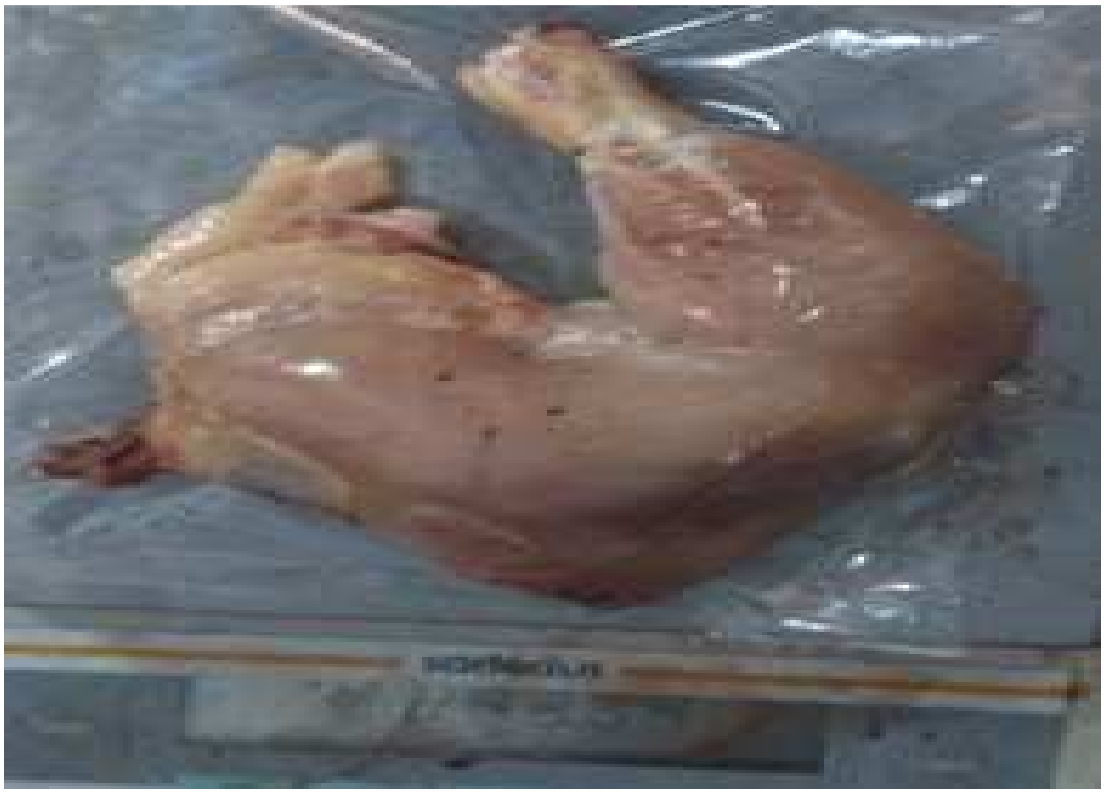

Plate 10: Weighing of leg muscle

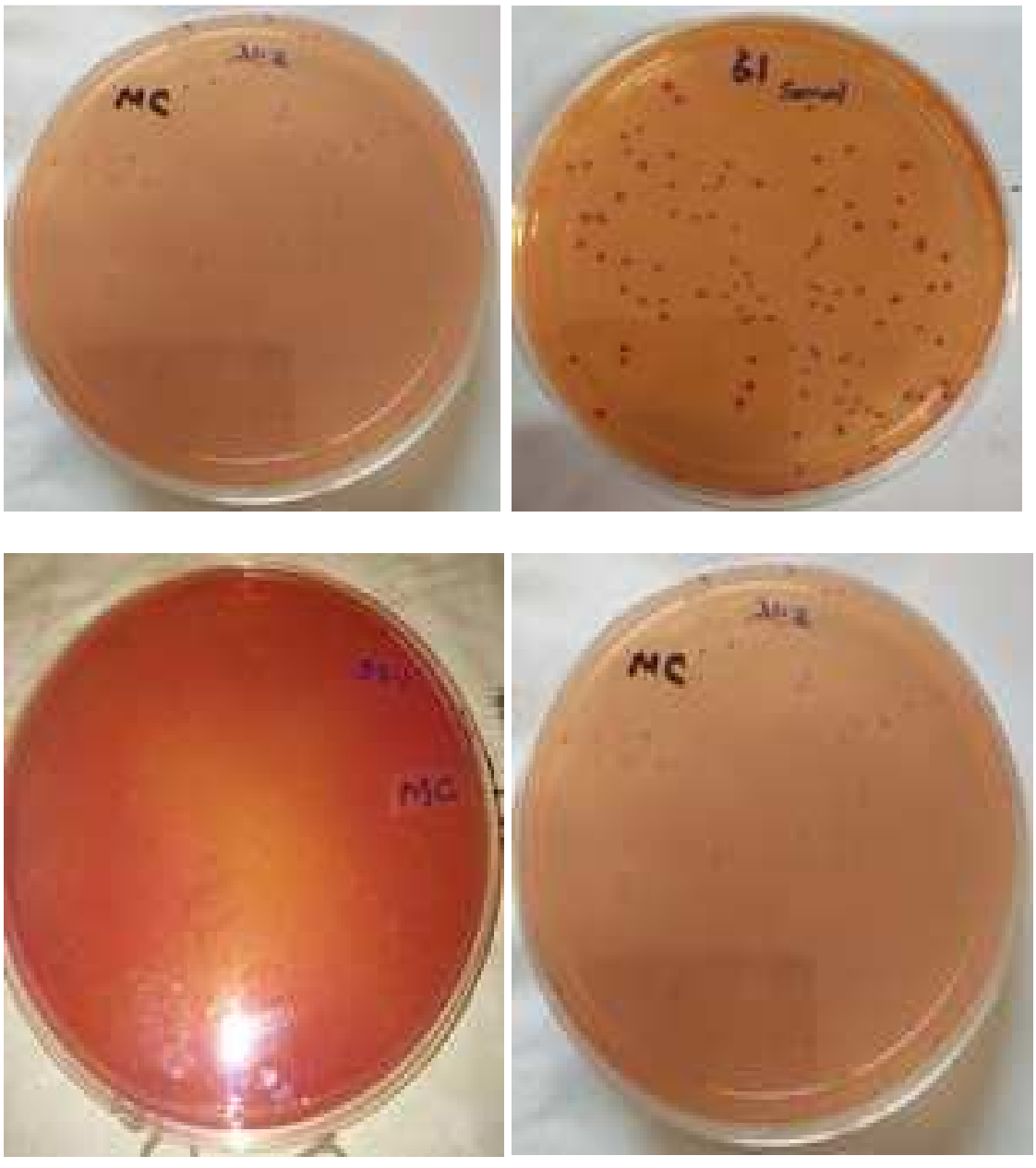

Plate 11: Colony forming units on Mac Conkey agar