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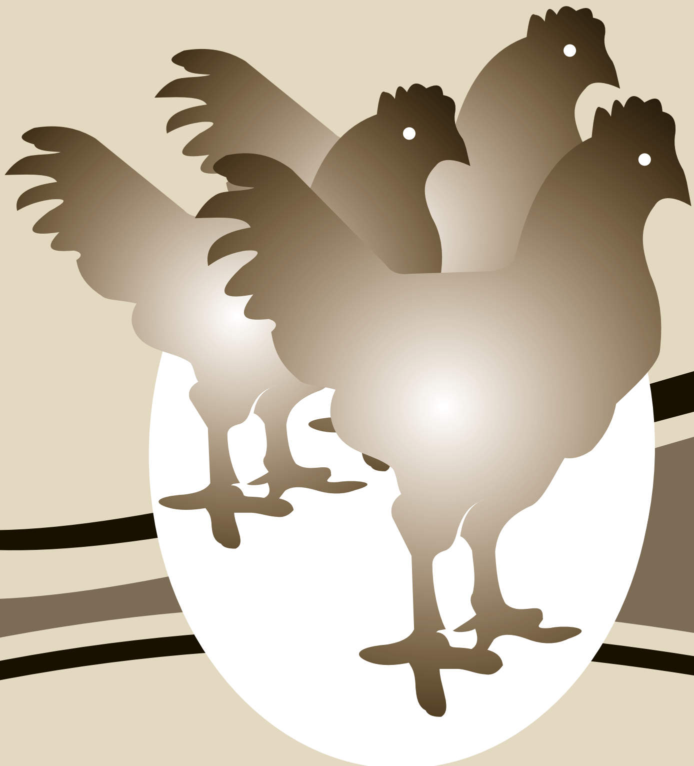

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A review of mycotoxin contamination in the feed of poultry: An impact towards immunosuppression

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ABSTRACT

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Mycotoxins, important secondary metabolites, are grown on feedstuffs and cause toxicologic syndromes in poultry. Those play a significant role in causing mortality and decreased production performance. Ingestion of mycotoxins results in altered immune response and decreases resistance to infectious disease. Feeds, used for poultry, are contaminated with various types of moulds that are responsible for the production of mycotoxin. Mycotoxins cause different kinds of toxicities such as acute and chronic toxicities, genotoxicity, mutagenicity, carcinogenicity, immunotoxicity, and teratogenicity in poultry. So, those cause immunosuppression through alteration of T or B activity and macrophage-effector cell function, declined production of antibody and immunoglobulin, and inhabited DNA, RNA, and protein synthesis.

Keywords: Mycotoxicosis, immunopathology, poultry, Molecular signalling

INTRODUCTION

Mycotoxins, a secondary metabolite found in the moulds, and can be seen in food**.** The mould generally grows on a various types of crops and foodstuffs including nuts, cereals, dried fruits, spices, coffee beans, and apples often in warm and humid conditions. Mycotoxins can play a major role in causing a variety of adverse health effects and pose a serious health threat to livestock, and poultry birds. It damages the capillary membrane through alteration of the permeability of the membrane resulting in haemorrhages and immunosuppression (Corrier, 1991). It has synergistic interactions with other infectious agents, nutritional deficiencies, and natural toxins. It plays a major role in causing mild to severe toxicity in poultry. It has been documented that about 25% of poultry feeds are generally contaminated with mycotoxins during the application of physical and chemical adsorbents in the feeds (Patil *et al.,* 2014). These mycotoxins are normally biologically active, toxic metabolite products produced by toxigenic fungi mainly belonging to Aspergillus, Fusarium, and Penicillium species, that invade crops in the land and may grow on foods during storage under favourable conditions of temperature as well as humidity (Bennett and Klich, 2003). The economic losses are primarily due to the decreased growth rate, feed conversion efficacy, carcass yield, carcass quality, and increased susceptibility to infection and pathogens (Patil *et al.,* 2014). Among several mycotoxins, aflatoxins (AF), citrinins (CIT), fumonisins (F), ochratoxins (OT), and trichothecenes are the most important and important in poultry feeds (Bakeer *et al.,* 2013). The present study emphasizes the role of mycotoxin in inducing immunosuppression in poultry birds.

Predisposing factors associated with mycotoxicosis in poultry

Generally, mycotoxins are generally of a low molecular weight secondary metabolites geneated by filamentous fungi *viz*., Aspergillus, Fusarium, and Penicillium, which invade crops in the field and may grow on foods during storage under favourable conditions of temperature as well as humidity (Corrier *et al.,* 1991; Hoerr *et al.,* 2010) (Table 1). The most important mycotoxins include aflatoxins, ochratoxin A, trichothecenes, zearalenone, and fumonisin (Bakeer *et al.,* 2013). Mycotoxins are produced from fungi, are dividing into two groups *viz.,* a) field fungi (Fusarium and Alternaria spp.), which invades into the grains in the field condition and second one occurs during storage with high moisture (*Aspergillus and Penicillium spp*) grow in the grains with low moisture (Bakeer *et al.,* 2018). There are various physical, chemical and biological factors associated with mycotoxicosis in feeds. Physical factors include humidity, temperature, microflora zones, and physical integrity of the grains whereas chemical factors constitute pH, the composition of the substrate, and mineral nutrients (Girh *et al.,* 2018). Biological factors *viz*., insects and specific strains are closely associated with mycotoxicosis in poultry. Free water or combined water are important criteria for the development of fungi as well as the production of mycotoxins. Free water is present in different tissues or cells whereas combined water is bounded with proteins and

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carbohydrates, forming an integral part of the cells that compose the tissues (Liew and Mohd-Redzwan, 2018). Quantity of water is also a predisposing factor for the development of fungi as well as the development of mycotoxins in grains. Contaminated energy feedstuffs *viz*., maize, oats, millets, barley, wheat meal, wheat, rice polish are also an important sources for mycotoxicosis in poultry due to poor agricultural practices, poor storage conditions and little knowledge on mycotoxins (Barati *et al.*, 2018). Protein supplementing feeds including maize gluten meal, sesame cake, soybean meal, groundnut cake, sunflower meal coconut meal is a rich source of fungi as well as mycotoxins as those are very prone to infection by any fungal attack and are generally to be contaminated with mycotoxins under suitable temperature and humidity conditions, during both the growing and storage period (Bennett, 1987; Liew and Mohd-Redzwan, 2018). Documentary evidence suggested that avian species, age, breed, strains, habitat, health and nutritional status of the poultry bird, exposure of poultry birds during ingestion of mycotoxin contaminated/infested feeds (Rawal and Kim, 2010).

Mycotoxin's target in broiler production

The most important mycotoxin producing genera are Aspergillus, Fusarium, and Penicillium. It has been demonstrated that mycotoxins play a significant role in lowering overall production of poultry through reduction of body weight, feed conversion efficiency, egg production, and disease resistant capacity (Bakeer *et al.*, 2013).

Aflatoxins in broiler production performance

Aflatoxin is secondary metabolites, produced by *Aspergillus flavus* and *Aspergillus parasiticus* fungi. It has been reported that this toxin causes metabolic damages and liver lesions, reduction of digestive enzymes, and immune suppression in poultry (Abedi and Talebi, 2015). It also hampers metabolic pathways resulting in the alteration of serum profiles, deduction of serum protein, glucose, phosphorus, and calcium. In addition, aflatoxin B1, the most abundant aflatoxin, affects the liver and causes reduced performance, digestive, and hematopoietic functions, carcinogenesis, and immunosuppression (Rawal and Kim, 2010). It has been documented that excessive intake of aflatoxin causes the reduction of production performance including reduced feed intake, body weight gain, feed conversion ratio, carcass yield, pigmentation, egg production, hatchability, and fertility (Bakeer *et al.*, 2013). Aflatoxin contamination can cause reduction of feed quality as well as deduced animal efficiency either through poor conversion of nutrients or problems such as reproductive abnormalities. Clinical signs of aflatoxin include increased morbidity, unthriftiness as well as mortality. Necropsy findings depict necrosis, congestion, lipid accumulation. On examination of necropsy, the lesions are found mainly in the liver, which can be due to necrosis and congestion or yellow due to lipid accumulation (Murugesan *et al.*, 2015). Haemorrhages may occur in the liver and other tissues. Poultry, especially turkeys, are very much sensitive to the various toxic as well as carcinogenic action of AFB1. It affects hepatic cytochrome P450-mediated bioactivation and deficient detoxification by glutathione S-transferases (GST). Therefore, the liver becomes yellowish-grey and atrophied (DΔnicke, 2002).

Ochratoxin in the performance of poultry

Ochratoxin is generally found in the field is in the form of ochratoxin A (OTA), which has a primary target organ on the kidneys as it is known to be nephrotoxic in poultry (Bennett and Klich, 2013). It has a major role in lowering the productive performances of poultry birds. These are a group of structurally related metabolites that are produced by fungi belonging to the genera Aspergillus, Penicillium. Ochratoxin A (OTA) is the most prevalent mycotoxin of this group. It causes carcinogenic effects in poultry. Documentary evidence suggested that signs of ochratoxin A toxicity in poultry include anaemia, reduced feed consumption weakness, declining growth rate, poor feathering, egg production, , and excessive mortality at high dietary concentrations (Bakeer *et al.*, 2013). It also damages the liver, bone marrow, and immune system. Hypothermia, diarrhoea, spontaneous activity and huddling, lowered egg production, mucosal erosions of gizzard, proventriculus mucosal haemorrhages, catarrhal enteritis, dehydration, and lowered carcass yield and declined feed conversion ratio are important signs of ochratoxicosin toxicity in poultry (Hoerr, 2010). Documentary evidence narrated that these toxins lowered growth, rate, and skeletal integrity, impaired phagocytosis, and coagulation of blood. It has been reported that these toxins are also genotoxic immunotoxic carcinogenic, embryotoxic, hepatotoxic, neurotoxic, and teratogenic (Puvaèa *et al.*, 2018). These are important toxins in domestic fowl and Japanese quail. The liver as well as kidney have become haemorrhagic, pale, and swollen. Girh *et al.* (2018) reported that lowered serum albumin and total protein are important diagnostic biomarkers for ochratoxicosis in poultry. In addition, it can cause gut dysbiosis, including increasing gut permeability, immunity, and bacterial translocation, and can eventually lead to gut and other organ injury also.

Fumonisin in production of poultry

Fumonisin B1 (FB1) is the important and the most thoroughly investigated mycotoxin because of its toxicological importance. It hampers production of birds. Fumonisins (B1, B2, and B3) are important mycotoxin produced by Fusarium fungi namely *F. moniliforme* and *F. proliferatum*, a natural contaminant of cereals, especially corn and its by-products (Corrier *et al.*, 1991). These play a significant role in reducing performance and immunosuppression in poultry. It causes electrical regulation of epithelial cells, liver functions, and reduction in sphingolipid biosynthesis resulting in blocking of the mitotic cycle phases of epithelial cells and decreasing its proliferation (Liew and Mohd-Redzwan, 2018). In an experimental study, Antonissen *et al.* (2015) demonstrated that fumonisins hampers the intestinal microbial homeostasis with necrotic enteritis. These play a major role in causing necrosis and ulceration of the oral mucosa, gastro-intestinal mucosa, mottling of the liver, atrophy of the spleen and other lymphoid organs, and visceral haemorrhages (DΔnicke, 2002). Laying birds depict declined egg production, recumbency, feed refusal, depression, and cyanosis in the comb and wattles. The blood chemistry exhibited lowered blood glucose and protein. It is reported that these mycotoxins have an affinity towards the liver as well as kidney and cause biliary hyperplasia, hepatic necrosis and affect proximal convoluted tubule (PCT) lining cells (Murugesan *et al.,* 2015). In an experimental study, it has been proven that these toxins cause a decreased production of immunoglobulins and thereby alter immune function (Bakeer *et al.,* 2013). Fumonisins have a significant role in lowering the efficiency of immune system of poultry. These have a negative impact on the intestinal tract as well as those elevate the incidence of intestinal pathogens and act as carcinogens also.

Trichothecenes in poultry production

Trichothecenes (croticin, diacetoxyscirpenol, and T-2 toxin) are important mycotoxin produced by the genus Fusarium. It inhibits the production of broiler. These toxins are severely contaminated with poultry feeds. These toxins also cause a health hazard and productivity of poultry characterized by ulceration and necrosis of the buccal cavity and lowered body weight (Corrier, 1991). T-2 is considered as most pathogenic mycotoxin and causes inhibition of phagocytic capacity, peroxidation of lipid, and decreased level of vitamins (Patil *et al.,* 2014). Trichothecenes toxins play an immense role in the disruption of gut epithelium permeability, lowered absorption of protein, reduced growth rate, lowered feed intake, alterations in blood parameters, and neurotoxicity (Bennett *et al.,* 2013). It can cause economic losses in productivity by reducing the feed intake, deduced body weight and declined egg production as well as oral lesions.

Zearalenone in poultry production

Zearalenone, a nonsteroidal estrogenic mycotoxin, is produced by various species of Fusarium fungi namely Fusarium graminearum (teleomorph Gibberella zeae) and Fusarium culmorum are most pathogenic in poultry (Chi *et al.,* 1980). It has a major role in restricting the growth and production of poultry birds. It has potential inhibitory role in reducing bird performance and egg yield. Branton *et al.* (1989) demonstrated that these toxins play a major role in the hemopoietic system resulting in declined white blood cells. These also cause comb shrinkage and hypertrophy of oviducts, declined body weight, and hepatotoxicity. It lowers the egg specific gravity as well as eggshell thickness wit interior egg quality. Interestingly, It also reduces serum calcium with an elevation of phosphorus content. On the other hand, it has capability to reduce feed intake with declined egg production. Furthermore, it takes part in the inflammation of the Bursa of Fabricius with swelling of hormone-induced cloacal swelling.

Moniliformin in poultry production

Moniliformin, a water-soluble secondary metabolic product of Fusarium species, is a common contaminant of corn, wheat, barley, and other crops that are generally used for poultry feed (Bakeer *et al.,* 2013). It has an inhibitory role in broiler production. It causes cardiotoxicity that is characterized by cardiomyopathy. It also plays important role in damaging the liver and kidneys in poultry species (Hoerr, 2010).It causes cardiomyocyte cross striations, inclined cardiomyocyte nuclear size, and an elevated number of cardiomyocyte mitotic in necrotic findings.

Citrinin in poultry production

Citrinin (CIT), a nephrotoxic mycotoxin produced by *Penicillium citrinum*. It is occurred by Penicillium and Aspergillus and is a natural contaminant of rice, corn, and other cereal grains. It is a common co-contaminant with OTA in the feed of poultry species. It is nephrotoxic characterized by endemic nephropathy, degenerative and necrotic changes in the enlarged renal tubular epithelial cells in poultry (Puvaèa *et al.,* 2018). Documentary evidence suggested it is to be neurotoxic, teratogenic, embryotoxic, immunotoxic mycotoxin in poultry (Girh *et al.,* 2018). Citrinin causes watery faecal droppings, diuresis, and deductions in weight gain.

Immunopathogenesis associated with mycotoxin

The immune system in the poultry is considered to be a crucial defensive mechanism against invading pathogenic bacteria and foreign cells. It is reported that mycotoxins hamper the immune system depending on the time, duration, and dose of exposure (Bakeer *et al.,* 2013). These exert various toxicity at biochemical and cellular levels leading to cellular injury or cellular deregulation.

Mycotoxins, when ingested through contaminated feeds could cause severe metabolic disorders *viz.,* alteration in cell membrane integrity, capillary hemorrhage, and immunosuppression (DΔnicke, 2002). The most important issues caused by mycotoxins are: hepatotoxicity (aflatoxin B1), atrophy of immune organs (e.g. aflatoxin B1, ochratoxin A, T-2 toxin), suppression of cell-mediated immunity (e.g. aflatoxin B1), and nephrotoxicity (e.g. ochratoxin A) (Rawal and Kim, 2010). Aflatoxin targets the liver, kidney, and lymph tissues in which lymphoid organs including the thymus, and bursa fabricius degenerate and disrupt the development of bursa fabricius and reduces the production of immunoglobulins (Rawal and Kim, 2010). Cellular and humoral immunosuppression are also affected by aflatoxins that cause aplasia of thymus and bursa of fabricius, lowered activity and the number of T cells, declined phagocytes, and reduced humoral immune components such as complements, interferons, and immunoglobulins (Abedi and Talebi, 2015). It has been also reported that poultry is more susceptible to viral and bacterial diseases due to the ingestion of aflatoxins (Barati *et al.*, 2018). These can be considered as the mostimportant immunosuppressive agent as these exert its toxic effects by binding to both DNA and RNA, blocking transcription, and thereby inhibiting protein synthesis (Bennett, 1987). The most important immunosuppressive effects of aflatoxins are declined complement activity, depressed macrophage effector cellular function, suppressed antibody production, impaired proliferation, and altered production of cytokines by T-cells. In addition, aflatoxin B1 plays a major role in damaging cell-mediated immunity by lowering albumin and globulin which inhibited protein synthesis. It has been narrated that aflatoxins cause declined hepatic gene expression of superoxide dismutase, glutathione S-transferase, and superoxide hydrolase and increased gene expression of Interleukin 6 and cytochrome p450 1A1 and 2H1 which are associated with phenomenon characterized by lowered energy production and fatty acid metabolism (carnitine palmitoyl transferase), growth and development (insulin-like growth factor 1), antioxidant protection (glutathione S-transferase), detoxification (epoxide hydrolase), coagulation (coagulation factors IX and X), and, immune protection (interleukins) (Girh *et al.,* 2018).

On the other hand, Ochratoxin A (OTA) is constituted of an isocoumarin moiety linked through the 7-carboxy group to the amino acid L-â-phenylalanine (Liew and Mohd- Redzwan, 2018). At a cellular level, OTA interferes with DNA, RNA, and protein synthesis by inhibiting the enzyme phenylalanine t-RNA synthetase (Puvaèa *et al.,* 2018). It plays a significant role in the alteration of renal carbohydrate metabolism through a deduction of the renal mRNA coding for phosphoenolpyruvate carboxykinase (PEPCK), a key enzyme in gluconeogenesis (DΔnicke, 2002). The toxic effects of OTA on DNA, RNA, and protein synthesis are meant to be due to the phenylalanine moiety of the toxin competing with phenylalanine in the enzyme-catalyzed reaction (Hoerr, 2010). It also exerts its toxic effects by causing hypercarotenaemia with increased mortality in broilers.

The main important mechanism of Fumonisin toxicity is the disruption of sphingolipid metabolism through inhibition of ceramide synthase (sphinganine/ sphingosine N-acyltransferase) a key enzyme required for the synthesis of ceramide and more complex sphingolipids (Puvaèa *et al.,* 2018). Reverse action of this enzyme system causes to an elevation in tissue concentrations of the sphingolipids sphingosine (SO) and sphinganine (SA), and a change in the SA: SO ratio. An increase in the SA: SO ratio, has been observed in tissues of turkeys, broilers, and ducklings fed with feed contaminated with fumonisin (Antonissen *et al.,* 2015). Fumonisins disrupt sphingolipid metabolism and block the synthesis of complex sphingolipids from sphinganine (Sa) and sphingosine (So). As a consequence, Sa and So accumulate in tissues and inhibit many cellular activities. It has been documented that the main immuno-suppressive effects of fumonisins are: impaired efficacy of vaccines, deduced antibody response, declined macrophage numbers and effector cell function, reduced lymphocyte count, and activity (Girh *et al.,* 2018).

Trichothecenes cause immune suppression through reduction of proliferation and activity of B- and T-cells, suppression of antibody production, depression of macrophage effector cell function, declined mucus production, and weakened intestinal cell tight junctions (Corrier, 1991). Interestingly, these inhibit the translation portion of protein synthesis by blocking phenylalanine tRNA synthetase. These play a significant role in the depletion of cellular integrity of lymphoid organs, decrease in antibody production, and suppression of macrophage and heterophil effector cell activities (Patil, 2014). T-2 toxin is also considered to be cytotoxic to chicken macrophages. As a result, it causes lowered immunoglobulin concentration resulting in increased susceptibility of poultry animals towards infection and vis-à-vis lowering the response to vaccination programs (Bennett *et al.,* 2003).

Documentary evidence suggested that zearalenone, an estrogenic mycotoxin, is an immunotoxic agent by causing impairment of lymphoid organs, and the modulation of immune responses and (Branton *et al.,* 1989; Hueza *et al.,* 2014). It plays an immense role in causing atrophy of the thymus, alteration of phenotypic characterization of spleen lymphocyte, decrease in peroxide production by peritoneal macrophages. It also impairs T cell-dependent humoral immune response (Bakeer et al., 2013).

On the other hand, moniliformin is generally cardiotoxic and causes ventricular hypertrophy. It plays a significant role in inhibiting the activity of pyruvate dehydrogenase complex of respiratory reaction, which prevents pyruvic acid, the product of glycolysis, to convert to acetyl CoA (Rabie *et al.,* 1982; Hoerr, 2010). Documentary evidence depicted that it damages damage myofibers, mitochondria, Z and M lines, and sarcoplasmic reticulum as well as increased extracellular collagen deposition (Chelkowski *et al.,* 1990; Puvaèa *et al.,* 2018).

Table 1: Depicts toxic effects of mycotoxin in poultry.

	System involved	Changes involved
Alfatoxin	• General structures • Reproductive system • Haemopoietic system • Immune system • Digestive system • G.I tract	• metabolic damages • liver lesions • digestive enzymes • immune suppression • serum protein • glucose • Phosphorus • calcium • body weight gain • feed intake • feed conversion ratio • carcass yield • pigmentation • egg production • hatchability and fertility
Ochratoxin	• Urogeniital system • Haemoietic system • Reproductive system • Muscular system • Immune system • G.I. tract	• Nephrotoxicity and anaemia • feed consumption and growth rate • Weakness and poor feathering • mucosal erosions of gizzard • mucosal haemorrhages mortality • egg production, carcass yield • catarrhal enteritis • dehydration • skeletal integrity • Phagocytosis • coagulation of blood. • Genotoxicity • immunotoxicity • carcinogenicity
Fumonisin	• G.I tract • Immune system • Haemopoietic system • Renal syatem	• intestinal microbial homeostasis • Imunosupprssion • liver functions • sphingolipid biosynthesis • ulceration of the oral mucosa • ulceration of gastro intestinal mucosa • mottling of the liver hyperplasia, hepatic necrosis • atrophy of the spleen • visceral hemorrhages • blood glucose and protein • affect proximal convoluted tubule (PCT) • immunoglobulins
Trichothecenes	• General system • G.I. Tract • Neurosystem • Haemopoietic system • Immune system	• health hazard • productivity of poultry • gut epithelium permeability • absorption of protein • feed intake and growth rate • alterations in blood parameters • neurotoxicity

	System involved	Changes involved
Zearalenone	• Haemopoietic system	• white blood cells
	• Hepatic system	• comb shrinkage
	• Reproductive system	• hypertrophy of oviducts
	• Immune system	• body weight
		• hepatotoxicity
Moniliformin	• Cardiac system	• cardiotoxicity and immune toxicity
	• Hepatic system	• cardiomyopathy
	• Renal system	• liver and kidneys
	• Immune system	• Nephrotoxic, nephropathy
Citrinin	• Renal system	• Neurotoxic
	• Immune system	• Teratogenic
	• Reproductive ssyetem	• Embryotoxic
		• immunotoxicity

Citrinin also acts as an immune suppressive agent by causing a significant elevation in micronucleus (MN) frequency, alterations in humoral and cell-mediated immunity, and modifications of complex cellular biotransformation and mutagenesis (Ahamad, 2018; Girh, 2019).

The most important toxin namely ochratoxin A, T-2 toxin, aflatoxin B1 plays a key role in inhibiting the synthesis of DNA, RNA, and protein and may damage DNA leading to cell transformation, abnormal cellular proliferation and finally tumor formation (Liew and Mohd- Redzwan S, 2018). They are also responsible for causing lipid peroxidation, damage of membrane structures, and their functions, induces apoptosis (programmed cell death) leading to cellular necrosis (Bennett, 1987).

CONCLUSION

Mycotoxins, a toxic fungal metabolite exert alterations in mucosal immunity with lowered animal performance, and susceptible to various pathogens that typically invade these surfaces. . These inhibit protein biosynthesis and cause DNA damages through alteration of immune cell proliferation, accelerated production of polyunsaturated fatty acids, and thereby decreased phagocytosis of macrophages. These suppress natural killer cell activity via inhibition of interferon production, cytotoxic T cell activation, gamma interferon production, lymphocyte stimulation, and consequently the alteration in pro-inflammatory cytokine. Therefore mycotoxin causes immunosuppression of poultry.

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Chicken productive performance in Ethiopia: review

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ABSTRACT

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To upgrade the country's poultry production and to satisfy the rising demand for poultry products, several studies have been conducted in different parts of Ethiopia on the productive performance of indigenous, crosses, and exotic chicken breeds. However, there were inconsistencies between these studies. Therefore; this review was conducted to assess the productive characteristics of indigenous, exotic, and their crosses. The contribution of exotic and crossbreed chickens to the total poultry population in Ethiopia is lower than the contribution in other African countries. The day-od weight for indigenous, cross, and exotic breeds ranges from 22.2-37, 27.0-40.1, and 28.7 to 42.0 g, respectively. The indigenous, crosses and exotic male and female chicken age at sexual maturity body weight reported in different parts of Ethiopia range from 1044.7-2010.0 and 642-1116.5, 1050-1780 and 1054-1600 and 1300-3020 and 1000-1940 g, respectively. The indigenous chickens are performing lower in egg number (34-65 eggs/year/hen) and egg weight (38-42.2 g) than the crossbreed and exotic chickens. The annual egg production potential of exotic and crossbreed chickens under backyard conditions and intensive systems ranges between 117.3 and 276 eggs. The crossbreed and exotic chickens are better for their egg number, egg weight, and weight at day-old and mature age than indigenous chickens. From this review, it is concluded that there is variation in chicken productive performance in different parts of the country.

Keywords: Chicken, Egg, Productive Performance, Ethiopia

INTRODUCTION

Poultry production is one of the key livestock subsectors of Ethiopia. It plays important role in terms of generating employment opportunities, improving family nutrition, and empowering women. It is a suitable business for poor households due to the small quantity of land needed and the low investment costs required to start up and run the operation (FAO, 2019). The Ethiopian poultry population is estimated to be 56.06 million, which consists of indigenous (88.19%), exotic (5.36%), and hybrid (6.45%) (CSA, 2017/18**)**. This population of chickens plays a crucial role in the livelihood of resource-challenged families and it has socio-cultural and economic benefits, especially in rural communities (Mamo *et al*., 2013). The dominant proportion of the national poultry meat and eggs are produced under the scavenging family poultry production systems using low-producing indigenous breeds. However, exotic breeds in intensive production systems are contributing to an increasing share of production. Exotic breeds contributed to more than 27% of the total number of eggs produced nationally; despite constituting only 9% of the total national flock (FAO, 2019). Poultry products are considered one of the most important sources of cheap protein as compared with other livestock. As well, chicken production is characterized by a higher conversion rate in comparison with other animals which is evidenced by lower feed need (2 to 2.5 kg) to produce one kg of poultry meat however other livestock needs more than seven kg of feed to yield the same amount of meat (Wahyono and Utami, 2018).

The village chicken's production system is dominated by indigenous chickens which possess desirable characteristics such as better egg and meat flavor, hard eggshell, and high dressing percentage (Abdelqader *et al*., 2007). However, they are very poor layers and lay around 34-80 eggs annually per hen on average with a very small size egg of about 45g (Alganesh *et al.*, 2003; Melkamu and Andarge, 2013). The lower production performance of indigenous scavenging chickens is related to their low genetic potential, high mortality, and longer reproductive cycle, such as slow growth rate, late sexual maturity, and broodiness for extended periods (Besbes, 2009). For this reason, attempts have been made by several scholars in different parts of the country to improve the productive performance of indigenous birds directly through the introduction of exotic chickens to the smallholder farming systems (Tadelle and Ogle, 2001; Hailu *et al*., 2012) and a crossbreed of exotic breeds to the local chickens since the 1950s (Solomon 2008). Since then, the productive performance of indigenous, crossbreed, and introduced exotic chickens are characterized by different management systems in different parts of the country. However, there were discrepancies between the various studies and it was, therefore, this review was carried out to assess the production performance of indigenous, exotic and crossbreed chickens.

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Table 1: Trends and population distribution of chickens in Ethiopia

Parameters	Year, CSA (2009 to 2018)							
	2009/10	2010/11	2011/12	2012/13	2013/14	2014/15	2016/17	2017/18
Total Population (mill)	42	49.3	44.90	50.38	51.35	56.87	59.50	56.06
Proportions by type (%)								
Chicks	36.44	37.12	40.11	38.91	38.30	37.68	37.93	35.42
Hens	33.50	32.25	33.00	32.77	33.32	33.10	33.47	34.93
Pullets	9.76	9.90	DNF	9.65	9.83	10.40	10.50	11.00
Cocks	11.38	11.39	DNF	10.30	10.24	10.21	9.82	10.10
Cockerels	5.45	5.62	DNF	5.34	5.29	5.65	5.54	5.85
Non-laying hens	3.47	3.72	DNF	3.03	3.01	2.95	2.74	2.70
Breed (%)								
Local	96.61	97.30	96.46	96.90	96.83	95.86	90.85	88.19
Hybrid	0.55	0.38	0.57	0.54	2.37	2.79	4.76	6.45
Exotic	2.84	2.32	2.97	2.56	0.80	1.35	4.39	5.36

CSA-Central Statistical Agency, DNF-Data is not found

Trends of the country's chicken breed and population

The world's chicken population was estimated at 16.2 billion, of which 71.6% were found in developing countries that were producing 67, 718, 544 metric tons of chicken meat and 57, 861, 747 metric tons of hen eggs (Bushra, 2012). The country's Central Statistical Agency (CSA 2009/10-2017/18) reported a consecutive increase in the total chicken population year over year (Table 1) despite the low per capita consumption. The population trend of indigenous chickens indicates decreasing as compared to hybrid and exotic breeds which is in line with the national poultry development program that aimed breed improvement though it is not to the expected level. The percentage contribution of exotic breeds of chickens (Table 1) to the total poultry population of Ethiopia is lower than the contribution in other African countries such as Cameroon (35%), Gambia (10%), Kenya (20%), Malawi (10%), Nigeria (9%) and Zimbabwe (70%) (Matawork, 2016). As seen in Table 1, the percentage of chicks decreased whereas laying hens increased from the year 2012 onwards and this high reduction in chick population resulted in to decline the total chicken population in 2017/18. The breed composition of the country's exotic and hybrid chickens increased while indigenous decreased (Table 1) and this implies that smallholder farmers are adopting exotic or hybrid and replacing their local chickens.

Production performances

Body weight at hatchling: The hatchling weight for Ethiopian indigenous chickens ranges from 22.2 g for those managed under smallholder farmer conditions to 37 g managed under intensive systems in Jimma College of agriculture (Table 2). Whereas, the day-old body weight for cross and exotic breeds ranges from 27.0 - 40.1 g and 28.7 to 42.0 g, respectively. Similarly, in some African countries such as Tanzania (31.9 g; Magonka *et al.*, 2016), Kenya (23-26 g; Kingori *et al.*, 2010) and Sudan (24.68 -27.83 g; Binda *et al.*, 2012) the day-old chick's weights for indigenous chickens were within the range reported for Ethiopian chickens. Besides, Faruque *et al. (*2013) reported 29.12-31.08 g for Bangladesh day-old native chicken. The lower weight at the hatch for Ethiopian native chickens as compared to the exotic and their crosses might be related to their lower egg weight (38 to 42.2g) as illustrated in Table 6. This view point was in agreement with to earlier scholars who reported differences in hatch weight due to egg weight (Tona *et al.*, 2004; Sahin *et al.*, 2009). Besides, Ewonetu and Kasaye (2018) reported as egg weight influence the post-hatch weight of chicken from day-old to 50 weeks of age. The day-old crossbred chicks were heavier than day-old chicks of local breeds and this implied that crossbreeding improves the day-old body weight of chickens. This was concurred to Yeboah *et al.* (2019) who reported higher live weight of the foreign chicks (42.84 g) than that of the locally hatched (38.11 g).

Body weight at maturity: The indigenous, crosses and exotic male and female chicken mature body weight reported in different parts of Ethiopia range from 1044.7-2010.0 and 642-1116.5, 1050-1780 and 1054-1600 and 1300-3020 and 1000-1880 g, respectively (Table 3). The mature weight of male and female for all chicken breed in Ethiopia is fallen within the range reported for the adult male (1200-3200 g) and female (700-2100g) in Africa (Gueye, 2000). A review compiled by Gueye (1998) for mature male weights of local fowl in some African countries (1600g in Mali, 1100g in Ghana, 1500 g in Chad, 1500 g in Burkina Faso, 1600g in Lesotho, 1200g in Morocco) were reported within the range for Ethiopian local male chickens. Likely, in his review compiled the mature female weights of local fowl recorded in Mali (1020g), Ghana (900-1100g) and Chad (1000g) were

Table 2: Review of hatching weight of chickens in different parts of Ethiopia

Breed type	Sex	Hatchling weight (g)	Study site	Authors
		Local		
Local	SU	27.3	North-west Ethiopia	Halima, 2007
Local	Male	28.8	Guraghe Zone	Misba and Aberra, 2013
Local	Female	26.2	Guraghe Zone	Misba and Aberra, 2013
Local	SU	22.2	Fogera district	Fisseha *et al.*, 2010
Local	SU	36-37	JCA	Solomon, 2004
Local (Various ecotype)	SU	25.8-32.8	DZARC	Tadelle *et al.*, 2003
Local (Various ecotype)	SU	25.5-29.3	ALRC	Halima *et al.*, 2007
		Exotic		
RIR	SU	35.2	ALRC	Halima *et al.*, 2007
RIR	SU	35.4	Mekelle	Kumar *et al.*, 2014
White leg horn	SU	33.46	HUPF	Ewonetu, 2017
White leg horn	SU	42.0	JCA	Solomon, 2004
Bovans White	SU	31.8	Mekelle	Kumar *et al.*, 2014
DR	SU	34.3	DZARC	Ibrahim *et al.*, 2019
DS	SU	34.1	DZARC	Ibrahim *et al.*, 2019
KK	SU	28.7	DZARC	Ibrahim *et al.*, 2019
LB	SU	31.8	DZARC	Ibrahim *et al.*, 2019
Fayoumi	SU	29.39	HUPF	Ewonetu, 2017

fall within the range reported for Ethiopian local female chickens. This means the productive performance of local chickens in terms of mature body weight in Ethiopia is almost similar to that of some other African countries. As seen in table 3, the weight of male and female local chickens at maturity age is lower than the weight of cross and exotic chickens in different regions of the country even under the same management system. This is probably related to the genotype. Besides, the heavier egg weight of crossbreed and exotic chickens (table 6) contributed to the heavier body weight at maturity. This is in agreement to (Tadelle *et al.*, 2003) who reported the difference in egg weight was reflected in chicken weight difference at later age.

Likewise, Ewonetu and Kasaye (2018) reported as egg weight influenced the post-hatch weight of chicken in the study conducted on egg weight difference from day-old to 50 weeks of age. Moreover, Iqbal *et al.* (2017) has been proved a direct relationship between a chick weight at hatch with mature weight. In general, the differences observed among regions with respect to the mature body weight can be also attributed to genetic variability, environment and rearing conditions in various agro-ecologies of the country. This perspective was agreed with Brhane *et al*. (2017) who reported the influence of both genetic and non-genetic factors on body weight of chickens.

Egg production: The annual egg production potential of exotic and crossbreed chickens reviewed in Ethiopia under backyard conditions and intensive system ranges between 133 and 276 eggs (Table 4). This corresponds to Khalid (2015), who reported 180-200 eggs/year for hybrid laying hens in hot and humid climate areas, whereas 250 to 300 eggs on average for the same breed in more temperate climates. The exotic breeds of chickens performed well under the intensive management system but still not at its satisfactory level as compared to other African countries. The egg output from traditional production sector is very low though they constitute the largest proportion of the country's chicken population. The egg production potential of indigenous chickens is between 34-65 eggs per year per hen in different parts of the country (Table 5). Thus, there exist a wide variability in the egg production capacity and this may be attributed to the genetic makeup and the management system. Besides, the lower egg output from the indigenous chickens is the results of uncontrolled breeding between various local chicken ecotypes, which have not been selected by systematic breeding methods (Alganesh *et al.*, 2003).

The lower performance in egg supports the reports of Tesfa *et al.* (2013) who reported 30-60 eggs per year per hen for indigenous chickens in Ethiopia. This review showed that the egg production performance of local chicken in Ethiopia is almost similar to findings reported in other African and tropical countries that was 37-95 in Africa (Gueye, 2000), 44 in Bangladesh (Barua and Yoshimura, 2005), 40-50 in Uganda (Ssewannyana *et al.*, 2008), 40-100 in Kenya (Kingori *et al.*, 2010), 52 in Ghana (Dankwa *et al.*, 2000), 30-60 in Rwanda (Mahoro *et al.*, 2017), 40-80 in Sudan (Sulieman, 1996), 70 in Zambia (Songolo & Ketongu 2001), 30-80 in Zimbabwe

Table 3: Review of body weight at sexual maturity of local, cross and exotic chickens in different parts of Ethiopia

Breed type	Sex	Mature body weight (g)	Study site	Authors
		Local breed		
Local	Cock	1753.0	Central Tigray	Alem, 2014
Local	Cock	1050.0	Southern Ethiopia	Mekonnen, 2007
Local	Cock	1273.0	Guraghe Zone	Misba and Aberra, 2013
Local	Cock	2010.0	Hawassa	Yonas, 2020
Local	Cock	1300.0	Tigray region	Abraham and Yayneshet, 2010
Local	Cock	1362.5	North west Amhara	Fisseha *et al*., 2010
Local	Cock	1340-1600	South Wollo	Mengesha *et al.*, 2008
Local	Cock	1044.7-1292	ALRC	Halima *et al*., 2007
Local	Hen	1042.5	Central Tigray	Alem, 2014
Local	Hen	987.0	Guraghe Zone	Misba and Aberra, 2013
Local	Hen	1100.0	Tigray region	Abraham and Yayneshet, 2010
Local	Hen	1116.5	North west Amhara	Fisseha *et al.*, 2010
Local NN	Hen	952.0	Hawassa University	Aberra, 2011
Local	Hen	642-873.5	ALRC	Halima *et al.*, 2007
Local	Hen	1030-1050	South Wollo	Mengesha *et al.*, 2008
		Cross breed		
Local X Fayoumi	Cock	1310.0	Guraghe Zone	Misba and Aberra, 2013
Local X RIR	Cock	1682.0	Guraghe Zone	Misba and Aberra, 2013
Crossbreed	Cock	1050-1780	South Wollo	Mengesha *et al.*, 2008
Local X Fayoumi	Hen	1054.0	Guraghe Zone	Misba and Aberra, 2013
Local X RIR	Hen	1227.0	Guraghe Zone	Misba and Aberra, 2013
Local NN X LW	Hen	1169.0	Hawassa University	Aberra, 2011
Local NN X NH	Hen	1259.0	Hawassa University	Aberra, 2011
Crossbreed	Hen	1450-1600	South Wollo	Mengesha *et al.*, 2008
		Exotic breed		
Sasso	Cock	3020.0	Hawassa	Yonas, 2020
Bovance Brown	Cock	2920.0	Hawassa	Yonas, 2020
Fayoumi	Cock	1300.0	Tigray region	Abraham and Yayneshet, 2010
Rhode Island Red	Cock	2200.0	Tigray region	Abraham and Yayneshet, 2010
Rhode Island Red	Cock	1735.7	Amhara Region	Halima, 2007
Fayoumi	Cock	1500.0	ATARC	Tesfa *et al.*, 2013
Rhode Island Red	Cock	1970-2100	South Wollo	Mengesha *et al.*, 2008
Fayoumi	Hen	1000.0	Tigray region	Abraham and Yayneshet, 2010
Rhode Island Red	Hen	1600.0	Tigray region	Abraham and Yayneshet, 2010
Rhode Island Red	Hen	1263.3	ALRC	Halima, 2007
Rhode Island Red	Hen	1880-1940	South Wollo	Mengesha *et al.*, 2008
Bovans Brown	Hen	1550.0	East Shewa	Desalew, 2012
KoekKoek	Hen	1640.0	East Shewa	Desalew, 2012
Fayoumi	Hen	1200.0	ATARC	Tesfa *et al.*, 2013
Fayoumi	Hen	1215.0	MRVO	Samson and Endalew, 2010

ATARC= Adami Tulu Agricultural Research Center; NN-naked-neck; LW- Lohmann White; NH-New Hampshire; MRVO- Mid Rift Valley of Oromia

(Muchenje and Sibanda, 1997), 40 in Nigeria (Ikeobi *et al.*, 1996), 20-100 eggs in Botswana (Gueye, 1998). This similarity of egg production performance for local chickens in various countries indicates the resemblance in their genotype and management given in all African countries. This is concurring to Sonaiya *et al.* (1999) who reported that over 70 percent of indigenous chickens in Africa are managed under free ranging system. This review also found that the local chickens are poor by their productivity. This is why many attempts have been made to introduce different exotic chicken breeds in to African countries to improve local chickens through crossbreeding (Hailu *et al.*, 2012). Moreover, the lower egg production performance of indigenous chickens is

Table 4: Review of egg production performance of exotic chickens in Ethiopia under different management system

Breed type	Eggs/hen/year	Study site	Keeping system	Author/s
Fayoumi	144	Tigray Region	SHF	Abraham and Yayneshet, 2010
RIR	185	Tigray Region	SHF	Abraham and Yayneshet, 2010
WL	173	Tigray Region	SHF	Abraham and Yayneshet, 2010
RIR	133	Central Tigray	SHF	Alem Tadesse, 2014
Fayoumi	159.9	Adami Tulu RC	IS	Tesfa *et al.*, 2013
BB	134.5	North western Amhara	SHF	Sisay *et al.*, 2017
Koekoek	135.9	North western Amhara	SHF	Sisay *et al.*, 2017
BW	154.3	North western Amhara	SHF	Sisay *et al.*, 2017
PK	156	Southern Tigray	SHF	Atsbaha *et al.*, 2018
PK	213	Adami Tulu RC	IS	Tesfa and Usman, 2018
IB	276	Ada'a and Lume Districts	SHF	Desalew, 2012
BB	266	Ada'a and Lume Districts	SHF	Desalew, 2012
PK	178	Ada'a and Lume Districts	SHF	Desalew, 2012
Sasso	133.2	Boricha district, Sidama zone	SHF	Serkalem *et al.*, 2019
BB	117.3	Boricha district, Sidama zone	SHF	Serkalem *et al.*, 2019
Koekoek	138.3	Boricha district, Sidama zone	SHF	Serkalem *et al.*, 2019

RIR= Rhode Island Red; WL= White Leghorn; IB= Isa Brown, BB= Bovan Brown; BW= Bovance White; PK= Potchefstroom Koekoek; RC-Research center; SHF=small holder farmer; IS= Intensive system

related to the practice that broody hens give days for natural incubation (21 days after each clutch) and rearing chicks (at least 60 days to wean after each clutch) and it could be also due to long pauses, periodic broodiness in addition to feed and management limitations.

Table 5: Egg production performance of indigenous chickens under smallholder's management system in different parts of Ethiopia

Eggs/ hen/ year	Author/s	Study Site
59.5	Solomon *et al.*, 2013	Northwest Ethiopia
60.0	Fisseha *et al.*, 2010	Bure districts
53.0	Fisseha *et al.*, 2010	Fogera districts
55.0	Fisseha *et al.*, 2010	Dale districts
54.3	Abraham and Yayneshet, 2010	Northern Ethiopia
38.5	Matawork *et al.*, 2019	Dawro Zone
34.0	Alemu, 1995	Asela
53.0	Yadessa *et al.*, 2017	Mezhenger zone
53.3	Yadessa *et al.*, 2017	Sheka zone
57.3	Yadessa *et al.*, 2017	Benchi-Maji zone
65.0	Melkamu and Andargie, 2013	Eastern Gojjam
40.0	Tadelle *et al.*, 2000	Haramaya
40.8	Assefa *et al.*, 2019	Sheka zone
48.0	CSA, 2017/18	Ethiopia

CSA-Central Statistical Agency

Egg weight: The average egg weights reported by many scholars in different parts of the country under various management systems is within the range of 43.0 to 63.0g for exotic whereas 38 to 42.2g for the indigenous (Table 6). This review found that the egg weight reported for indigenous chickens (Shi *et al.*, 2009) are less than the egg weight categorized to small size in Canadian egg size standards: small (42.0–48.9 g), medium (49.0–55.9 g), large (56.0– 63.9 g), extra-large (64.0–69.9 g) and jumbo (70.0 g or higher) and in USA egg size grades: jumbo (e"70 g), extra-large (65–70 g), large (56–65 g), medium (49–56 g) and small (42–49 g) (FAO, 2003). The lower egg weight for indigenous chickens in Ethiopia might be due to their small body size including the non-hereditary and genetic factors. Overall, in Ethiopia the local chicken egg weight is below the standard of eggs categorized to small size in Canada and USA whereas the eggs of exotic and their crosses most commonly fallen in the category of small and medium size reported in Canada and USA (FAO, 2003). However, this review showed as the egg weight performance of local chickens in Ethiopia is nearly similar to the findings reported in other African countries such as 29-46g in Ghana (Dankwa *et al.*, 2000), 30.7g in Guinea (Mourad *et al.*, 1997), 33-48g in Kenya (Kingori *et al.*, 2010), 30-50 g in Botswana (Gueye, 1998), 36.8 g in Nigeria (Adetayo and Babafunso, 2001), 48.9g in South Africa (Nhleko *et al.*, 2003) and 37.95-39.89 g in Sudan (Mohammed *et al.*, 2005).

From this review (Table 4) it can be concluded that exotic and crossbreed chickens produce eggs with large size than the indigenous chickens. It is an established

Table 6: Review of egg weight performance of local, cross and exotic chickens in Ethiopia under different management system

Breed type	Egg weight(g)	Study site	Keeping system	Authors
		Local Breeds		
Local	42.2	Tigray region	Smallholder farmers	Abraham and Yayneshet, 2010
Local (NN)	42.0	DNF	Improved management	Aberra *et al.*, 2005
Local	41.2	Boricha district	Traditional management	Serkalem *et al.*, 2019
Local	38.3	Guraghe zone	Farmer management condition	Melesse *et al.*, 2013
Local	38.0	JCA	Rural household conditions	Solomon, 2004
Local	38.0	JCA	Intensive management	Solomon, 2004
Local	38.0	Manasibu district	Under village management	Alganesh *et al.*, 2003
Local	38-38.18	South Wollo	Under village management	Mengesha *et al.*, 2008
		Crossbreeds		
Fayoumi-cross	40.0	Guraghe zone	Farmer management condition	Melesse *et al.*, 2013
RIR- Cross	44.2	Guraghe zone	Farmer management condition	Melesse *et al.*, 2013
Crossbreed	50.27-50.67	South Wollo	Under village management	Mengesha *et al.*, 2008
NN x NH	49.0	DNF	Improved management	Aberra *et al.*, 2005
		Exotic Breeds		
WL	52.0	JCA	Rural household conditions	Solomon, 2004
WL	53.0	JCA	Intensive management	Solomon, 2004
Fayoumi	44.3	ATARC	Intensive management	Tesfa *et al.*, 2013
Koekoek	52.5	Tigray region	Under farmer management	Shumuye *et al.*, 2018
Exotic	60-63	South Wollo	Under village management	Mengesha *et al.*, 2008
Bovans brown	49.5	Boricha district	Traditional management	Serkalem *et al.*, 2019
Sasso	53.8	Boricha district	Traditional management	Serkalem *et al.*, 2019
Keokeok	43.9	Boricha district	Traditional management	Serkalem *et al.*, 2019
WL	52.1	Tigray region	Smallholder farmers	Abraham andYayneshet, 2010
RIR	52.5	Tigray region	Smallholder farmers	
Fayoumi	43.0	Tigray region	Smallholder farmers	

ATRC=Adami Tullu Agricultural Research Center; JCA=Jimma College of Agriculture; na= not available; NH=New Hampshire; NN=necked neck; RIR= Rhode Island Red; WL= White Leghorn; DNF=data is not found.

fact that the weight of an egg is significantly varies between strains of hen (Pandey *et al.*, 1986). The egg evaluated under different management system is vary by their weight and largely affected by environmental factors, food restriction and parental age and average body weight; and the differences in this review could be attributed to these factors which is in conformity with what was reported by Mohammed *et al.* (2005).

CONCLUSION

This review indicated as variations exist in chickens' productive performances in different areas of the country. The productivity of indigenous chicken is rated low with an annual egg production per hen being 34-65, and a very small egg size of about 38-42.2g compared to exotic and cross breeds. There is some evidence that there is a large amount of genetic variance in chicken production performance in Ethiopia.

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Molecular cloning and characterization of partial promoter of *Acetyl Coenzyme A Carboxylase B* gene in chicken

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ABSTRACT

Nayak, N., Bhattacharya, T.K., Shukla, R., Divya, D., Prasad, R. and Chakurkar, E.B. 2022. Molecular cloning and characterization of partial promoter of Acetyl Coenzyme A Carboxylase B *gene in chicken. Indian Journal of Poultry Science, 57(02): 113-117.*

Poultry industry has achieved its present position from a mere backyard venture mainly due to selection for rapid early growth, optimum nutrition and rearing environment. However, intensive genetic selection for growth inadvertently selected for increased carcass fat. Hence, study was undertaken to identify partial promoter sequence and expression profile of *ACACB* gene as it mainly responsible for fat synthesis. An upstream part of *ACACB* gene with 697bp fragment was chosen and cloned in to a promoter less pAcGFP1-1 vector. The recombinant-vector was transfected in to cell culture of chicken embryo fibroblast cells. After two days of transfection, total RNA, DNA and protein were isolated and examined for expression of reporter gene. The 697bp fragment displayed a considerable amount of fluorescence under fluorescent microscope along with preferred band of 250bp of GFP on gel. The mRNA expression depicted the magnitudes of ΔC_t of 19.92 in transfected cells where there was no expression of GFP in control cells for GFP gene. The expression was also checked in western blot having presence of GFP in transfected cells. It is concluded that 697bp upstream fragment of *ACACB* gene had potential to be the promoter to regulate gene expression in the *in-vivo* cell culture medium.

Keywords: Acetyl coenzyme-A carboxylase, Broiler chicken, Cloning, Fatty acid, Promoter

INTRODUCTION

The *de novo* fatty acid synthesis occurs in extra-mitochondrial system (Botham and Mayes, 2015), which is responsible for the complete synthesis of palmitate from acetyl-CoA. Production of malonyl-CoA from irreversible carboxylation of Acetylm-CoA by Acetyl-CoA carboxylase is the rate limiting step in lipid biosynthesis in chicken. Two major isoforms of Acetyl-CoA Carboxylase are Acetyl-CoA Carboxylase A (*ACACA*) and Acetyl-CoA Carboxylase B (*ACACB*) with different tissue distribution (Ha *et al*., 1996). Most importantly, *ACACA* and *ACACB* genes are responsible for fat synthesis and expressed more in fatty birds as compared to lean ones (Prasad *et al*., 2018). The *ACACA* is predominately expressed in lipogenic (liver and adipose) tissues, whereas *ACACB* in oxidative tissues like skeletal muscle and heart (Brownsey *et al*., 2006). *ACACA* gene is responsible for de novo fatty acid synthesis by producing malonyl-CoA and a rate-limiting enzyme in the regulation of fatty acid synthesis. The ACACB is a phosphoprotein which is mainly responsible for fatty acid oxidation by inhibiting carnitine palmitoyl transferase-1 (CPT-1) which controls the long chain fatty acyl-CoA transport into mitochondria (Mcgany and Brown, 1997). It is localized on the outer mitochondrial membrane in proximity to carnitine palmitoyl transferase 1, where it presumably functions as a negative regulator of fatty acid oxidation (Abu-Elheiga *et al*., 1997; Abu-Elheiga *et al*., 2000).

In chicken, the predominant site for adipose deposition is abdominal fat (20% of total body fat) and subcutaneous fat to a less extent (Zerehdaran *et al*., 2004). The contribution of portomicrons (similar to chylomicrons in mammals) from dietary fats to triglyceride is low, since there is only 5% of a dietary lipid in regular feed (Hermier, 1997). Triglyceride deposits in chicken adipose tissue are mainly through the uptake of fatty acids. In birds, liver is the predominant site (95%) of *de novo* fatty acid synthesis and to a lesser extent (<5%) in adipose tissue.

The promoter sequence of a gene plays vital role in its expression, so a little change in the transcription factor binding sites in the promoter site may cause significant change in expression. Transcription of the gene for *ACACB* is under the control of two promoters (P-I and P-II). In liver, P-I is inactive (Oh *et al*., 2005), but activity is initiated by binding of sterol regulatory element binding protein-1 (SREBP-1) to steroid response elements in P-II (Oh *et al*., 2003). Sequence variation in the *ACACB* gene promoter might affect expression in liver inûuencing net levels of malonyl-CoA, with possible implications for energy balance dependent on genotype.

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There is scanty information on promoter function of the *ACACB* gene and its expression during embryonic development as well as post hatch stage unlike ACACA gene. Better understanding about the expression of this gene will be useful in the future for lean meat production along with improvement in the production efficiency in poultry. The purpose of this study was to identify, characterize the ACACB gene followed by molecular cloning, expression, which could laid a foundation for further exploring its transcriptional regulation and function.

MATERIALS AND METHODS

PCR amplification of ACACB upstream sequence

The 5' upstream region of transcription start site of 697 bp was selected for *ACACB* gene to identify the partial functional promoter sequence of the gene. The primers were designed based on the sequence upstream to the transcription start site of *ACACB* gene. The region was selected based on predicting possible transcription factors binding sites for the expression of each target gene. The primer sequences used to amplify the fragment of promoter were F: GAATTCAGGGATGGCA GCAGCTC and R: GTCGACGGACGGCAATCATCAGC with amplicon size of 697bp with annealing temperature of 56°C.

The amplification by polymerase chain reaction (PCR) was carried out in 0.2 ml PCR tubes containing 2.5 µL of 10X PCR buffer (1X), 1 µL of dNTP mix (2.5 mM), 1.5 µL (30 ng) each of forward and reverse primers, 0.3 µL (1.5 U) of Taq DNA polymerase and 1 µL of genomic DNA (50 ng) and nuclease free water to make the volume up to 25 µL. The thermal cycling conditions for both the fragments were as follows: initial denaturation at 95°C for 10 minutes, denaturation at 95°C for 40 seconds, annealing at 56°C for 40 seconds and extension 72°C for 40 seconds for 35 cycles each, then final extension at 72°C for 10 minutes and 4°C thereafter.

Cloning of 5' upstream fragments and transformation

The amplified product was run in 1% agarose gel electrophoresis and the desired band was excised from the gel. The sliced gel pieces were purified by Qiagen's QIAquick® Gel Extraction Kit (#K0791). The partial product was cloned into a reporter vector, pAcGFP1-1 (Clonetech) for studying the functionalities of the fragment following standard cloning protocol (Bhattacharya *et al*., 2015) mentioned in Table 1. The positive clones were selected with kanamycin antibiotic. The positive clones were screened with colony PCR, plasmid PCR and sequencing of recombinant DNA.

CEF culture and transfection

The recombinant clone (pAcGFP1-1 vector with respective insert) of target gene was transfected into the chicken primary embryo fibroblast (CEB) in duplicate by using Gene Pulser XcellTM Electroporation system (Biorad) (Shigekawa and Dower, 1998).

Expression analysis

The transfected and control cells were harvested after 48 hours of transfection and total RNA was isolated from cell lysate using Trizol (Amresco). Isolated RNA samples were made DNA free by treating with DNaseI (Fermentas) and cDNA was synthesized using High-Capacity cDNA Reverse transcription Kit (Hi-cDNA synthesis kit, MBT076-10R) in thermo cycler (Mastercycler, Eppendorf, Germany) with final volume of 20 µL containing Reverse Transcription (RT) buffer (2 µL), 10X solution (2 µL), M-MuLV Reverse Transcriptase (0.8 µL), Nuclease-free H_2O (8.2 µL) and 1 ng RNA (5 µL). Quantification of mRNA expression levels of target gene (*ACACB*) and reference/constitutive gene (*GAPDH*) genes were done by using thermal cycler Applied Biosystems® Step One Real-Time PCR (Life Technologies) with SYBR® Green JumpStart™ Taq ReadyMix™ (Sahu *et al*., 2019). Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was used as an internal control for normalizing different amounts of input RNA. The primers used in real-time PCR of ACACB gene were F: GCT CCT GCT GCC CAT ATA TTA and R: GTC CGT GAT GAC ACC TTT CT with 94bp amplicon size, annealing temperature of 58°C. GAPDH was used as housekeeping gene with amplicon size of 119bp with same annealing temperature of 58°C.

The threshold cycle (C_t) value of the target and reference genes were determined for all qPCR reactions. The mRNA expression of target genes was analyzed by comparative C_t method of relative quantification. The gene quantification was expressed as "n-fold up/down regulation of transcription" in relation to a reference sample, called the calibrator (mock transfected hepatocytes). The expression of target gene was calibrated by that of the reference gene, *GAPDH*, at each time point and converted to the relative expression (fold of expression), as follows: Fold of expression = $2^{-\Delta\Delta Ct}$.

Table 1: Reaction mixture for RE digestion of promoter fragments and reporter vector, and their ligation to pAcGFP1-P1 vector

RE digestion of promoter fragments	Amount (µL)	pAcGFP1-P1 vector ligation	Amount (µL)
Plasmid	10	GFP1 vector	5 µI
Sal I	0.5	Buffer	2.5 µI
EcoRI	0.5	Insert	15 µI
Buffer O	1.5	Ligase	3 µI
DNA/ RNase free water	2.5	Total	25.5 µI

Where, ΔC_t = Average C_t of target gene – Average C_t of reference gene (*GAPDH*).

$\Delta\Delta C_t$= Average ΔC_t of target (shRNA) – Average ΔC_t of calibrator sample (control).

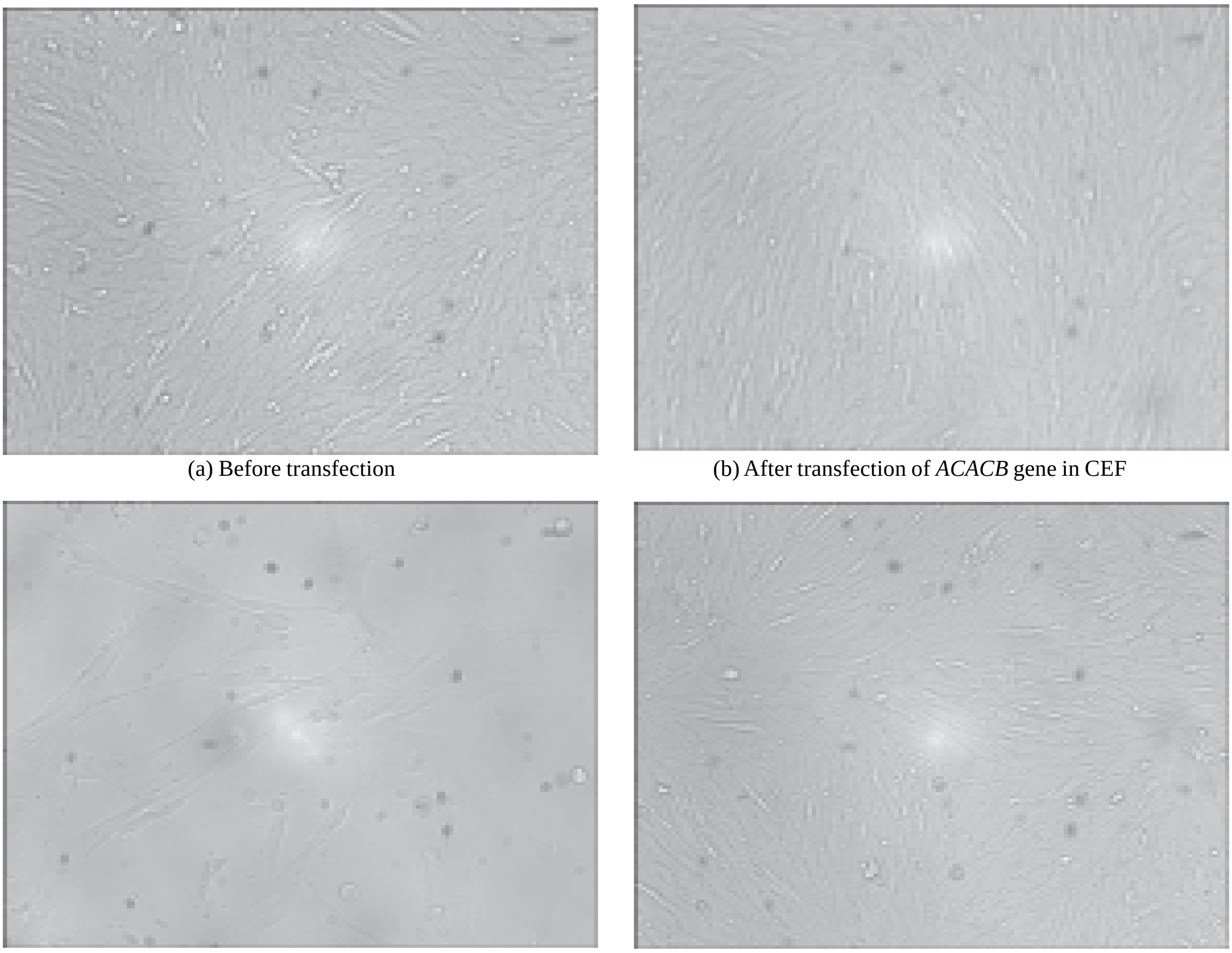
The isolated protein was analyzed with 12% SDS-PAGE of the tissue lysate (Laemmli, 1970) and was detected by western blotting.

RESULTS AND DISCUSSION

The 697 bp upstream part of *ACACB* gene as a partial promoter was cloned in a transfection vector to determine its efficiency as promoter to regulate expression of reporter gene (GFP). The 697 bp fragment was cloned in to a promoter less pAcGFP1-1 vector. Then the positive recombinant clone of 697 bp was transfected in to chicken embryo fibroblast cells (Fig. 1). The change in colour of chicken embryo fibroblast cells after transfection may be due to pulsation through electroporation in a dose dependent manner and transient /stable incorporation of the construct.

The 697 bp fragment displayed a considerable amount of fluorescence under fluorescent microscope (Fig. 2) along with desired band of 250 bp of GFP on gel. The mRNA expression of cell lysate was assessed by real time PCR using cDNA as a template. The magnitude of 19.92 ΔC_t value was observed in the transfected cells and there was no expression of GFP gene in control cells. Finally, SDS-PAGE (Fig. 3) and western blot (Fig. 4) were performed using the isolated crude protein of respected cell lysate with consequential appearance of protein band pointing the occurrence of tagged GFP protein in transfected cells.

The expression results showed the functional efficiency of the promoter which is located upstream of the 5' region of the gene. Transcription of any gene will be controlled by the promoter sequence. Transcription factors binds to this promoter region and recruits the RNA polymerase for the production of the mRNA from the coding region of the gene. To get the knowledge about how promoter region regulating transcription process, identification and characterization of the promoter region is necessary. Promoters bear the regulatory mechanism of genes; therefore, their correct annotation and characterization is vital to understand gene function (Suzuki *et al*., 2001). So, an attempt was made in the present study, to identify a partial promoter region



(a) Before transfection

(b) After transfection of *ACACB* gene in CEF

(c) After 24hrs transfection of *ACACB* gene

(d) After 36hrs transfection of *ACACB* gene

Fig. 1: Chicken embryo fibroblast (CEF) under inverted microscope

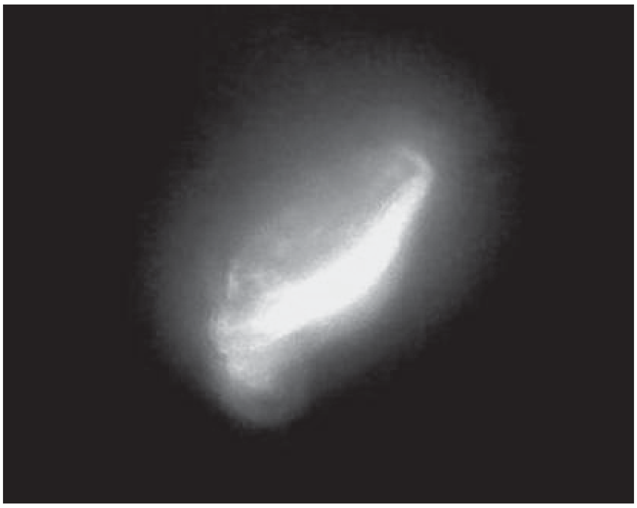


Fig. 2: Fluorescent microscopy of CEF transfected recombinant promoter less vector (pAcGFP1-P1)

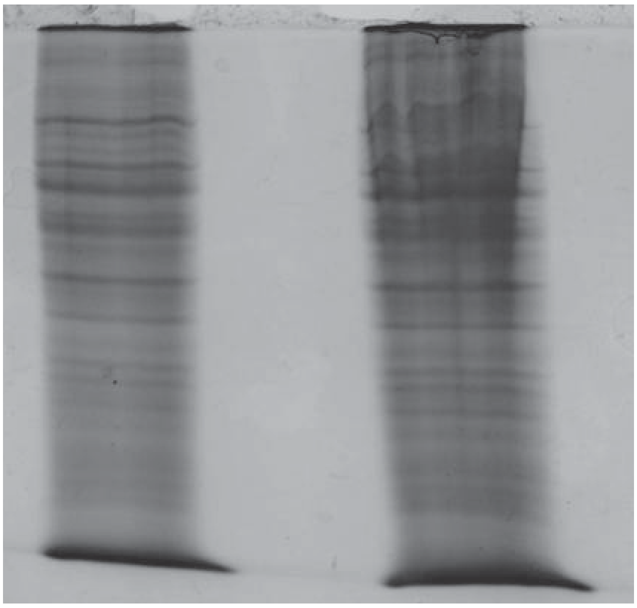


Fig. 3: SDS PAGE of GFP protein in CEF of ACACB gene

Fig. 4: Western blot of GFP protein in CEF of ACACB gene

that would be sufficient to carry out their activity together with the account of possible transcription factor binding sites identified and presented in the upstream 5' region of transcription start site of *ACACB* gene. The transcription factors are exerting differential roles in growth, differentiation and finally in transcription processes.

Gene expression under the control of 5' upstream fragment was determined here in terms of presence of fluorescence from the cells and protein bands in the western blot. These findings indicated that the smaller fragment of 697 bp may be considered as the minimal promoter as compared to 1kb fragment. These promoter fragments may be used to express desired genes under *in vitro* cell culture system for cellular experiments. There are very scanty reports on *ACACB* gene promoter characterization in chicken. Abu-Elheiga *et al*. (2003) investigated the role of ACAC2 in mutant mice fed high fat/high carbohydrate diet but ACC2$^{-/-}$ mutant mice weighed less than their WT cohorts due to the essential role of *ACACB* in controlling fatty acid oxidation (). It has also been reported that the *ACACB* gene involved in lipid metabolism and fatty acid oxidation in chicken (Ji *et al*., 2012).

CONCLUSION

These findings indicate that the 697 bp 5' upstream fragment had potential to transcribe the gene under *in vitro* cell culture system. We also suggest to use this promoter to express any desired gene in muscle fibre of transgenic animals.

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Phenotypic characterization of indigenous chicken breeds under village production system for sustainable development in Ethiopia

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ABSTRACT

Dame, L., Tesema, B. and Dadecha, D. 2022. Phenotypic characterization of indigenous chicken breeds under village production system for sustainable development in Ethiopia. Indian Journal of Poultry Science, 57(02): 119-132.

This study was conducted to characterize Indigenous Chicken populations under village production system. A total of 180 households were randomly selected from two purposively selected woredas. Two mature chickens per household with a total of 360 (240 females and 120 males) were used for qualitative traits observation and quantitative traits measurement. Quantitative data was collected early morning to avoid the effect of feeding and watering. Further, qualitative traits were assessed through observation birds. The collected data were analyzed using SPSS (version 20) and General Linear Model procedure (PROC GLM) SAS. In general, nine qualitative and twelve quantitative traits were considered for this study. The results revealed that the indexed value of chicken selected based on the selection criteria shows, breeding hens were selected based on their egg production potentials, large body weight, diseases resistance ability, plumage color and growth rate. Moreover, cock selection was based on body weight, plumage color, disease resistance, comb type and growth rate. The average age at first egg-laying for indigenous chicken was 6.12±0.03 months. The study revealed that about 96.7% of the observed chickens were feathered while the rest 3.3% were naked necks. The dominant chickens' plumage colors identified in the study area were; brownish (34.7%), red (18.9%) and wheaten (15.3%). Shank color was predominant yellow (30%) and white (30.3%) followed by orange (21.9%), black (8.1%), red-brown (5.8%) and green (3.9%). The quantitative traits result showed there was a highly significant difference ($P<0.05$) between the sexes. The study revealed that the performance of economically important traits variation existing between sexes of chickens and study areas needs improvement through the management and genetic improvement programs.

Keywords: Indigenous chicken, phenotypic trait, production system, village.

INTRODUCTION

Globally, the indigenous chicken production system is recognized as a strategy means for capital build-up, poverty malnutrition and hunger reduction among the resources of poor households (Besbes, 2009). In Africa, village poultry production systems are mainly based on scavenging. The native chickens kept in nearly every home in rural areas are characterized by an integral component of the farming systems requiring low-inputs with outputs accessible at both within and between household levels (Kitalyi, 1997). In Ethiopia, the vast production system used for chickens is characterized by inadequate management, disease- and predator-related flock mortality, and low productivity (Melkamu, 2013).

According to CSA (2020/2021) report the total chicken population in Ethiopia was estimated to be 57 million, from which 78.85% were indigenous, 12.02% cross, and 9.11% were exotic breeds. It kept by all strata of society from the landless rural poor to the rich (Wilson, 2010). In Ethiopia, most of the rural communities keep indigenous chicken populations under a scavenging management system (Abiyu et *al*., 2019, Azage et *al*., 2010) due to the presence of various agro-climatic conditions which enables the rural societies to keep a wide variety of indigenous chicken population. In general, the village chicken production system is defined by poor performance in terms of egg output, small egg size, sluggish development rate, late maturity, an innate tendency to broodiness, and high chick mortality (Aberra, 2000; Nigussie *et al*., 2003; Solomon, 2003). However, local chickens are known for their ability to resist disease, thermo-tolerance, good egg and meat flavor, hard eggshells, high fertility and hatchability (Aberra, 2000). They are often part of 'balanced' farming systems, which have important roles in the rural household as a source of high-quality animal protein and emergency cash income, and play a significant role in the socio-cultural life of the rural community (Nigussie *et al.*, 2011).

According to the report of Aklilu (2007), village poultry is the first step on the ladder for poor households to climb out of poverty and is a source of self-reliance for women, since poultry and egg sales decided by women and provide women with an immediate income to meet household expenses. Despite the importance of indigenous breeds in rendering income, posse's cultural value and source of nutrition for household, they are under threat due to various factors such as changing production systems and indiscriminate crossbreeding (Besbes, 2009).

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The indigenous ecotypes of chickens are the most common breeds raised in Ethiopia, and they differ greatly in terms of body conformation, plumage color, comb type, and productivity (Halima *et al*., 2007; Abiyu *et al.*, 2019). Characterization serves as the foundation for all other animal development interventions and gives information for formulating effective breeding plans, making it the first step in long-term genetic improvement. Breed characterization encompasses all actions pertaining to the description of the origin, development, structure, population, quantitative, morphological, and molecular methods that can characterize qualitative traits of the breeds in defined management and climatic settings (FAO, 2012). Phenotypic characterization is a comparatively easy and cheap tool of breed characterization (FAO, 2012). Research on phenotypic and genetic as well as morphological characterization of indigenous chicken ecotypes was done in some selected areas of Ethiopia (Aberra and Tegene, 2011; Nigussie, 2011) identified sufficient genetic variations between groupings of locally raised chickens in terms of morphology, conformation, and body weights. However, there is a research gap on phenotypic characterization of indigenous chicken, production system, performance status and morphological characteristics in Girja and AdolaRede districts in the Guji zone. Further, the qualitative and quantitative traits of indigenous chickens across the study area have not yet been addressed. Therefore, the present study was designed to characterize Indigenous chicken population based on phenotypic under village production system in the selected study districts.

MATERIALS AND METHODS

Description of the study areas

This study was conducted in Girja and AdolaRede districts of Guji Zone, which is located in the southwestern part of Ethiopia 586 km far away from Addis Ababa. Guji zone is bounded by Southern Nation, Nationality and Peoples Regional State in north, Somalia Regional States in south, Borena zone in west and Bale zone in east. Geologically, the zone is located between 38–40° East longitude and latitude 4-5° on the North and the altitude ranges from 500 m up to 3500 m above sea level. Guji Zone has 18 districts from which this study involved two districts; namely Girja and AdolaRede.

Sampling techniques and sample size determination

The study was conducted at AdolaRede and Girja districts which were purposively selected based on chicken production potential and agro-ecology. Two kebeles (HaranfamaKeldina and GanaleKorca from Girja and AdamaDiba and Boke Bulala from AdoleRede) from each district were purposively selected based on village

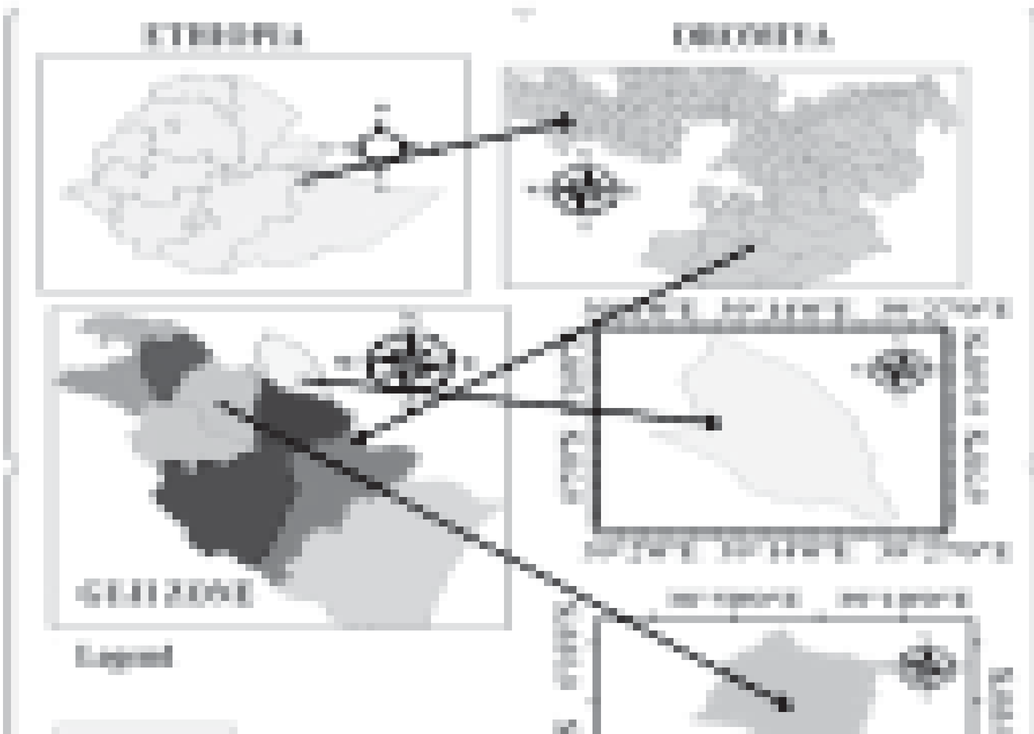

Fig. 1: Map of the study areas

poultry population density, chicken production potential, size of the area and accessibility of infrastructure. The households that own indigenous chicken were listed and sampled for interview. A total of 180 households (84 and 96 households) from Girja and AdolaRede districts, respectively were selected using the formula described by Cochran (1963). The households 40, 44; 50 and 46 households from Haranfama Keldina, Ganale Korca, Adama Diba and Boke Bulala kebeles, respectively were sampled for socio-economic data collection. The number of sampled households in the study area (districts and kebeles) were determined using

$$n_0 = \frac{Z^2pq}{e^2}$$

Where; No = required sample size

Z^2= is the abscissa of the normal curve

e^2= is the margin of error (±0.05%, margin of error for confidence level of 95%)

p= 0.136 is the degree of variability in the attributes being measured refers to the distribution of attributes in the population.

q= 1-p

$$n_0 = \frac{Z^2pq}{e^2}$$

= 3.8416 x (0.136) (0.864) ÷ 0.0025

= 180.56 ~180

According to Cochran (1963:75), the sample size (n) can be determined by using the following formula.

$$n = \frac{n_0}{1 + \frac{(n_0 - 1)}{N}}$$

Two chickens per household with a total of 360 (168 in Girja of which 88 from Ganale Korcha and 80 from Haranfama keldina; and 192 in Adola Rede of which 92 from Boke Bulala and 100 from AdamaDiba) were sampled for phenotypic data collection. The chickens sample consists of adults of both sexes: 360 chicken (240 female) and (120 male) with the ratio of 2:1 as per the recommendation of FAO (2011). Greater than 6-month-old chicken was selected from each district using a simple random sampling technique.

Data sources and collection method

Both primary and secondary data were employed for this study. The primary data were collected through semi-structured questionnaires, field observation and group discussion. Semi-structured questionnaires were used to collect data from the household. Besides, information on household characteristics, chicken production system, and productivity performance were collected using semi-structured questionnaires. While secondary data like livestock population, altitude, latitude, climate data (temperature, rainfall), and total household were collected from Livestock Resources and Development Office. Furthermore, Focus Group Discussions (FDG) with an average of 8 to 10 members were conducted at each selected kebele. The discussions were focused on the history of the flock, production system, purpose of keeping chicken, selection criteria of breeding hen and cock, farmers trait preference and challenge of village chicken production system.

Observational data and body measurements

A total of 360 six-month or older chickens from both districts were used to collect qualitative data through direct observation on sexually matured chicken and information from owners. The collected qualitative data were feather distribution, plumage color, comb type, shank feather, beak color, shank color, earlobe color, eye color and head shape based on standard format breed descriptor list (FAO, 2012). Further, linear body measurements were taken from the same chickens. However, the age of chickens selected for linear body measurement was determined by households. Data was taken in the early morning to avoid the effect of feeding and watering. The quantitative traits such as body weight, chest circumference, shank length, neck length, body length, wingspan, wattles width, wattles length, ear lobes length, beak length and comb length and width were measured by using the nearest unit centimeter, while the body weight was measured in kilogram (Kg) using a spring balance.

Effective population size and coefficient of inbreeding

The effective population size and inbreeding coefficients were calculated based on individual household flocks' size using:

Ne = (4 Nm × Nf) / (Nm + Nf)

Where: Ne = effective population size,

Nm = number of breeding males and

Nf = number of breeding females.

The rate of inbreeding coefficient (ΔF) was calculated from Ne as:

ΔF = 1/2Ne (Falconer and Mackay, 1996).

Data management and statistical analyses

The qualitative data were analyzed using descriptive statistics such as average, standard error of the mean and frequencies were applied for both quantitative and qualitative data. Achi-square (÷2) test was used to compare categorical variables for significance across the study area. General Linear Model procedure (PROC GLM) SAS were used for the analysis of liner body measurements with the fixed effects of districts, sex and their interactions. Means were compared using Duncan's multiple range test and values were considered at significance level P< 0.05. Correlation analyses were conducted to identify the relationship between quantitative morphological traits.

Ranking of farmers' trait preferences

Ranking analyses were used for computing data on farmers' traits preferences, selection criteria of breeding hen and cock, the purpose of keeping chicken and egg production. Indexes were used to calculate data collected from ranking using weighed averages using (Kosgey, 2004) formula.

RESULTS AND DISCUSSION

Demographic characteristics of the households

The characteristics households interviewed for village chicken were presented in (Table 1). The survey results indicated that 67.8% of the households were females and 32.2% of households were males. This result agreed with the reports of Meseret (2010) that 30% and 70% village chicken owners were male and female-headed households, respectively in Gomma woreda of Jimma zone. Further, Fikadu (2018) reported that 33.5 and 66.5% were male and female, respectively in Seka Chekorsa and Kersa districts of Jimma Zone. The higher percentage of female respondents in the study districts might been participation in outdoor activities like livestock herding and other agricultural activities.

The mean age of the respondents was 36.81±0.43 years. This indicated the presence of active labor forces, which has a positive impact in reducing the labor constraints that may face in chicken production. The overall average family size in the study area was 7.62±0.23 persons per household. The average family size per household was higher in Girja (7.12±0.23) than Adola Rede (8.19±0.38) district. The proportion of family size had a significant difference across the study area. The household with large family sizes well participated in chicken production. This showed that a large family size enables households to manage their chickens properly. The average family size in the current study was higher than the national average of 4.6 persons (CSA, 2011) and greater than the family size of 6.95 persons per household which was reported by Mekonnen (2007). Further, greater than the finding of Worku *et al.* (2012) reported 6.0±2.00 persons per household in the West Amhara region. This variation might be due to the variation of awareness on family planning.

Chicken flock structure and composition

Flock Size

The overall mean chicken flock size per household was presented in (Table 2). The overall mean flock size of chicken per household was 5.75±0.2, 3.13±0.1, 1.77±0.1, 3.28±0.1, and 2.44±0.1 chicks, pullets, cocks, hens and cockerels, respectively in the study areas. The proportions of flock size per household were 6.46±0.3 and 4.8±0.3chicks; 3.18±0.2 and 3.06±0.1 pullets; 1.91±1.0 and 1.62±0.8 cocks; 3.37±0.2 and 3.17±0.1 hens; and 2.48±0.1 and 2.37±0.14 cockerels in AdolaRede and Girja district, respectively. The current result was higher than 12-13 chickens/household reported from Bure district (Fisseha *et al*., 2010; Hunduma *et al*., 2010), and 9.22 chickens/household reported from South Ethiopia (Mekonen, 2007). However, the current result was lower than with the mean flock size per household of chick (6.86±5.5), chicken flock size per household of pullet (4.81±5.0), hen (5.39±3.4), cocks (2.37±1.7), and cockerel (3.94±2.4) reported by Alemayehu (2017) in East Shoa Zone Lume District. This variation might be due to the differences in feeds availability, diseases, predators and climatic condition.

Sources of chickens

The majority (58.9%) of the respondents were obtained a flock of chickens for replacement from the market followed by hatching at home (31.1%) and a gift from relatives (10.0%). This might be the farmers prefer to buy chickens from the market to get better-performed breeds and get chicken at low price from marketplace. This result was agreed with Meareg (2016) and Ayana (2020) report 78.90 and 60% households were bought foundation stock from the market to obtain starter poultry flocks in central Tigrayand Awi zone, Amhara region, respectively.

Table 1: Household characteristics in study area

Parameter	**Districts**			
	AdolaRede(N=96)	**Girja(N=84)**	**Overall (N=180)**	**P Value**
Sex of the respondents (%)				
Female	68.8	66.7	67.8	
Male	31.2	33.3	32.2	
Occupation (%)				
Farmers	86.5	88.1	87.2	
Gov't employee	3.1	3.6	3.3	
Merchant	6.3	4.8	5.6	
Ot hers	4.2	3.6	3.9	
Average age (Mean±SD)	36.27±0.5	37.42±0.7	36.81 ±0.4	NS
Average family size (Mean±SD)	7.12±0.2	8.19±0.4	7.62 ±0.2	*
Male	3.48± 0.1	3.91±0.2	3.68±0.1	NS
Female	3.64±0.2	4.26±0.2	3.93±0.1	*
Average land holding (Mean±SD)	1.58±0.5	1.84±0.1	1.71 ±0.6	*

NS= non-significant, *****= significant,

Table 2: Chicken flock size and source of foundation stock

Variables	Districts			
	AdolaRede Mean±SE	Girja Mean±SE	Overall Mean±SE	P Value
Chickens' categories				
Chicks	6.46±0.3	4.8±0.3	5.75±0.2	***
Pullet	3.18±0.2	3.06±0.1	3.13±0.1	NS
Cocks	1.91±1.0	1.62±0.8	1.77±0.1	*
Hen	3.37±0.2	3.17±0.1	3.28±0.1	NS
Cockerel	2.48±0.1	2.37±0.1	2.44±0.1	NS
Total chickens per household	17.4 +0.4	15.02+0.3	16.37+ 0.1	
Source of foundation flock (%)				*
Purchased from markets	50.0	69.0	58.9	
Gift from relatives	12.5	7.1	10.0	
Hatched at home	37.5	23.8	31.1	

NS= non-significant, *= significant, ***= highly significant

Chicken management practices

The major feeds and feeding practices of chickens in the study areas were reported in (Table 3) were practiced the scavenging system of feeding with small supplementary feed while the remaining (9.4%) of the surveyed household were practiced pure scavenging. Feed supplementation is to increase egg production, meat yield, growth performance and improve the hatchability of broody hens. The finding of the current study was higher than the report of Nebiyu *et al.* (2013) and Zemelak *et al.* (2016) who reported that 81.8 and 78.7% of households provided supplementary feeds for their chickens in Halaba district and Ethiopia, respectively. However, the current result was lower than the report of Fisseha *et al*. (2010), Birhan (2018), Abiyu et *al.* (2019) and Demissu *et al.* (2019) who reported that 97.5, 99.4, 98.7, and 94.7% of the households provided supplementary feeds for their chickens in Bure district, western Amhara, Kaffa Zone and Western Ethiopia, respectively.

The major chicken supplementary feeds identified in the study areas were maize, wheat, kitchen leftover and a mixture of them. This result was agreed with the report of Zemelak *et al.* (2016) that 78.7% of the respondents provided supplementary feed for chicken was cereals like; maize, wheat and barley across agro-climatic zones in Ethiopia. Similarly, Worku *et al.* (2012) reported different types of grain used as supplementary feeds across varied districts and 50.4% of the household's used maize as the major source of feed supplementation, while 39.3 and 10.3% of them used wheat and barley, respectively in West Amhara Region.

The status of chicken feed was assessed in the study area reported that feed shortage happened during the rainy (summer) season whereas 6.7% of the respondent reported feed shortage during the dry season. Feed was a critical problem due to cereal crops were not produced throughout the year and used for human consumption. The result showed significantly different ($P<0.05$) across the study kebeles. This result was comparable with the report of Samson and Endalew (2010) that 95% of the respondents indicated that the critical time of supplementary feeding was from summer (June-August) while the remaining 5% indicated that winter (March-May) was the critical time of feed supplementation in the mid-rift valley of Oromia.

Moreover, 23.3% of respondents were provided feed for chickens using feed trough (plastic and /or metal made) and 67.2% without feed trough. The provision of feeds on the feeding trough was used for reducing the contamination and wastage of feed. This result agreed with the finding of Meareg (2016) that 93.4% of the household were not use feeding trough, and the remaining 6.6% of the households were used plastic made, wooden and stone made materials to feed their chicken in the Central zone of Tigray.

Water plays an important role in the digestion, metabolism of fowl and serves as a medium to administer some important vaccines. The entire respondents were provided water for their chickens in the study areas. A similar result was reported by Addisu *et al.* (2013) and Mearg (2016) that all of the respondents provide water for their chickens in the north Wollo and central zone of Tigray, respectively. Regarding watering frequencies, about 52.2%, 36.7% and 11.1% of the respondents were provided water for their chicken in adlibtum (free access), twice a day and once a day, respectively. This result was comparable with the finding of Mearg (2016) finding that the majority of the respondents (62.4%) were provided water for their chicken in adlibtum (free access). In addition, the finding of Addis and Malede (2014) showed that almost all of the respondents provide water adlibtum (free access) for their chickens.

Table 3: Chicken feed and water resources and feeding frequencies in the study areas

Parameter	Districts			
	AdolaRede(n=96)	**Girja(n=84)**	**Overall (n= 180)**	**P Value**
Provision of supplementary feed (%)				NS
Yes	92.7	88.1	90.6	
No	7.3	11.9	9.4	
Type of supplementary feed				NS
Wheat	9.4	7.1	8.3	
Maize	50	64.28	56.7	
Mixture	18.8	9.52	14.4	
Kitchen leftover	14.6	7.1	11.1	
Form of feed provision n (%)				*
In the feeding trough	31.2	14.3	23.3	
On the bare ground	61.5	73.8	67.2	
Season of feed shortage happened				***
Rainy season	89.6	97.6	93.3	
Dry season	10.4	2.4	6.7	
Classes of chickens supplementary feed given				NS
Chicks	52.1	46.4	49.4	
Pullet	7.3	7.1	7.2	
Hen	30.2	36.9	33.3	
Cocks	6.2	4.8	5.6	
Cockerel	4.2	4.8	4.4	
Frequency of water provisions (%)				
Free access	47.9	57.1	52.2	
Only morning	10.4	11.9	11.1	
Morning and evening	41.7	31.0	36.7	

N= Number of respondents; NS= non-significant, *= significant, ***= highly significant

Housing systems

Housing is one of the most important factors which influence the productivity of chickens and protect chickens from predators, theft, disease transmission, bad weather conditions and provide an egg-laying place. The result showed that majority of the respondents housed their chickens. The chickens housing system practiced in the study areas were; separate shelter, perch in the main house, perch in another animal house, perch in the kitchen and perch under veranda from locally available materials. The current result was in line with the result of Agide (2015) reported 31.85% of the respondents in the North Shewa used separate poultry houses. However,

Table 4: Housing condition and reason for non-housing of chicken in the study area

Parameter	Districts		
	Adola Rede N=96	**GirjaN=84**	**OverallN (180)**
Availability of chicken house (%)			
Yes	90.6	84.5	87.8
No	9.4	15.5	12.2
Types of houses (%)			
Separate Shelter	38.5	35.7	37.2
Perch in the house	15.6	19	17.2
Perch in another animal	6.2	2.4	4.4
Perch in the kitchen	24	23.8	23.9
Perch under veranda	6.2	3.6	5
Reasons for not constructing chicken houses separately (%)			
Lack of knowledge (awareness)	33.3	30.8	31.8
Less attention given for chickens	22.2	15.4	18.2
Risk predator	44.4	23.1	31.8
Risk of theft	-	30.8	18.2

N= Number of respondents; NS= non-significant, *= significant; Poultry diseases in the study area lower than the report of Addis and Malede (2014), Mearg (2016) and Alemayehu (2017) reported that 90%, 65.7% and 61.1% of the respondents in North Gondar, the central zone of Tigray and Lume district were separate poultry houses, respectively. The types of chicken houses were presented in (Table 4).

Poultry disease in the study areas

Poultry disease is one of the challenges in poultry development in the study areas. Diseases cause severe economic loss in poultry production. In the study area, about 88.9% of the respondents reported that there was a disease outbreak (Table 5). The major poultry diseases reported by households were Newcastle disease (NCD), ectoparasite, fowl typhoid and cholera and *Gumboro* disease in their order of importance. The poor housing and a low level of management contributed to a high incidence of poultry diseases in the areas. Further, insufficient veterinary service and the absence of a scheduled vaccination program seemed the major bottlenecks that need to be solved in the study areas. Newcastle disease was the major that causes chicken loss among identified chicken diseases which was reported by Matiwos *et al*. (2015) that Newcastle (Wararshe/Fengel) and fowl cholera (cholera) were the major diseases affecting chickens in Amaro district, SNNPRS of Ethiopia. Similarly, Bikila (2013) also reported that the major diseases in order of their importance were 85% Newcastle disease (NCD) and 15% other diseases (Coccidiosis, Fowl pox and Fowl typhoid) in the Chelliya district. External and internal parasites were another chickens' health problem in the study areas. The external parasite identified was mites and lice which associated with seasonal variations, higher rates of infestation being occurred during the rainy seasons. Internal parasites like a round worm and tapeworms existed in the study area.

Mechanisms of diseased chicken treatments and places of treatment were veterinarian, and traditional remedies, in the study area. The majority of the respondents were treated sick chickens at home by traditional treatment methods using mixtures of chill, garlic and ginger, lemon juices, local beverages (araqee/ Katikala) in both study districts. According to respondents using traditional treatment was due to low prices and unavailability of veterinary services at their locality. The result was in line with the report of Samson and Endalew (2010) that farmers in the Mid-Rift valley of Oromia, use garlic, different green leaves like 'Bala Ganate', lemon, local alcohol, paper powder, butter as drenching, nasal application and smoking to treat sick chickens. Moreover, according to Fisseha et al. (2010), the most popular form of folk medicine for NCD was giving sick birds a mixture of local alcohol (called "Arekie"), lemon, and onion.

Objectives of chicken production

The households practiced multiple breeding objectives of chicken production. The main objectives of keeping chicken in the study area were for cash income from the sale of live chicken, for meat consumption, for

Table 5: Major chicken diseases and their control measures in the study areas

Variable	Districts			
	Adola Rede N=96	**Girja N=84**	**Overall**	**P Value**
Availability of disease (%)				NS
Yes	90.6	86.9	88.9	
No	9.4	13.1	11.1	
Types of diseases mostly available (%)				NS
Newcastle Disease	57.3	56	56.7	
Fowl Typhoid and Colera	11.5	13.1	12.2	
Gumboro	8.3	2.4	5.6	
Ectoparasite	13.5	15.5	14.4	
Chicken treatment method (%)				NS
Medication	27.5	19.2	23.8	
Use traditional remedies	45.9	40.2	46.9	
Place of sick chicken treatment (%)				*
Animal health post	28.1	11.9	20.6	
Veterinary clinic	2.1	2.4	2.2	
At home by traditional medicine	60.4	72.6	66.1	
Types of traditional medicine used for treatment (%)				NS
Lemon juice	11.5	14.3	12.8	
Arake	12.5	14.3	13.3	
Mixtures of chill, garlic and ginger	36.5	44.0	40.0	

N= Number of respondents; NS= non-significant, *= significant,

Table 6: Ranking of keeping chicken and egg production in AdolaRede and Girja districts

Variable	Districts										Overall
	AdolaRede					Girja					
	R1	R2	R3	R4	I	R1	R2	R3	R4	I	
Purpose of keeping chicken											
Generate cash income from sale live chicken	64.6	16.7	10.4	8.3	0.3	61.9	16.7	21.4	-	0.3	0.3
For egg production	27.1	58.3	12.5	2.1	0.3	21.4	50.0	21.4	7.1	0.3	0.3
Meat production	4.2	18.8	58.3	18.8	0.2	9.5	23.8	45.2	21.4	0.2	0.2
Cultural/ceremonies	4.17	6.3	18.8	70.8	0.1	7.1	9.5	11.9	71.4	0.2	0.2
Purpose of egg production											
For cash from egg sale	66.7	18.8	14.6		0.4	59.5	31.0	9.5		0.4	0.4
For hatching	8.3	45.8	45.8		0.3	19.1	19.1	61.9		0.3	0.3
For home consumption	25.0	35.4	39.6		0.3	21.4	50.0	28.6		0.3	0.3

Index (I) = the sum of (4 times 1^{st} order + 3 times 2^{nd}order + 2 times 3^{rd}order + 1times 4^{th}order) for individual variables divided by the sum of (4 times 1^{st}order + 3 times 2^{nd}order +2 times 3^{rd}order + 1 times 4^{th}order) for all variables; R1 = first rank; R2 = second rank; R3 = third rank, R4 = fourth rank

egg production and cultural/ceremonies with an overall index value of 0.3, 0.2, 0.3 and 0.2, respectively (Table 6). This result was in agreement with the report of Fisseha *et al.* (2010) that the sale of live chicken for cash income was the first important function of rearing chicken in Fogera (77.8%) and Dale (43.7%) districts. Similarly, Fikadu (2018) reported that the source of income ranked first (0.64, 0.60 and 0.60) in highland, midland and lowland, respectively in Seka chekorsa and kersa districts of Jimma zone, southwest Ethiopia.

On the other hand, the main purposes of egg production were to generate cash income from sale, home consumption and hatching (for replacement) with an overall index value of 0.42, 0.31 and 0.27, respectively. This result was similar to the report of Fikadu (2018) that the source of income was ranking first in highland (0.64), in midland (0.62) and lowland (0.65), respectively. The main production goal for raising chickens, according to Tadelle (2003) and Halima (2007), was to generate cash, followed by consumption.

Effective population size and inbreeding in village chickens

The effective population size (Ne) is influenced by the actual number of breeding males and females in the flock at a given time. The change was due to variation in the flock size, and type of rearing practice. The rate of inbreeding coefficient per generation changes with any change in the effective population size. Effective population size is a measure of genetic variability within a population. The large values of Ne indicating more variability and small values indicating less genetic variability (Cervantes *et al.*, 2008). The effective population size (Ne) and the rate of inbreeding (ΔF) mean calculated for the indigenous chicken flock of the study areas were 4.57 and 0.12, respectively. The rate of inbreeding in the free-range chicken population of AdolaRede was almost similar to Girja district.

This result agrees with the findings of Feyera (2016) and Getachew (2014) that 4.41 and 0.12 in Western Oromia, 4.13 and 0.122 Southern Tigray, respectively. The level of inbreeding in the current finding was higher than the maximum acceptable level of 0.06 reported by Armstrong, (2006). The utilization of breeding cock hatched within the flock and lack of awareness about inbreeding may lead to the accumulation of problems associated with inbreeding and decrease genetic diversity. In addition, the variations of effective population size might be due to the ratio of males to females in the flocks, longevity of the chickens (productivity) and the number of flocks contributing genetic material into the next generation and variation in flock size.

Table 7: Effective population size and level of inbreeding in village chicken flock in the study areas

Variables	Districts		
	AdolaRede	Girja	Overall
Nf	3.37	3.17	3.27
Nm	1.91	1.62	1.76
Ne	4.87	4.28	4.57
“F	0.11	0.12	0.115

Effective population size=Ne; Number of breeding female=Nf; Number of breeding male=Nm; Rate of inbreeding=ΔF

Selection criteria used for breeding hen and cock

The farmers' decisions on the selection of breeding stock were considered both morphological traits for production. The criteria used for the selection of breeding female were egg production, body weight, disease resistance, plumage color, growth rate, broodiness, hatchability and mothering ability, respectively. The egg number was the major selection criteria of breeding female ranked first in AdolaRede and Girja. This result was similar to the report of Birhan (2018) that the production of the high number of eggs was the first preferred trait in western Amhara, Ethiopia. Similarly, Nigussie *et al*., (2010) reported that egg production was the most important selection criterion in different parts of Ethiopia.

The main criteria for the selection of breeding cock in the study area were body weight, plumage color, disease resistance, comb type and growth rate, respectively. The body weight was the major selection criteria of breeding cock ranked first in study areas. The preference of body weight indicated that households were interested in chicken that can grow faster and have large body sizes for both home consumption and cash income. Therefore, economically important traits such as body weight for male and egg production potential of female chicken were important for chicken breeding improvement in the study area.

Trait preference of farmers for village chicken production

The main traits that respondents preferred indigenous chicken in the study areas were; egg production potential, body weight, disease resistance, hatchability, mothering ability, plumage color and comb type, with an overall index value of 0.24, 0.17, 0.17, 0.11, 0.07, 0.15 and 0.11, respectively. The current result was agreed with the report of Fikadu (2018) that egg production, body weight and disease resistance (adaptability traits) were preferred traits that ranked first, second and third to be improved in seka chekorsa and kersa districts of jimma zone, southwest Ethiopia. Similarly, Addisu *et al.* (2013) and Shishay *et al*. (2016) reported that the mean egg laid/clutch (egg production/ hen) (1st), body weight (meat yield) (2nd) and adaptations (disease resistance) (3rd) were the major preferred traits to be improved through breeding in North Wollo and Tigray region, respectively.

Color and comb were other chicken traits preferred by respondents. Double combed chickens were most preferred than single combed because households considered chicken with double comb as high meat and eggs yielders. Bodyweight is the most important trait to decide the marketing price of the chicken (high weight is more costly than low weight). This result was similar to the reports of Fisseha *et al.* (2010) and Alem and Yayneshet (2013) that most chicken owners considered plumage color and comb type as the main determinant factors in the selection of chicken for production, consumption and marketing purposes in Bure and Fogera districts and central Tigray, respectively.

Productive and reproductive performance of indigenous chicken

Age at first mating of male and female chicken

The result of the survey indicated that the overall mean age of sexual maturity of males and females of indigenous chicken was 5.26±0.03 and 5.56±0.04 months, respectively. The mean age of sexual maturity of male chickens in Girja was (5.43±0.05 month) and AdolaRede was (5.12±0.04 months) (Table 8). Ages at sexual maturity of male and female chicken were significantly different ($P<0.05$) across the study area. A significantly higher mean age of sexual maturity of indigenous female chicken was obtained from the Girja district (5.68±0.05 months). The current result was comparable with the finding of Gebreegziabher and Tsegay (2016) that the overall mean ages at sexual maturity for male and female chickens were 5.6 and 5.5 months, respectively in Wolaita Zone, Southern Ethiopia. However, it was lower than the report of Shishay et *al.* (2015) that the overall mean age at sexual maturity of female was 7.19±0.04 months while the overall mean age at sexual maturity of male was 5.71±0.03 months in Western Zone of Tigray. The difference might be due to environmental conditions, breed difference and management of chicken**.**

Age at first egg laying (months)

The overall mean age at first egg-laying for female indigenous chicken in the study area was 6.12±0.03 months (Table 8). Age at first egg-laying was significantly different ($P<0.05$) across the study area. The current result was similar to the report of Melkamu and Wube (2013) that 6 months of age at first egg-laying for local chicken breeds in Debsan Tikara kebele at Gondar Zuria Woreda, North Gondar. However, the current result was lower than the finding of Shishay *et al.* (2015) that the overall age at first egg-laying of pullet was 7.19±0.04 months in Western Tigray. This variation in age at first egg-laying might be due to lack of proper supplementary feeds, availability of scavenging feed resources and disease outbreak.

Annual egg production

The overall mean annual egg production per hen in the study area was 55.03±0.49 (Table 8). The annual egg production was significantly different ($P<0.05$) across the study areas. A significantly higher mean annual egg yield per hen was obtained from AdolaRede chicken (57.29±0.04) than Girja (52.67±0.76) chicken. The main reason for this difference might be weather conditions and management aspects of the households' chicken rearing in AdolaRede.

The current result was agreed with the report of Markos *et al.* (2015), Eskinder (2013) that annual egg yield per hen of indigenous chicken in the western zone of Tigray, Horro and Jars were 52.68 eggs, 54.48 eggs and 55.23 eggs, respectively. The current result was higher than the finding of Addisu *et al.* (2013) and Abiyu *et al.* (2019) that the mean annual egg yield per hen of indigenous chickens in North Wollo and Kaffa Zone, 49.51±0.38 eggs and 44.0±6.0 eggs, respectively. However, the current result is lower than the report of

Table 8: Productive and reproductive performance of indigenous chicken at study districts (Mean±SE)

Variable (Mean±SE)	Districts			
	AdolaRedeN=96	GirjaN=84	Overall	P Value
Number of eggs per hen per clutch	14.92±.22	14.35±0.22	14.64±0.16	NS
Clutch length in days	22.54±.07	22.34±0.09	22.45±0.06	NS
Number of clutches per hen per year	3.84±0.04	3.67±0.06	3.76±0.04	*
Annual egg production	57.29±0.59	52.67±0.76	55.03±0.49	***
Age at first mating of (cockerel) in month	5.12±0.037	5.43±0.05	5.26±0.03	***
Age at first mating of pullets in month	5.47 ±0.03	5.68± 0.05	5.56±0.04	*
Age at first egg laying	6.01±0.04	6.26±0.05	6.12±0.03	***
Incubated egg	12.61±0.28	11.66±0.21	12.17±0.18	*
No of eggs hatched	11.04±0.19	10.06±0.15	10.58±0.13	***
No of eggs wasted	1.57±0.073	1.6±0.105	1.59±0.063	NS
Hatchability percentage (%)	87.55	86.27	86.94	

Hatchability % = (No of chicken hatched / No of eggs incubated) *100; N= Number of respondents; NS= non-significant, *= significant, ***= highly significant

Mearg (2016), and Alemayehu (2017) that the overall annual egg production per hen in the central zone of Tigray in northern Ethiopia, and Lume district, East Shoa zone were 69.6±15.57 eggs, and 76.4±3.4 eggs, respectively. The management aspects of the household all chicken rearing and breed of chicken might be the reason contributing to the observed variations in annual egg production of indigenous chicken in the country.

Clutch number

The overall mean number of clutches per hen per year of local chicken was 3.67±0.06 with 3.84±0.04 in Girja and 3.76±0.04 in AdolaRedes. Relatively large clutch size coupled with a large number of cycles/hen/years contributed to the larger estimated number of eggs per year for AdolaRede than Girja districts. The current result was in line with the finding of Mekonnen (2007) and Addisu *et al*. (2013) who reported 3.8 and 3.62/year of clutch numbers for chickens in three districts of SNNPRs and North Wollo zone, respectively. However, it was lower as compared with the report of Markos *et al.* (2015) who reported clutch numbers of 4.57 in midland, 4.35 in highland and 4.34 in lowland with an overall mean of 4.42 cycles per hen per year in the western zone of Tigray. The differences observed between the study areas for the number of clutches per year might be due to genetic and environmental conditions.

Number of eggs per clutch

The overall number of eggs/hens per clutch in the study was 14.64±0.16. However, there were no significant differences observed between study areas for this variable. The current result was in line with the finding of Endale *et al*. (2017) that 14.3 eggs per hen per clutch in Sheka and Benchi-Maji zones of southwestern Ethiopia. However, the current result was higher than the result reported by Meseret (2010), Addisu *et al.* (2013), Wondu *et al.* (2013) and CSA (2016) that 12.92, 12.64, 11.53, and 12 eggs per clutch in Gomma woreda, North Wollo Zone, North Gondar and Ethiopia, respectively. The current result of the current finding was lower than 17.7 and 16.6 eggs per clutch reported by Tadelle (2003) and Bogale (2008) in five agro-ecological zones of Ethiopia and Fogera district, respectively. This difference in the number of eggs per hen per clutch might be due to lack of proper supplementary feeds, breeds of chickens handled by farmers, availability of the scavenge feed resources, disease outbreak and provision of clean water by the households.

Hatchability percentage

The overall mean hatchability percentage of eggs of indigenous chicken in the study area was 86.94% (Table 8). The hatchability percentage of eggs was significantly different (P<0.05) across study areas. A Significantly higher hatchability percentage was recorded at AdolaRede (87.55%) than Girja (86.27%). The current result was comparable with the finding of Alem (2014) and Birhan (2018) indicated that the overall mean hatchability percentage of eggs in Halaba district, Central Tigray and western Amhara was 85.8% and 85.75%, respectively. However, the current result was higher than the report of Gebreegziabher and Tsegay (2016) that the overall hatchability percentage in the Wolaita Zone and Southern Ethiopia was 79.1% and 77.9%, respectively. The reason for this variation might be due to high temperature, storage condition of the egg, quality of eggs, to some extent the hen factors, outbreak disease, predator attacks, availability of scavenging feed resources and feed supplements.

Phenotypic characteristics of local chickens

Feather distribution and plumage colour

In the current study, indigenous chickens were predominantly normal feathered while some of them were naked neck. The result was agreed with the finding of Getachew *et al.* (2015) that 95% of feather distribution of local chicken was normally feathered while 5% of them were naked neck in Bench Maji Zone of South Western Ethiopia. One of the main tropical indigenous chicken genes with favorable impacts on heat tolerance and adult fitness is the naked neck gene, according to (Aberra, 2000; Aberra and Tegene, 2011). The data showed that the naked neck chicken breeds were not frequently distributed in the study areas due to the farmer's preferences.

The plumage color of chickens was dominant with plumage color identified in the study areas was brownish followed by red and wheaten. The plumage color of chickens was varies based on their sex. Accordingly, in the AdolaRede district, the female chickens had predominantly brownish and wheaten plumage whereas the male chickens had red, libewar/wesera and Ambesma plumage color. Similarly, in the Girja district, the female chickens were predominantly characterized by brownish, wheaten and black plumage color while, the major plumage color identified for male chicken were red, Brownish and Ambesema. Getachew *et al.* (2015) reported that the majority of the chicken population in sheko district of Bench Maji Zone was characterized by red (68.33%), Gebsima (15%) and White (8.33%) plumage. The pigmentation of hair and feathers is mainly determined by the distribution of two kinds of melanin; eumelanin and pheomelanin that produce coloration ranging from brown to black and yellow to red, respectively. Each melanin is synthesized by melanocytes located in the proximal region of hair and feather follicles (Mingke *et al*., 2004). Feather colors and feather patterns are the results of genetic differences (feather color is sex-linked) and the presence of gonad tropic hormones (Bell. 2002).

Comb type

The surveyed chickens had double, pea and single comb types. Accordingly, the dominant comb types identified for male chicken was double comb types whereas the female chickens had pea comb-type in both districts (Table 9). A similar finding was reported by Bogale *et al*. (2019) that double comb-type was predominant followed by single and pea comb in the west Hararghe zone. In contrast, Habtamu *et al*. (2019 reported that (82%) of the indigenous chickens of Benishangul-Gumuz Regional state were mostly single combed. The difference might be due to the breed types existing in the study area. Combs are important structures for heat loss in birds (Kampen, 1974) and since the tropical climate is predominantly characterized by high ambient temperature, large combs would provide an efficient means of heat dissipation through the process of vasodilatation.

Quantitative traits

Bodyweight and other body measurements are useful parameters that are used to describe a breed or type jointly with the breed's morphological characteristics and the environment it inhabited. The body weight and other linear measurements of the sampled chickens were summarized in (Table 10). From the total 360 matured local chickens sampled in both sexes twelve measurable parameters such as body weight (BW), chest

Table 9: Qualitative traits of indigenous chicken in the study area

Variable	Districts					
	AdolaRede		Girja			
	Female (%)	Male (%)	Female (%)	Male (%)	Overall (%)	P Value
Feather distribution						*
Normal	100	96.9	96.4	89.3	96.7	
Naked Neck	-	3.1	3.6	10.7	3.3	
Plumage colour						***
Completely White	1.6		8.9	3.6	3.9	
Completely Black	6.2		14.3		6.7	
Completely Red	6.2	46.9	1.8	50.0	18.9	
Multicolor/Ambesma	7.8	12.5	7.1	10.7	8.9	
Red Brownish	46.1	9.4	39.3	28.6	34.7	
Wheaten	24.2		21.4		15.3	
Grayish/ Gebsima	7.8	6.2	1.8	7.1	5.6	
Libewar/Wesara	-	25.0	-	-	4.4	
Black with white tips/teterma	-	-	5.4	-	1.7	
Comb type						NS
Rose (Double Comb)	20.3	67.2	35.4	67.9	45.8	
Pea	46.9	6.2	42.5	7.1	32.2	
Single	18.8	26.6	21.2	25.0	21.9	

N= Sample Size; NS= non-significant, *= significant, ***= highly significant

Table 10: The Mean±SD for Quantitative traits of indigenous chicken in study district, sexes and interaction of the study area.

Variable	Districts				Sex			Sex*
	AdolaRede	Girja	Overall	P value	Female	Male	P value	districts
CC	26.4± 0.1	26.5 ±0.1	26.47± 0.1	NS	25.83±0.1	27.74±0.1	***	***
BDL	37.53±0.3	36.30±0.4	36.96±0.2	*	35.25±0.3	40.37±0.2	***	*
CW	1.23±0.1	1.68±0.1	1.45±0.1	NS	0.76±0.0	2.66±0.1	***	***
CL	3.12±0.3	3.62±0.1	3.37±0.2	*	2.04±0.2	5.51±0.2	***	***
NL	12.35±0.1	11.27±0.1	11.85±0.1	***	11.13±0.1	13.27±0.1	***	NS
BW	1.46±0.1	1.34±0.0	1.40±0.0	***	1.26±0.0	1.69±0.0	***	*

CC= chest circumference, BW= body weight, BDL= body length, CL=comb length, CW= comb width, NL= neck length. N= Sample Size; NS= non-significant, *= significant, ***= highly significant.

circumference (CC), body length (BL), comb length (CL), comb width (CW), and neck length (NL) were measured in cm.

The GLM least-squares means and interaction effects of sex with the district of body weight and other linear body measurements of chickens from AdolaRede and Girja districts were analyzed.

The average chest circumference was 26.47±0.13cm across the study districts and there was no significant difference (P>0.05) in chest circumference across the districts. Chest circumference comparison of chicken based on their sex revealed that male chicken (27.74±0.13cm) has wider chest circumference than female chicken (25.83±0.8cm). In the study areas, chest circumference was significantly different (P<0.05) between the male and female chickens.

The overall mean body length of chicken was 36.96±0.24 cm in the study areas. This finding implies the body length of indigenous chicken was a significantly different (P<0.05) across districts. Similarly, there was a significant difference in body length of male and female chickens was 40.37±0.22 and 35.25±0.29 cm recorded, respectively. Thus, the male chicken had a higher body length than the female chicken.

The overall mean neck length of indigenous chicken was 11.85±0.09cm in the study areas. The neck length of indigenous chicken was significantly different (P<0.05) between districts. The difference might be due to genetic and environmental factors. The average neck length of male and female chickens was 13.27±0.10 and 11.13±0.10 cm in the study areas, respectively. The neck length was significantly different (P<0.05) between the sexes of chicken.

The mean body weight of chicken was 1.46±0.02kg inAdolaRedeand1.34±0.02kg in Girja with an overall mean of 1.40±0.02 kg. The result revealed the average body weight of indigenous chicken was significantly different (P< 0.05) between districts of the study area. There was a variation in average body weight between the male and female chickens. Hence, the average body weight of males was 1.69±0.02kg and the female was 1.26±0.02 kg. This result agreed with the report of Addis *et al.*, (2013) that the average body weight of male and female chicken was 1.63±0.03 and 1.37±0.02 kg in North Gondar Zone, respectively. Similarly, Aberra (2018) reported that the average body weight of male and female chickens was 1.42±0.2 kg in Sheka Zone of South-Western Ethiopia. This variation of the live body weight might be due to individual differences in measuring accuracy, age of the bird, and season of the year in which the chicken is weighed (during seasons of relatively better feed supply most likely chickens have higher live body weight).

The lower body measurement values observed for females than for male chickens in this study are also consistent with the findings from other studies (Msoffe *et al.*, 2004; Alabi *et al.*, 2012; Semakula *et al.*, 2011; Olawunmi *et al.*, 2008), suggesting that sexual dimorphism in chickens is manifested to a large number of body attributes and in most breeds. This may be attributed to sex hormones which may promote larger muscle development in males than in females. The effect of sex in favor of males can be attributed to anatomical and physiological differences. Physiologically, the sex-related differences might be partly a function of the sex differential hormonal effect on growth (Semakula *et al.*, 2011).

Correlation between body weight and other linear body measurements.

The correlation coefficient (r) of the sampled chicken population in the study area between the live body weight and other linear body measurements were presented in (Table 11). The correlation coefficients between live body weight and other body measurement traits were found positive with the presence of highly significant (P<0.05) correlation between body weight with comb length, comb width, chest and circumference. The current result was similar to the report of Abebe *et al.*, (2017) that live body weight was positively correlated (r= 0.73, 0.61, P<0.01) with chest circumference. Body weight is a trait of economic importance to livestock farmers, and therefore, selection for bodyweight to improve the productivity of indigenous chicken (Peters *et al.*, 2007). In animal breeding, predicting body weight from linear measurements is a common practice (Ogah *et al.*, 2009).

Generally, linear body measurements information for a particular poultry species or breed is important for breed or species identification and economic valuation in its utilization. Furthermore, the relationships between body weight and linear body measurements are important for predicting body weight and can also be applied speedily in selection and breeding programs in indigenous chickens.

Table 11: Correlation coefficient between body weight & other linear body measurements

Traits	BW	WL	BL	CC	CW	CL	NL
BW							
WL	.263**						
BDL	.408**	.275**					
CC	.430**	.256**	.189**				
CW	.607**	.297**	.335**	.446**			
CL	.611**	.495**	.441**	.501**	.772**		
NL	.491**	.307**	.470**	.274**	.459**	.547**	

**. Correlation is significant at the 0.01 level (2-tailed), *. Correlation is significant at the 0.05 level (2-tailed)
CC= chest circumference, BW= body weight, BDL= body length, CL=comb length, CW= comb width, NL= neck length.

CONCLUSION

The study revealed that the proportion of chicken flock size per household was significantly different across the study areas. The management systems like housing, feeding, watering and healthcare that were provided for chickens were low attention. The pullet was started egg-laying at the age of 6.12±0.03 months andthe number of eggs produced per hen was low (57.81±.49 eggs/hen/ year). egg production performance, body weight, disease resistance ability, plumage color and growth rate were prioritized indexed value that respondents selected hens for breeding and the breeding cocks were selected based on their body weight, plumage color, disease resistance ability, comb types and growth rate. The dominant plumage color of chicken identified in the study areas was brownish color followed by red, wheaten, multicolor/ ambesma, black, grayish/Gebsima, libera/wesara, white and black with whitetips/teterma. The result of the current study revealed that there were significant variations in linear body measurement between male and female chickens and across the study areas. The observed and measured qualitative and quantitative traits variability provide ample opportunity to the breeder for genetic improvement by crossing indigenous chicken genetic resources through selection. The performance of economically important traits variation existing between sexes of chickens and study areas needs improvement through the management and genetic improvement programs.

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Effects of genotype and age on egg quality traits in crossbred chickens developed for backyard poultry farming

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ABSTRACT

Padhi, M.K., Chatterjee, R.N. and Rajkumar, U. 2022. Effects of genotype and age on egg quality traits in crossbred chickens developed for backyard poultry farming. Indian Journal of Poultry Science. 57(02): 133-138.

The experiment was conducted to study the effect of genotype and age on egg quality traits in four different crosses viz. PD1 X PD4 (PA), PD1 X PB2 (PB), PD1X PD3 (PR), PD1X IWI (PW). The egg qualities were measured in 20 eggs each of each cross at 28, 40, 52 and 64 weeks of age. The genotype significantly (P<0.05) affected egg weight and shell thickness at all the ages of measurements, whereas, significant (P<0.05) differences for shape index, albumen index and Haugh Unit were observed for 28, 52 and 64 weeks of age only. Colour index and yolk index were significant (P<0.05) between crosses at 52 and 64 weeks of age. Albumen, yolk and shell % differed significantly (P<0.05) between crosses at 28, 40, 52; 28, 40 and 28 weeks of age, respectively. Age effect was significant (P<0.05) for all the egg quality traits in all the crosses except for yolk colour where it was significant (P<0.05) for PW birds only. Egg weight increased as the age advanced and most of the egg quality parameters decreased at older ages. Yolk % and shell % increased at older ages but albumen % decreased. Shell thickness increased at older age of measurement. PA recorded better shell thickness which may be a useful trait to lower breakage during handling of eggs compared to other crosses. The study concluded that genotype and age significantly (P<0.05) affected different egg quality traits measured at different ages in all the four crosses.

Keywords: Age effects, Backyard poultry, Crosses, egg weight, Egg qualities, Genotype

INTRODUCTION

In rural, tribal and remote locality of our country where industrial poultry is difficult to practice, the farmers are practicing backyard poultry farming. Both heavy and light chicken breeds along with local indigenous birds are being used for backyard poultry farming. They have low egg production, slower growth rates and have broodiness with good mothering ability. Due to their better adoptability to the harsh environment the poor farmers preferred the local birds. However, there is always demand by the farmers for a bird having colour plumage like indigenous birds with better growth and production and requires very minimum or nil change in husbandry and housing practice that are being followed for the indigenous birds. Different crossbreds, synthetic hybrids and indigenous birds are being developed for backyard poultry farming and also evaluated for different traits (Padhi *et al.*, 2003; Khan, 2008; Padhi *et al.*, 2012; Padhi *et al.*, 2014a; Padhi *et al.*, 2015; Rajkumar *et al.*, 2021). However, there is still needs to develop and evaluate different crosses for backyard poultry farming. Quality of eggs is an important attribute for any breeds or variety to be developed. Egg quality of different crosses developed for backyard poultry faming are reported (Gupta *et al.*, 2007; Niranjan *et al.*, 2008; Sola-Ojo and Ayorinde, 2011; Alewi *et al.*, 2012; Padhi *et al.*, 2014b). Many factors influence the egg quality i.e., breed/strain/ variety, temperature, relative humidity, rearing practice and season (Sauter *et al.*, 1954; Washburn, 1990). Effect of genotype and age on egg quality in chicken was reported (Suk and Park, 2001; Tona *et al.*, 2004; Niranjan *et al.*, 2008; Padhi *et al.*, 2013, Padhi *et al.*, 2014b). Age effect on egg quality in other avian species like Duck also reported (Padhi *et al.*, 2021) It is always important to measures the egg quality at different ages whenever any new crosses are being developed for the backyard poultry faming. So the objective of the present study was to determine the effect of genetic groups and age on different egg quality parameters measured in four crossbreds developed for backyard poultry farming.

MATERIALS AND METHODS

Eggs for the present study was collected from four different crosses viz, PD1 X PD4 (PA), PD1 X PB2 (PB), PD1X PD3 (PR), PD1 X IWI (PW). The male parent used in all the four crossbreds is being developed from a low performing Cornish population (Ayyagari, 2003) and used as male line for backyard poultry. PB2, PD3 and IWI were all female line and developed from synthetic colored meat type stock, Dhalem Red and White Leghorn, respectively. PD4 was derived from Aseel a native breed of India. Eggs quality traits were measured at 28, 40, 52 and 64 weeks of age and from each cross 20 eggs were collected at particular age. Eggs for the present study were collected randomly late afternoon and were kept at room temperature overnight for evaluation

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in the next day. The eggs were numbered first and then weighed on an electronic balance to determine their weights in g. Thereafter, egg length (mm) and width (mm) were measured by digital vernier calipers for determining shape index. Then the eggs were broken on a clean flat glass surface. Measurements like yolk height (mm), albumen height (mm) were recorded using spherometer. Yolk width (mm) and albumen width (mm) were recorded using a digital Verniers Calipers. The yolk colour was measured with a Roche yolk colour fan scale (Roche scale). Shell thickness was randomly measured from the broad end, narrow end and the middle of the shell using a micrometer, and the average of the three measurements was taken as shell thickness in millimeter (mm). Weight of yolk was recorded and the shell weight was recorded after drying the egg for 48 hours. Albumen weight was determined by subtracting the yolk and shell weight from the gross egg weight. On the basis of the above measurements different egg quality traits were obtained using the following formulae. Shape index = egg width/egg length X 100; albumen index= albumen height/albumen width, yolk index= yolk height/yolk width; Haugh unit (HU) = 100log (H+7.57-1.7 $W^{0.37}$), where H= albumen height in mm, W=weight of egg in g, according to Haugh (1937); yolk % = yolk weight/egg weight X 100; albumen % = albumen weight/egg weight x 100 and shell % = shell weight/egg weight x 100. The data were analysed as per Snedecor and Cochran (1989) to see the significance between different genetic groups and also effect of age on different egg quality traits in each genetic group.

RESULTS AND DISCUSSION

The findings were presented according to genotype wise at different age of measurement in Table 1 for egg weight, shape index, yolk colour, yolk index, albumen index, Haugh unit and shell thickness and in Table 2 for yolk, albumen and shell %.

Egg weight

Egg weights differ significantly(P<0.05) between different crosses at each age of the measurements. PB recorded higher egg weight at all ages of measurements amongst the crosses except at 52 weeks (Table 1). It may be due to use of meat type parents for this cross. In PA 28 weeks egg weight was lowest this being due to use of indigenous birds as one parents. The egg weight of PA, PR and PW are comparable at 40 and 52 weeks of age. At 52 weeks PW recorded significantly (P<0.05) highest egg weight than other crosses. Age effect was significant (P<0.05) in each cross and as the age advances the egg weight increases. However, in PR and PW crosses the egg weight significantly (P<0.05) increases up to 52 weeks of age but it decreases at 64 weeks of age, this may be due to higher egg production in this two crosses during that period thus reducing the egg weight towards later part of the measurements. Significant (P<0.05) effect of genotype on egg quality were reported by Padhi *et al.* (1998), Gupta *et al.* (2007), Islam and Dutta (2010) and Alewi *et al.*(2012). Effect of age on egg weight in different crosses of chicken and pure line was reported (Leadur *et al.*, 2002; Niranjan *et al.*, 2008; Rajkumar *et al.*, 2009; Zita *et al.*, 2009). The egg weight obtained at 40 weeks of age was comparable to the weight obtained in crosses developed for backyard poultry farming as reported by Niranjan *et al.* (2008). However, it is to be mentioned here that for the backyard poultry farming egg number and shell colour are more important than the egg weight.

Shape index

Shape index differ significantly (P<0.05) between the crosses at all the ages of measurements except at 40 weeks of age (Table 1). Shape index values were high for PA cross for most of the time and lowest value were observed in PW irrespective the age of measurement. Shape index values ranges from 73.09 to 78.30 at the four ages of measurement in the four crosses. Significant (P<0.05) effects of genotype on shape index were reported (Zita *et al.*, 2009; Rajkumar *et al.*, 2009; Alewi *et al.*, 2012). None significant (P<0.05) effect of genotype on shape index was reported by Gupta *et al.* (2007) and Haunshi *et al.* (2013) which were in agreement with the shape index at 40 weeks of age. The egg shape index obtained in the study was comparable to the findings of Zita *et al.* (2009) and Alewi *et al.* (2012). Effects of age was not significant (P<0.05) in PA and PW where as it was significant (P<0.05) in PB and PR. Significant (P<0.05) reduction of shape index as the age advances was reported by Zita *et al.* (2009), Tumov and Gous (2012). Variation in shape index in different ages were also reported (Niranjan *et al.* 2008, Rajkumar *et al.*, 2009) which was also observed in the present study for PA and PB.

Yolk colour

Yolk colour was only significant (P<0.05) between different crosses at 52 weeks of age where PB and PR recorded lower value compared to PA and PW (Table 1). Significant (P<0.05) effect of genetic groups on yolk colour was reported by Niranjan *et al.* (2008), Rajkumar *et al.* (2009) and Alewi *et al.* (2012). However, Haunshi *et al.* (2013) reported none significant (P<0.05) effect of breed on egg colour which was in agreement with the yolk colour at 28, 40 and 64 weeks of age. Yolk colour value obtained in the present study comparatively lower than the reports of Niranjan *et al.* (2008) and Rajkumar *et al.* (2009) and higher than the report of Haunshi *et al.* (2013). Age effect was significant (P<0.05) only for PW, which was in agreement with the report of Rajkumar *et al.* (2009). Variation of yolk colour in a genetic group at different ages was obtained by Niranjan *et al.* (2008).

Yolk index

At 28, 40 and 52 weeks of age no significant (P<0.05) difference was observed for yolk index between the crosses, however, at 64 weeks of age PW recorded significantly (P<0.05) low index than the other three crosses (Table 1). Significant (P<0.05) effect of genetic groups for yolk index were reported (Niranjan *et al.*, 2008; Sola-Ojo and Ayorinde, 2011; Alewi *et al.*, 2012) which was in agreement with the present findings at 64 weeks of age. None significant (P<0.05) difference between genetic groups for yolk index was reported by Haunshi *et al.* (2013) which was comparable to the findings in the present study for egg quality at 28, 40 and 52 weeks of age. The yolk index values ranged from 0.406 to 0.497 irrespective the age and genetic groups. Wider variation in yolk index values was report by Niranjan *et al.* (2008) than the present study. Lower yolk index was reported by Rajkumar *et al.* (2009) in naked neck genotype than the present findings. Age effect on yolk index was significant (P<0.05) in all the crosses and as the age advances the yolk index values decreases in each cross and lowest values were obtained at 64 weeks of age. Significant (P<0.05) effect of age on yolk index and as the age advances the yolk index value decreases were reported (Rajkumar *et al.*, 2009; Zita *et al.*, 2009) which were in agreement with the present findings.

Albumen index

Albumen index was significant (P<0.05) between different crosses at 28, 52 and 64 weeks of age and none significant (P<0.05) at 40 weeks of age. At 52 and 64 weeks of age PR showed lowest index amongst the four crosses indicating at older age the albumen quality of PR comparatively poor than the other three genetic groups (Table 1). Significant (P<0.05) effect of genotype on albumen index was reported (Gupta *et al.*, 2007; Kaur *et al.*, 2008). Significant (P<0.05) effect of genotype for albumen height which was used for calculation of albumen index were also reported (Rajkumar *et al.*, 2009; Sola-Ojo and Ayorinde, 2011). Albumen index ranged from 0.069 to 0.131 irrespective the age and genetic groups which was falls in the ranges report in literature (Gupta *et al.*, 2007; Kaur *et al.*, 2008; Zita *et al.*, 2009). Age effect was significant (P<0.05) for all the crosses for albumen index and the index values at 40 weeks of age significantly (P<0.05) decreased compared to index values at 28 and 52 weeks of age. This may be due to decrease of albumen quality in the peak summer season. The best albumen index values were obtained at 52 weeks of age in all the crosses and it significantly (P<0.05) decreased at 64 weeks of age. Significant (P<0.05) age effect on albumen and lower albumen index at older age was reported by Zita *et al.* (2009). Significant (P<0.05) effect of age on albumen height and lower height at older age was reported by Silverside *et al.* (2006). The albumen index values indicated that PA and PB albumen quality at 64 weeks of age better than the other two crosses and the quality decreases at older ages.

Haugh unit

Significant (P<0.05) differences between crosses were obtained for Haugh unit measured at 28, 52 and 64 weeks of age. Haugh unit was not significant (P<0.05) between crosses at 40 weeks of age (Table 1). Significant (P<0.05) differences for Haugh unit between different genetic groups were reported (Niranjan *et al.*, 2008; Zita *et al.*, 2009; Sola-Ojo and Ayorinde, 2011; Alewi *et al.*, 2012). The Haugh unit score in the present study ranges from 70.54 to 93.67 and the best Haugh unit was obtained at 52 weeks of age. Variations in Haugh unit score between different genetic groups were reported (Gupta *et al.*, 2007; Kaur *et al.*, 2008; Niranjan *et al.*, 2008; Alewi *et al.* 2012) and the Haugh unit obtained in the present study falls in the reported range and even better at 52 weeks of age. Effect of age was significant (P<0.05) in all the crosses for Haugh unit and, in PR and PW the Haugh unit significantly (P<0.05) decreased at 64 weeks of age compared to other ages of measurements. Significant (P<0.05) effect of age on Haugh unit were reported where there was reduction of score at the older age (Tona *et al.*, 2004; Zita *et al.*, 2009; Tumova and Gous, 2012).

Shell thickness

Shell thickness differs significantly (P<0.05) between different crosses irrespective the age of measurements (Table 1). Except at 52 weeks of age the shell thickness was better in PA (PD1 X PD4) indicating the better shell thickness of this cross may be of use in the backyard to reduce the breakage of eggs. The shell thickness ranges from 0.334 to 0.386 in all the ages and crosses. Shell thickness ranging from 0.38 to 0.44, 0.29 to 31, 0.38 to 0.41 and 0.302 to 0.309 mm were reported by Suk and Park (2001), Gupta *et al.* (2008), Niranjan *et al.* (2008) and Alewi *et al.* (2012), respectively. Significant (P<0.05) effects of genetic groups for shell thickness were reported (Gupta *et al.*, 2008; Niranjan *et al.*, 2008; Rajkumar *et al.*, 2009; Alewi *et al.*, 2012). Effect of age was significant (P<0.05) for shell thickness in PA, PB and PW which was in agreement with the reports of Suk and Park (2001), Rajkumar *et al.* (2009) and Zita *et al.* (2009). PR recorded similar shell thickness at different age of measurements. Shell thickness was increased at 64 weeks of measurements in PA and PB compared to 52 weeks which is in agreement with the report of Suk and Park (2001).

Yolk %

Yolk % differs significantly (P<0.05) between different crosses at 28, 40 and 52 weeks of age and was not significant (P<0.05) at 64 weeks of age (Table 2). At all the ages of measurements PA recorded higher % of yolk compared to other crosses except at 52 weeks where PA, PB and PR recorded none significant (P<0.05) yolk % between themselves. This may be due to presence of

Table 1: Effect of age on egg quality parameters in different crosses

Traits	PA	PB	PR	PW
Egg Weight (g)				
28week	46.78^{c}±0.55B	50.67^{c}±0.71A	49.78^{c}±0.80A	49.51^{d}±0.77A
40week	54.11^{b}±0.84B	57.63^{bc}±1.12A	55.49^{b}±0.75AB	54.70^{c}±0.96B
52 week	59.97^{a}±0.69B	59.51 ^{b}±0.96B	60.47^{a}±1.13B	62.92^{a}±0.46A
64week	60.14^{a}±1.30AB	63.01 ^{a}±0.99A	57.92^{b}±0.97B	59.89^{b}±1.31B
Shape Index				
28 week	78.29±0.53A	78.53^{a}±1.22A	78.46^{a}±1.16A	74.40±0.81B
40 week	76.29±0.87	75.73^{bc}±0.91	76.12^{b}±0.43	73.90±0.43
52 week	77.05±0.59A	75.00^{c}±0.45BC	75.83^{b}±0.46AB	73.89±0.70C
64 week	78.3±1.04A	77.55^{ab}±0.81A	74.71^{b}±1.02B	73.09±0.85B
Yolk Colour				
28 week	7.10±0.20	7.10±0.20	6.85±0.18	7.10^{ab}±0.24
40 week	6.90±0.17	6.90±0.16	6.60±0.15	6.70^{bc}±0.16
52 week	7.15±0.15A	6.6±0.197B	6.55±0.22B	7.35^{a}±0.18A
64 week	6.50±0.30	6.94±0.25	6.45±0.17	6.35^{c}±0.18
Yolk Index				
28 week	0.480^{a}±0.007	0.497^{a}±0.005	0.486^{a}±0.006	0.482 ^{a}±0.015
40 week	0.448^{bc}±0.005	0.454^{b}±0.005	0.460^{b}±0.005	0.445^{b}±0.006
52 week	0.455^{b}±0.005	0.459^{b}±0.003	0.449^{b}±0.004	0.444^{b}±0.005
64 week	0.438^{c}±0.009A	0.433^{c}±0.004A	0.429^{c}±0.006A	0.406^{c}±0.009B
Albumen Index				
28 week	0.106^{b}±0.006B	0.132^{a}±0.005A	0.109^{b}±0.005B	0.106^{b}±0.006B
40 week	0.091^{c}±0.006	0.098^{b}±0.005	0.105^{c}±0.005	0.093^{c}±0.005
52 week	0.131^{a}±0.003A	0.131^{a}±0.003A	0.116^{a}±0.004B	0.126^{a}±0.004A
64 week	0.103^{b}±0.006A	0.102^{ab}±0.007A	0.069^{d}±0.004B	0.070^{d}±0.004B
Haugh Unit				
28 week	84.76^{b}±1.83B	92.58^{a}±1.33A	84.81^{b}±1.46B	88.46^{a}±1.79AB
40 week	78.59^{c}±1.95	80.72^{b}±1.58	83.59^{b}±1.75	79.19^{b}±1.50
52 week	93.67^{a}±0.63A	93.72^{a}±0.58A	90.24^{a}±0.67B	92.35^{a}±0.58A
64 week	83.82^{b}±2.47A	83.24^{b}±2.43A	71.77^{c}±2.51B	70.54^{c}±1.76B
Shell thickness (mm)				
28 week	0.386^{a}±0.004A	0.362^{a}±0.066B	0.349±0.005BC	0.340^{bc}±0.005C
40 week	0.371^{ab}±0.014A	0.322^{b}±0.008B	0.334±0.006B	0.334^{c}±0.005B
52 week	0.348^{b}±0.005AB	0.343^{b}±0.006B	0.342±0.004B	0.361^{a}±0.004A
64 week	0.378^{a}±0.008A	0.372^{a}±0.009A	0.346±0.008B	0.351^{ab}±0.005B

PA=PD1 X PD4, PB= PD1 X PB2, PR= PD1 X PD3 and PW= PD1 X IWI
Means having even one common superscript in lower case in a column for a particular trait did not differ significantly (P>0.05) at different ages.
Means having even one common superscript in upper case in a row for a particular trait did not differ significantly (P>0.05) between different genetic groups.

PD4 which was an indigenous breed as one parent in PA than other crosses. Significantly (P<0.05) higher yolk % in indigenous birds compared to exotic breed was reported by Padhi *et al.* (1998). Yolk % varies from 28.03 to 33.96 %irrespective of ages in different crosses which were in accordance to the available literature in different genetic groups (Zita *et al.*, 2009; Sola-Ojo and Ayorinde, 2011; Tumova and Gous 2012). Significant (P<0.05) difference between genotypes for yolk % was reported by Zita *et al.* (2009) and Sola-Ojo and Ayorinde (2011). Age effect was significant (P<0.05) for yolk % for all the crosses and as the age of measurements increases the yolk % increased and maximum % was obtained at 64 weeks of age and minimum at 28 weeks. Significant (P<0.05) age effect on yolk % and increase yolk % at older age was reported in literature (Silverside and Scott, 2001; Zita *et al.*, 2009). These reports were in agreement with the present findings.

Albumen %

Significant (P<0.05) differences between crosses for albumen % were observed at 28 and 40 weeks of age (Table 2). At both the age lowest albumen % was obtained in PA. This may be due to use of one parent as indigenous breed in this cross. Significant (P<0.05)

Table 2: Effect of age on yolk, albumen and shell % in different crosses

Traits	PA	PB	PR	PW
Yolk %				
28 week	28.84^{c}±0.52A	26.03^{c}±0.55C	28.27^{b}±0.35AB	27.11^{c}±0.24BC
40 week	31.25^{b}±0.41A	28.03^{b}±0.62B	28.87^{b}±0.31B	28.50^{b}±0.32B
52 week	31.21^{b}±0.27A	31.28^{a}±0.74A	31.74^{a}±0.47A	29.41^{b}±0.36B
64 week	33.96^{a}±1.09	32.90^{a}±0.39	32.68^{a}±0.55	33.81^{a}±0.55
Albumen %				
28 week	61.20^{a}±0.58B	64.99^{a}±0.58A	62.46^{a}±0.41B	63.83^{a}±0.26A
40 week	59.60^{a}±0.40B	62.60^{b}±0.67A	61.58^{a}±0.38A	61.83^{b}±0.40A
52 week	59.89^{a}±0.31	59.79^{c}±0.83	59.27^{b}±0.56	61.47^{b}±0.38
64 week	57.13^{b}±1.45	58.81^{c}±0.50	58.83^{b}±0.51	57.47^{c}±0.59
Shell %				
28 week	9.97^{a}±0.17A	9.78^{a}±0.14A	9.28^{ab}±0.14B	9.06^{b}±0.13B
40 week	9.15^{b}±0.17	9.38^{a}±0.13	9.55^{a}±0.12	9.67^{a}±0.13
52 week	8.89^{c}±0.16	8.94^{b}±0.24	8.99^{b}±0.19	9.13^{b}±0.16
64 week	8.91^{bc}±0.42	8.29^{c}±0.25	8.49^{c}±0.17	8.71^{c}±0.13

PA=PD1 X PD4, PB= PD1 X PB2, PR= PD1 X PD3 and PW= PD1 X IWI

Means having even one common superscript in a column for a particular trait did not differ significantly (P>0.05).

Means having even one common superscript in upper case in a row for a particular trait did not differ significantly (P>0.05) between different genetic groups.

lowest albumen % in indigenous fowl compared to exotic breeds was reported (Padhi *et al.*, 1998). Albumen % observed in different crosses at different ages of measurement ranged from 57.13 to 63.83 %. Significant (P<0.05) effect of genotype on albumen % were in agreement with the reports of Zita *et al.* (2009) and Sola-Ojo and Ayorinde (2011). Effect of age was significant (P<0.05) on albumen % in all the genetic groups and at older age the albumen % decreased compared to early age of measurement. Similar results were reported by Silverside and Scott (2001) and Zita *et al.* (2009).

Shell %

Shell % differ significantly (P<0.05) between different crosses at 28 weeks of age, however, at 40, 52 and 64 weeks of age no significant (P<0.05) difference between crosses were found (Table 2). The shell % ranges from 8.29 to 9.97 % in different crosses measured at different ages. Significant (P<0.05) effects of genotype for shell % were reported by Zita *et al.* (2009), Islam and Dutta (2010) and Haunshi *et al.* (2013). Effects of age on egg shell % were significant (P<0.05) in all the crosses and the shell % decreased at 64 weeks of age significantly (P<0.05) than at 28 weeks of age. Significant (P<0.05) age effect on shell % was reported in literature (Silversides and Scott, 2001; Zita *et al.*, 2009) which agrees with the present findings.

CONCLUSION

The study concluded that genotype and age significantly (P<0.05) affected different egg quality traits measured at different ages in all the four crosses. As the age advanced the egg weight increased but the egg quality parameters decreased. Further, the shell thickness was found better in PA (PD1 X PD4) indicating the eggs of this cross may withstand breakage compared to other crosses. The presence of PD4 as one parent which is developed from Aseel a native breed of India may be contributing to the better thickness in PD1 X PD4 compared to other crosses.

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Growth performance and nutrient metabolizability of Japanese Quails (*Coturnix coturnix japonica*) orally administered varying levels of Lemon grass (*Cymbopogon citratus,* Stapf) aqueous extract

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ABSTRACT

*Banjo, A.A., Alabi, J.O., Out, B.O., Kolo, S.P. and Omotola, M. 2022. Growth performance and nutrient metabolizability of Japanese Quails (*Coturnix coturnix japonica*) orally administered varying levels of Lemon grass (*Cymbopogon citratus, *Stapf) aqueous extract. Indian Journal of Poultry Science, 57(02): 139-143.*

A 42-days trial was conducted to evaluate the efficacy of lemon grass aqueous extract (LGAE) on growth performance and nutrient metabolizability of Japanese quails (*Coturnix coturnix japonica*). The 10 days old Japanese quails (n = 240) used in this study were allotted to 5 groups and were fed a common corn-soybean meal-basal diet unrestrictedly for a period of 42 days. The five treatments include the control group fed basal diet with no additive, the second group received antibiotics (Embacox® at 8g per litre) while the remaining three (3) treatments were administered LGAE in drinking water at varying levels of 0.20, 0.40, and 0.60 ml per litre once a week. The quails which received 0.2ml LGAE per litre had higher ($P<0.05$) body weight gain, and daily feed intake was increased by 3.88% when compared with antibiotic-treated group. FCR was higher ($P<0.05$) in quails exposed to 0.4 and 0.6ml LGAE while the least value was obtained in quails which received antibiotics (Embacox®). Significant increase in water intake ($P<0.05$) and water to feed ratio ($P<0.001$) was observed in growing quails which received 0.2ml LGAE when compared to other treatments. Japanese quails which received lemon grass extract in water showed higher ($P<0.05$) nutrient metabolizability, except crude fibre, than those in control group. Nutrient metabolizability increases (except for fibre) as the LGAE inclusion level increases. The study concluded that Japanese quail served 0.2ml LGAE per litre had similar growth rate with those on control groups while additional dosage resulted in negative effects. LGAE administered in drinking water up to 0.6ml per litre enhance nutrient metabolizability and utilization.

Keywords: Japanese Quails, lemon grass, antibiotics, growth parameters, nutrient digestibility

INTRODUCTION

Poultry farming has been experiencing progressive growth rate in production volumes over the last few decades compared to other livestock counterparts due to increasing demand for safe, affordable poultry products (Oni *et al.*, 2020). Protein supply from poultry meat and eggs contribute immensely to achieving food and nutritional security due to short production cycle, higher yields, and highly efficient feed-food ratio (Fafiolu *et al*., 2020). Besides the domestic chickens (broilers and layers), Japanese quails (*Coturnix coturnix japonica*) farming is on the increase nowadays as source of specialty egg and meat (Talsani *et al.*, 2021). They are currently the smallest poultry species with unique features including short life cycle, rapid growth, lower feed consumption, early sexual maturity, high rate of lay, good meat taste, and disease resistance (El-Katcha *et al.*, 2014; Bansod *et al.*, 2021).

The need to satisfy the increasing interest of end-users in natural food, and with an outlook to meet up the growing rate of demand, the poultry industry is paying attention on how to amplify the growth performance and focused at bringing up new foods that would be harmless to the health of humans (Alishah *et al*., 2013). In view of this, a wide range of phytobiotics such as spices, herbs and essential oils is currently being used as alternative to antibiotic growth promoter in poultry rearing (Parade *et al.*, 2019; Fafiolu *et al.*, 2020; Alagawany *et al.*, 2021). Phytobiotics contain substantial quantity of various phenolic compounds. The combined effects of these bioactive substances in such plants usually produce the characteristic scent or flavour peculiar to each plant (Fafiolu *et al*. 2020). Generally, the mode of action of phytobiotics are basically taste modifying, digestion stimulating, and immunomodulatory effect. They also exert antioxidant, antimicrobials, anti-proliferate, hypolipidemic, and detoxifying properties (Khattak *et al.*, 2014).

Lemongrass (*Cymbopogon citratus*, Stapf) is a widely distributed perennial herb belonging to Poaceae family which has been used by man and animals due to its numerous health benefits. It has the citrusy fragrance like that of lemon with which it gives pleasant taste and aroma to food and contains a very high amount of vitamin C. The bioactive substances in Lemongrass comprise of

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flavonoids, phenolic compounds, terpenoids as well as essential oils which include citral, isoneral, myrcene and linalool. These phytoactive constituents are responsible for various biological activities earlier documented in poultry birds (Parade *et al.,* 2019; Alagawany *et al.,* 2021). Various methods of extracting the bioactive compounds from lemongrass and the efficacy of such essential oils and extracts in both *in vivo* and *in vitro* studies have been documented (Oboh *et al.,* 2010; Shah *et al.,* 2011; Olorunnisola *et al.,* 2014; Ogbonna *et al.,* 2017; Mukarram *et al.,* 2022). However, the paucity of available information on the influence of administering lemongrass aqueous extract (LGAE) in drinking water of Japanese quails necessitates this study.

The comparative advantages of using LGAE include, but not limited to, the following: 1). Availability: Selected phytobiotics are readily available. They can be grown or harvested in the vicinity or sourced at the local markets with little cost. 2). Ease of processing: lemon grass can be air dried and milled using household grinder or milling machine. 3). Ease of administration: Quail farmers who use commercial feeds can explore the medicinal and health benefits in lemon grass by applying the aqueous extract in drinking water. Therefore, the purpose of this study was to assess the effect of lemon grass aqueous extract on the growth performance and nutrient metabolizability of growing Japanese quails.

MATERIALS AND METHODS

Study Ethics

This study was conducted at the Poultry Unit, Animal Production Teaching and Research Farms of the Federal University of Technology Minna, Niger State, Nigeria, and the experimental procedures were performed in accordance with the approved research protocols by the University's Animal Care and Use Review Committee.

Management of experimental birds

The conventional cage system placed in a pen were used to rear the Quails where they got unbiased care and supervision. The quail pen used was properly cleaned and disinfected with the use of germicides a week before the birds arrived. Openings (windows) of the quail pen were appropriately enclosed with tarpaulin as well as the cage floor with newspapers to facilitate heat conservation during the brooding stage. Brooding was done following recommended ambient temperature regimens while proper animal husbandry management and biosecurity measures were strictly adhered to (Pourakbari *et al.,* 2016). All birds were reared under the same management practices for the 42-days experimental period with free access to the water and feed (mash form).

Preparation of Lemon Grass extract

Lemon Grass aqueous extract was prepared using the method of Orrego *et al.* (2009). The plant materials underwent room-temperature air-drying to 10% moisture content and milled into fine powder using the hammer mill grinding machine. After grinding the lemon grass leaves, the grinded lemon grass leaves were weighed (10 g) and poured into weighed boiled water (1 litre) which was covered and left-over night. The lemon grass was sieved/filtered the next morning and was administered in drinking water at different inclusion levels while the residue was discarded. This method is also in accordance with the hot water extract method (Oboh *et al.,* 2010).

Study design and treatments

A total of 240 Japanese quails (10-day old) used for this experiment were randomly allotted to 5 groups of 48 chicks in 6 replicates (8 per replicate) in a completely randomized design. The five treatments include the control group fed basal diet with no additive (Table 1), the second group received antibiotics (Embacox® at 8g per litre) while the remaining three (3) treatments were administered lemon grass aqueous extract in drinking water at varying levels of 0.20, 0.40, and 0.60 ml per litre. The antibiotics and lemon grass aqueous extract were administered in drinking water to each bird once a week.

Table 1: Ingredients and nutrient contents of the Japanese quail diet

Ingredients	Composition (g/kg)
Maize	521.70
Soybean meal (45% CP)	357.00
Guar meal (42% CP)	32.50
Wheat offal	32.10
Soybean oil	18.00
Dicalcium phosphate (18%)	16.50
Limestone ($CaCO_3$)	13.50
Common Salt (NaCl)	3.00
L-Lysine HCl	1.00
DL-Methionine	1.20
Vitamins and trace minerals premix[*,1]	1.25
Toxin binder (T5X Binding)[2]	0.50
Nutrient composition	
Metabolizable energy (Kcal/kg)	2951.00
Crude protein	223.60
Crude fibre	39.60
Ether extract	43.00
Ash	30.40
Nitrogen free extract	572.40
Dry matter	909.00
Calcium	10.30
Available phosphorus	4.20
Lysine	11.50
Methionine	4.40

*Premix supplied contain; Vit. A: 12 MIU, Vit. D_3: 3MIU, Vit. E :30g, Vit. K_3: 2.5g, Vit. B_1: 2g, Vit. B_2: 5g, Vit. B_6: 3.5g, Vit. B_{12}: 20mg, Folic acid: 1g, Niacin: 40g, Calpan: 10g, Biotin: 80 mg, Antioxidant: 125g, Co: 250mg; Se: 250mg, Ý: 1.2g, Fe: 40g, Mn: 70g, Cu: 8g, Zn: 60g, ChCl: 200g. [1]Produced by Animal Care Services Konsult (Nig.) Ltd., Nigeria; [2]Produced by Wisium SA (PTY) Ltd., South Africa

Growth performance indices

Live weight of the Japanese quails was measured prior to the commencement of the experiment to avoid biasness in randomization. Thereafter, Birds were weighed per cage on a weekly basis in order to compute weight gain (Bansod *et al.,* 2021). Daily feed intake was also measured per replicate cage as the difference between the feed given and those not consumed. Number of dead birds were noted in each replicate (Oni *et al.,* 2020). The average of body weight gain per bird, feed intake per bird on daily basis, and feed conversion ratio were computed (Talsani *et al.*, 2021) as shown in the formulae below. A known quantity of water was served to each replicate daily, and quantity not consumed were measured with calibrated measuring cylinder to estimate water intake and water: feed ratio.

$$\text{Average body weight gain (ABWG)} = \frac{\text{Weight Changes (g)}}{\text{No. of Quails per replicate}}$$

$$\text{Average daily feed intake (ADFI)} = \frac{\text{Feed offered (g)} - \text{Feed left over (g)}}{\text{No. of Quails per replicate}}$$

$$\text{Feed conversion ratio (FCR) (ADFI)} = \frac{\text{Average body weight gain (g)}}{\text{Average daily feed intake (g)}}$$

$$\text{Average water intake (ml)} = \frac{\text{Water served (ml)} - \text{Water not consumed (ml)}}{\text{No of Quails per replicate}}$$

$$\text{Water: feed ratio} = \frac{\text{Average water intake}}{\text{Average daily feed intake}}$$

Nutrient metabolizability

Three replicate cages (24 birds per treatment) were selected at random at the last week of the research to measure nutrient metabolizability. Feed consumption and total excreta output were monitored per cage for 5 days. Droppings collected were oven-dried till a constant weight is achieved (Oni *et al.,* 2020). Proximate composition of feed and dried excreta samples was analyzed for dry matter (Method 934.01), crude protein by the Kjeldahl method (N×6.25; Method 990.03), crude ûbre (Method 978.01), ether extract (Method 920.39), and ash (Method 942.05) using standard methods of AOAC (2000). The metabolizability of nutrient constituents such as dry matter, crude protein, ether extract, crude fibre, ash and nitrogen free extract for the five treatment groups were estimated using the formulae below:-

$$\text{Nutrient metabolizability (\%)} = \frac{\text{(Nutrient in feed condumed} - \text{Nutrient in excreta voided)}}{\text{(Nutrient in feed consumed)}}$$

NFE= 100–(moisture + crude protein + fibre + ether extract + ash)

Statistical analysis

All data on growth performance, water consumption and nutrient metabolizability of growing Japanese quails were analyzed with a generalized linear model procedure in a One-way analysis of variance (ANOVA) of SPSS version 20.0 Statistical Software (IBM SPSS, 2017) where each replicate pen serve as the experimental unit. Differences among mean values were separated with Duncan Multiple Range Test with a $P<0.05$ indicating significance. Statistical model used was: $Y_{ij}= \mu+T_i+E_{ij}$. Y_{ij} is the experimental observation, μ is overall mean, T_i is the effect of lemon grass aqueous extract, and E_{ij} is random error.

RESULTS AND DISCUSSION

Growth performance

The effects of LGAE in drinking water of growing quails on growth performance are presented in Table 2. The birds which received 0.2ml LGAE per litre had higher ($P<0.05$) final weight and body weight gain which was similar to the control groups whereas those served 0.4ml LGAE had lower body gain. Body weight is regarded as one of the most important production indices due to its strong positive correlation to the meat (carcass) yield, and consequential influence on market value and farmers' profit margin. Previous studies by Alagawany *et al.* (2021) reported that dietary inclusion of lemon grass essential oil (150-600 mg/kg) resulted in linear and quadratic increase in body weight gain of growing Japanese quails. Daily feed intake was significantly ($P<0.05$) increased in the quails received 0.2ml LGAE compared with others, even of the antibiotic group by 3.88%. This could be attributed to the presence of appreciable amounts of phytochemicals including flavonoids which gives pleasant taste and aroma that enhance feed palatability. This was consistent with the reports of Mmereole (2010) and Khattak *et al.* (2014) who observed significant improvement in the growth rate of broiler chicks fed diets supplemented with Lemon grass (*Cymbopogon citratus*) compared to the control group. Similarly, Mukhtar *et al.* (2012) reported higher weight gain and increased feed consumption with the addition of Lemon grass extract oil in broiler chicks' diets.

Feed conversion ratio was higher ($P<0.05$) in quails exposed to 0.4 and 0.6ml LGAE while the least value was obtained in quails which received antibiotics (Embacox®). This contradicts the reports of Mukhtar *et al.* (2012), Khattak *et al.* (2014) and Alagawany *et al.* (2021) who reported improved FCR with lemon grass or its essential oil. Ogbonna *et al*. (2017) stated that the inclusion of lemongrass leaf meal (up to 2%) in aflatoxin-contaminated broiler chicks' diet produced similar weight gain and FCR with chicks fed non-contaminated diets. The variation in results findings implies that the efficacy

Table 2: Growth performance of Japanese quails administered varying levels of lemon grass aqueous extract

Parameters	T1	T2	T3	T4	T5	SEM	P value
Initial weight (g)	22.17	21.88	20.42	21.75	20.96	0.31	0.067
Final weight (g)	179.33[a]	179.29[a]	179.13[a]	172.25[c]	173.50[b]	0.81	0.006
Average body weight gain (g/day)	3.74[a]	3.75[a]	3.78[a]	3.58[c]	3.63[b]	0.10	0.005
Average daily feed intake (g/day)	10.60[c]	10.32[d]	10.72[a]	10.61[c]	10.66[b]	0.73	0.012
Feed conversion ratio (g/g)	2.83[b]	2.75[c]	2.84[b]	2.96[a]	2.93[a]	0.10	0.004
Water intake (ml)	956.06[b]	949.07[c]	989.38[a]	964.33[b]	945.42[c]	0.25	0.017
Water: feed ratio (ml/g)	2.15[c]	2.19[b]	2.21[a]	2.16[c]	2.12[d]	0.18	<0.001

[abcd]Means on the same row are significantly different (P<0.05); SEM: Standard error of meansT1: no additive; T2: 8g Embacox®; T3: 0.2ml LGAE; T4: 0.4ml LGAE; T5: 0.6ml LGAE

of phytobiotics on birds' performance depends on such factors as profile of bioactive components present in the extracts used, inclusion dose, duration of administration as well as physiological and health status of the birds (Diaz-Sanchez *et al.*, 2015; Fafiolu *et al.*, 2020). Significant increase in water intake (P<0.05) and water to feed ratio (P<0.001) was observed in growing quails which received 0.2ml LGAE when compared to other treatments. This probably explains the reason for increase feed intake. Increase in LGAE inclusion level resulted in linear decrease in water intake and water to feed ratio. This likely suggests that Japanese quails' taste bud is more sensitive to the citrusy flavour of lemon grass. More so, the water to feed ratio observed in this study ranged from 2.12 to 2.21 ml per gram of feed. This means the assertion that water requirement of birds is usually twice the amount of feed consumed is also applicable to Japanese quails.

Nutrient metabolizability

The results of nutrient metabolizability presented in Table 3 revealed that Japanese quails which received lemon grass extract in water showed higher (P<0.05) nutrient metabolizability, except crude fibre, than those in control group. Digestion and absorption of dietary nutrients increased (except for fibre) as the LGAE inclusion level increases. In poultry, the production and growth depend upon the digestion and efficient utilization of absorbed nutrients in the feed as accomplished by the intestinal health (Alagawany *et al.,* 2021). It has been documented that the pleasant flavour or fragrance of phytogenics stimulates oronasal and gastro-intestinal tracts thereby impacting palatability and feed intake positively. It also enhances digestive secretions as well as gut functions for better nutrient break-down and assimilation thus resulting in improved growth rate (Diaz-Sanchez *et al*., 2015; Fafiolu *et al*., 2020). Lemon grass contains citral that helps to digest food, while polyphenols increase the use of energy and enhances the oxidation of fatty acids in the body. This probably explains the higher protein, fat and ash digestions obtained in quails that drink LGAE-treated water. Furthermore, Silva *et al.* (2011) reported that Lemongrass essential oils promoted digestion and nutrient absorption due to its antimicrobial and antioxidant effects. The enhancement in dry matter metabolizability following LGAE in drinking water was in line with improvements in the metabolizability values of crude protein, crude fat, ash, and soluble carbohydrates (NFE).

CONCLUSION

Japanese quail served 0.2ml LGAE per litre produced similar growth performance as those on control groups while higher dose caused reduce weight gain. LGAE administered in drinking water up to 0.6ml per litre improves nutrient utilization. Further studies are needed to validate lemon grass aqueous extract dosage in drinking water to improve Japanese quails' growth performance.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Table 3: Nutrient metabolizability of Japanese quails administered varying levels of lemon grass aqueous extract

Nutrients (%)	T1	T2	T3	T4	T5	SEM	P value
Dry Matter (DM)	73.87[d]	74.31[d]	74.86[c]	75.79[b]	76.11[a]	0.25	0.002
Crude Protein (CP)	72.02[d]	72.49[d]	73.49[c]	74.18[b]	75.65[a]	0.34	<0.001
Crude Fibre (CF)	78.60[b]	79.38[a]	77.60[c]	76.82[d]	72.67[e]	1.29	0.005
Ether Extract (EE)	79.22[d]	79.49[d]	80.24[c]	81.14[b]	83.00[a]	0.36	<0.001
Ash	67.21[b]	66.18[c]	69.32[a]	68.51[ab]	69.26[a]	1.73	0.004
Nitrogen free extract (NFE)	56.55[d]	57.15[c]	57.11[c]	57.50[b]	58.12[a]	0.14	0.017

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Growth performance, meat yields and economics of broiler chickens fed diets containing selected feed additives

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ABSTRACT

Saini, J., Dhande, P.L., Ranade, A.S., Avari, P.E. and Desai, D. 2022. Growth performance, meat yields and economics of broiler chickens fed diets containing selected feed additives. Indian Journal of Poultry Science. 57(02): 145-149.

The present study was conducted on growth performance, meat yields and economics of broiler chickens fed diets containing feed additives. For this study 210 day-old broiler chicks were procured from the hatchery. The broiler chicks were randomly divided into seven groups of 30 chicks each *viz,* 1) control, 2) control + cocktail enzymes, 3) control + prebiotic, 4) control + probiotic, 5) control + organic acid 6) control + antioxidant and 7) control + saponin and inulin. Each group was further divided into two replicates of 15 birds each. Diets were formulated were formulated according to NRC (1994) recommendations. Variables measured consisted of BW gain, feed intake, feed conversion ratio, mortality, carcass weight and yield.The performance parameters revealed that the significant ($P<0.05$)differences in average feed consumption among various groups supplemented with different feed additives but the average gain in weights, feed conversion ratio of birds were similar. Moreover, economic improvementin performance traits was observed in broilers fed the cocktail enzyme followed by probiotic and prebiotic compared with control birds. This indicates thatthe supplementation of multiple / cocktail enzyme selection was best among other treatments,can be used as a growth promoter in broilerdiets and can improve the gut health. These feed additivesshow promising effects as alternatives for antibiotics in broiler chicken.

Keywords: Broiler, Growth performance, Economics, Feed additives

INTRODUCTION

Many feed substances obtained from nature belongingto the groups of prebiotics, probiotics, organic acids, enzymes, silicates, herbs and spices etc., havebeen vigorously tested and evaluated for their potential to replace antibiotic growth promoters (AGPs) in poultry diets(Panda *et al*., 2006; Khan *et al*., 2012). Such, alternative substances were referred as naturalgrowth promoters (NGPs). There are a number of such investigated NGPs that are mainly utilized for providing beneficial role for improving health of poultry against various infectious diseasesrather than regular nutrition. The involvement of these NGPs in improving intestinalmorphology and nutrient absorption may also encourage the scientists to include these compoundsin the diet to improve gut health, promote the growth and overall performance of birds.

Some of key features identified from the most well-knownhypothesized mechanism (Huyghebaert*et al*., 2011) thatfavours performance of gut are: (1) antimicrobial action; (2) reduces the incidence and severity ofsubclinical infections; (3) reduces the microbial use of nutrients; (4) improve absorption ofnutrients; (5) reduces the amount of growth-depressing metabolites; (6) control microbiotashifts; (7) inhibit the production and excretion of cytokines by immune cells (macrophages) and (8) shifting the microbiota composition towards one that is less capable of evoking an inflammatoryresponse (Humphrey and Klasing, 2003). Based on the suggested mechanism of action, none ofthe non-antibiotic NGPs is likely to compensate the loss of gut health. So, it must be emphasized that some strategies will only help to compensate partially by NGPs and will work through indirect mechanisms.

Recently, several enzymes have been developed, which have considerable potential for animal feed application (He *et al.*, 2010). These enzymes have special properties such as active over a broad pH range, exhibit thermostability, resistant to pepsin and trypsin and viable under simulated gastric conditions. Probiotics are strains of various microbial species currently has gained attention as a substitute to antibiotics for poultry production as growth promoters (Ahmad, 2006). Prebiotic has been defined as a non-digestible dietary supplement or feed ingredient that beneficially affects the host by selectively stimulating the growth by altering the composition and metabolism of the gut microbiota (Gibson and Roberfroid, 1995).Organic acid lower the pH, at which the activity of proteases and beneficial bacteria is optimized andproliferation of pathogenic bacteria is minimised by a direct antibacterial effects destroying their cell membranes (Chowdhury *et al*., 2009). Saponin and inulin (*Cyamopsis tetragonolobus*) improve intestinal microflora and gut morphology, anticoccidial effect by interfering with the cell membranes (Abbas *et al*., 2012).

The term gut health is currently gaining much more attentions in veterinary literature especially in poultry and has been applied to coordinate the working efficiency of gut (Cummings *et al*., 2004; Laudadio*et al*., 2012).

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Although, the term is restricted to gastro-intestinal (GI) tract only and does not involve other organs (Bischoff, 2011). The gut is the primary site for multitude of processes such as, digestion, fermentation, nutrient absorption, nutrient metabolism, intestinal integrity, immune recognition, immune regulation and development of immune tolerance (Sommer and Backhed, 2013).

Present broilers market demands with good meat yield, better feed conversion and early growth along with all other traits is increased. Based on this concept, the goal of the present study is to investigate the effects of different feed additives on broiler performance and their association with the economics.

MATERIALS AND METHODS

Birds and housing

A total number of 210 one day - chicks (Cobb 400) were obtained from a commercial hatchery and randomly divided into 7 groups (30 birds/group) and housed in pens of identical size (2 m^2) in a deep litter system with rice husk as litter material and equipped with feeders and waterers. Each group had 2 replicates (15 birds/pen).The birds had free access to water and feed. The climatic conditions and lighting program was followed as the commercial recommendations. Environmental temperature in the first week of life was 32°C and decreased to 25°C until the end of the experiment. During the first week, 22 h of light was provided with a reduction to 20 h afterward.

Medication and vaccination

Immediately after arrival, chicks were provided with anti - stress elements (vitamins and B complex) through drinking water. The immunization against Ranikhet Disease by B1 strain vaccine and New Castle Disease killed vaccine was carried out on 5th day. Infectious Bursal Disease (Intermediate plus strain) vaccination was carried out on 12th day.

Dietary treatments

Three basal diets, pre–starter (0-7 days), starter (08-21 days) and finisher (22-35 days) were formulated. The experimental diets were formulated according to NRC (1994) recommendations. Experimental treatments, composition of feed additives with its dose rate were mentioned in Table 1.

Table 1: Experimental treatments, composition of feed additive and its dose rates.

Feed additive	Dose rate
Control	
Basal diet + Cocktail enzymes	(350g/ton)
Basal diet + Prebiotic	(800g/ton)
Basal diet + Probiotic	(50g/ton)
Basal diet + Organic acid	(1.0 kg/ton)
Basal diet + Antioxidant	(250g/ton of oil)
Basal diet + Saponin and inulin	(300g/ton)

Cocktail enzyme: Cellulose: 6,000,000 µ/kg, Xylanase: 10,000,000 µ/kg, â-glucanase: 700,000 µ/kg, phytase: 1200,000 µ/kg, á-amylase: 700,000 µ/kg, pectinase: 70,000 µ/kg, protease: 3,000,000 µ/kg, lipase: 5,000 µ/kg, hemicellulase, amyloglycosidase and pentosanase.

Prebiotic: M*annanoligosaccharides (MOS) d*erived from yeast cell walls (*Saccharomyces cerevisae*)

Probiotic: Total viable count of 2 × 10^9 CFU/g *Lactobacillus plantarum, Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus salivarius subsp. thermophilus, Lactobacillus acidophilus, Lactobacillus rhamnosus, Enterococcus faecium, Bificobacterium bifidum.*

Organic acid: Fumaric acid, lactic acid, ground corn cob, amorphous silicon dioxide, benzoic acid, citric acid, propionic acid and ethyl butyrate.

Antioxidant: Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and propyl gallate (PG).

*Saponin and inulin: M*ixture of chicory plant and Sapindaceae & Saponariaplant.

Growth performance

All birds were weighed individually to the nearest gram after their arrival from the hatchery to the experimental farm (initial weight). The daily records for feed consumption and mortality were maintained. The body weight gain of the birds was recorded on weekly basis during the course of the whole experiment for each treatmentand the feed conversion ratio (FCR) was calculated for the period of 01 to 35 days during the trials. The growth performance traits were: average weekly live weights, average weekly gain in weights, average weekly feed consumption, average weekly feed conversion ratios, weekly mortality and economics of production at the end of fifth week

Statistical analysis

Data collected for growth performance parameters were statisticallyexamined by using interaction effect in two-way ANOVA in the SPSS statistical software package as per method of Snedecor and Cochran (1994). Results were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Weekly live weight (gm)

The initial body weight (BW) of chicks did not differ ($P>0.05$)between the dietary treatments (Table 2). At the end of the experiment (d35), birds supplemented with the cocktail enzyme had a greater ($P<0.05$) BW (2215.92 g) compared with controls (2118.75 g). Moreover, prebioticsupplemented birds had a greater BW (2125.25 g)than control birds (Table 2).The findings were in accordance with Abdel-Raheem *et al.* (2012) and Incharoen (2013) in broilers. At the end of the experiment (day 35), birds supplemented with the enzyme had a greater body weight as compared with control and other treatments (Table 2). Moreover, a slight improvement in body weight was observed in broilers fed the prebiotic,

Table 2: Performance parameters of broilers of different feed additives in all treatments.

Parameters	Week	Control	Enzyme	Prebiotic	Probiotic	Organic	Antioxid-ant acid	Saponin and inulin	% CV	"F" Value
Weight of bird / week	Day old	40.47±0.74^{u}	39.48±0.74^{u}	40.50±0.74^{u}	40.91±0.74^{u}	42.02±0.74^{u}	40.21±0.74^{u}	39.97±0.74^{u}	3.09	**4.08****
	1st	142.03±0.74^{av}	148.02±0.74^{bv}	149.70±0.74^{bv}	147.91±0.74^{bv}	146.98±0.74^{abv}	143.89±0.74^{av}	141.94±0.74^{av}		
	2nd	392.75±0.74^{aw}	422.52±0.74^{bw}	387.63±0.74^{bcw}	400.88±0.74^{bdw}	398.68±0.74^{bdw}	386.90±0.74^{bcw}	394.73±0.74^{aw}		
	3rd	795.22±0.74^{ax}	854.37±0.74^{bx}	806.45±0.74^{cdx}	819.40±0.74^{efx}	816.25±0.74^{fgx}	799.67±0.74^{hx}	805.58±0.74^{dix}		
	4th	1445.65±0.74^{ay}	1525.80±0.74^{by}	1412.13±0.74^{cdy}	1433.43±0.74^{ey}	1411.29±0.74^{fdy}	1419.22±0.74^{gy}	1394.42±0.74^{hy}		
	5th	2118.75±0.74^{az}	2215.92±0.74^{bz}	2125.25±0.74^{cz}	2069.13±0.74^{dz}	2103.88±0.74^{ez}	2087.17±0.74^{fz}	2027.00±0.74^{gz}		
Weight gain / bird / week	1st	101.83±0.74^{av}	107.04±0.74^{bv}	107.94±0.74^{bv}	106.00±0.74^{bv}	103.34±0.74^{av}	102.16±0.74^{ay}	100.89±0.74^{ay}	4.39	**2.06^{NS}**
	2nd	249.98±0.74^{aw}	274.24±0.74^{bw}	239.31±0.74^{cw}	253.19±0.74^{dw}	253.14±0.74^{adw}	242.19±0.74^{cw}	253.68±0.74^{dw}		
	3rd	403.02±0.74^{ax}	432.30±0.74^{bx}	418.80±0.74^{cx}	419.25±0.74^{cx}	417.71±0.74^{cx}	415.29±0.74^{cdx}	411.13±0.74^{fx}		
	4th	650.43±0.74^{ay}	671.44±0.74^{by}	605.68±0.74^{cy}	614.03±0.74^{dy}	595.04±0.74^{ey}	619.55±0.74^{gy}	588.83±0.74^{gy}		
	5th	673.10±0.74^{az}	690.12±0.74^{bz}	713.12±0.74^{cz}	635.73±0.74^{dz}	692.59±0.74^{bz}	667.95±0.74^{ez}	632.59±0.74^{dz}		
Feed consumption/ bird/week	1st	132.47±0.74^{av}	131.78±0.74^{abv}	133.72±0.74^{av}	133.94±0.74^{av}	129.26±0.74^{bcv}	131.50±0.74^{av}	132.96±0.74^{av}	2.65	**4.71****
	2nd	353.75±0.74^{aw}	359.52±0.74^{bw}	339.97±0.74^{cw}	344.54±0.74^{dw}	350.08±0.74^{ew}	341.21±0.74^{cfw}	350.19±0.74^{egw}		
	3rd	609.65±0.74^{ax}	648.51±0.74^{bx}	597.76±0.74^{cx}	605.30±0.74^{dx}	583.20±0.74^{ex}	601.17±0.74^{fx}	587.03±0.74^{gx}		
	4th	898.74±0.74^{ay}	956.12±0.74^{by}	879.80±0.74^{cy}	894.21±0.74^{dy}	871.93±0.74^{ey}	917.00±0.74^{fy}	929.44±0.74^{gy}		
	5th	1271.34±0.74^{az}	1286.29±0.74^{bz}	1203.84±0.74^{cz}	1217.88±0.74^{dz}	1198.38±0.74^{ez}	1248.09±0.74^{fz}	1258.00±0.74^{gz}		
FCR	1st	1.30±0.03^{v}	1.23±0.03^{v}	1.24±0.03^{v}	1.26±0.03^{v}	1.25±0.03^{v}	1.29±0.03^{v}	1.32±0.03^{v}	3.89	**1.75^{NS}**
	2nd	1.38±0.03^{vw}	1.31±0.03^{vw}	1.42±0.03^{w}	1.36±0.03^{vw}	1.38±0.03^{w}	1.41±0.03^{w}	1.38±0.03^{v}		
	3rd	1.42±0.03^{w}	1.42±0.03^{wx}	1.43±0.03^{w}	1.46±0.03^{wx}	1.40±0.03^{cw}	1.45±0.03^{w}	1.43±0.03^{v}		
	4th	1.51±0.03^{abw}	1.50±0.03^{abx}	1.45±0.03^{aw}	1.48±0.03^{abx}	1.47±0.03^{abw}	1.47±0.03^{abw}	1.58±0.03^{bw}		
	5th	1.68±0.03^{ax}	1.92±0.03^{by}	1.89±0.03^{bx}	1.89±0.03^{by}	1.73±0.03^{ax}	1.87±0.03^{bx}	1.71±0.03^{ax}		

Parameter 1 (a-i), 2 & 3 (a-g) and 4 (a-b) The means in row with different superscript differ significantly at 0.05.

Parameter 1 (u-z), 2 & 3 (v-z) and 4 (v-x) The means in column with different superscript differ significantly at 0.05.

organic acid and control as compared with other treatments. The statistical analysis revealed that, the differences in the weekly live weights of the birds were non-significant (Table 2).

Weekly gain in weight (g)

The average total weight gain (from day 1 to 35) was highest for birds supplemented with cocktail enzyme (713.12 g) as compared with control birds (673.1 g) and birds supplemented with other feed additives. These observations were similar to the findings of Sharifi*etal.* (2012) and Abdel-Raheem*et al.* (2012) in broilers and Zhang and Kim (2014) in Ross broilers. The statistical analysis revealed that, the differences in the weekly gain in weight of the birds was non-significant (Table 2).

Feed consumption

The average total feed consumption (from day 1 to 35) was again highest for birds supplemented with enzyme as compared with control birds and birds supplemented with other feed additives, The observations were similar to the findings of Sharifi*etal.* (2012) in broilers. The statistical analysis revealed that, the differences in the average weekly feed consumption of the birds was significant (Table 2).

Feed conversion ratio

Feed conversion ratio (FCR) was fair for birds supplemented with organic acid (1.73) and antioxidant (1.87) than control birds (1.68) and other supplemented birds. The observations were almost in line with the reports of Awad*et al.* (2009) and Sharifi *et al.* (2012) in broilers. In addition, enzyme (1.92) supplemented birds had a good FCR than control birds. The statistical analysis revealed that, the difference in the weekly feed conversion ratio (FCR) of the birds was non-significant (Table 2).

Mortality

The corresponding mortality percentages of the treatments were 3.33, 3.33, 3.33, 0.00, 0.00, 3.33 and 3.33, respectively. The results were similar to the findings of Awad*et al.* (2009) in broiler.The mortality rate was lower for the probiotic supplemented treatment than other supplemented treatment and control treatment. It is noted that the mortality of birds of different treatments was well within the limit and hence it is concluded that the use of feed additives did not have any ill effect on the health of the birds.

Economics

The economics of production of the present trial was worked out considering the prevailing prices of inputs in the market. The cost of day-old chicks, feed, medication, vaccination, labor and overheads and the amount of return on the sale of the broilers were considered while calculating the profit. Feed cost was the only variable due to differences in the cost and quantity of feed additives used in the trial (Table 3). The economics of broiler production for different treatmentswas calculated at the end of fifth week. The data pertaining to the same are presented in the table 3.

Table 3: Economics of production of broilers of different feed additives

Parameters	T1	T2	T3	T4	T5	T6	T7
Chick cost (Rs)	40.18	40.18	40.18	40.18	40.18	40.18	40.18
Feed intake (g)							
Pre-starter	200	200	200	200	200	200	200
Starter	1000	1000	1000	1000	1000	1000	1000
Finisher	2065.95	2182.22	1955.09	1995.87	1933.45	2033.97	2057.62
Total feed intake (g)	3265.95	3382.22	3155.09	3195.87	3133.45	3233.97	3257.62
Feed price per Kg							
Pre-starter	24.43	24.50	24.87	24.53	24.59	24.45	24.64
Starter	24.87	24.93	25.31	24.97	25.03	24.91	25.08
Finisher	24.65	24.72	25.09	24.76	24.81	24.71	24.86
Feed cost per bird							
Pre-starter	4.89	4.90	4.97	4.91	4.92	4.89	4.93
Starter	24.87	24.93	25.31	24.97	25.03	24.91	25.08
Finisher	50.93	53.94	49.05	49.42	47.97	50.26	51.15
Total feed cost per bird (Rs)	80.68	83.77	79.34	79.29	77.92	80.06	81.16
Miscellaneous cost per bird (Rs)	10	10	10	10	10	10	10
Net cost of production per bird (Rs)	130.86	133.95	129.52	129.47	128.10	130.24	131.34
Body weight at the end of 5thweek (g)	2118.75	2215.92	2125.25	2069.13	2103.88	2087.17	2027.00
Return on sale @ Rs 75 per kg body weight	158.91	166.19	159.39	155.18	157.79	156.54	152.03
Net profit per bird (Rs)	28.04	32.24	29.88	25.71	29.69	26.30	20.68
Net profit per kg (Rs)	13.24	14.55	14.06	12.43	14.11	12.60	10.20

It is observed that the cost of production per bird was lower for the treatment groups as compared to control. Average live weights of the birds from different treatments were the only variable factor affecting returns. The sale price for birds from different groups was considered Rs. 75/- per kg on live weight basis. Itis noted that the birds from treatment receiving diet with cocktail enzyme recorded highest net profit per kg followed by groups T3, T5, T1, T6, T4 and T7. Thus, it is concluded that supplementation of cocktail enzyme with the basal diet is helpful to get better returns. Also, helpful in obtaining more profit due to improved performance in terms of higher live weight gain and lower feed consumption.

The economics of production of broilers of different feed additive is displayed in Table 3. The present study was in agreement with Langhout *et al.* (1999) and Yasar and Forbes (1999) who showed that the poultry innards are affected by diet. From the above mentioned observations, the primary role of a diet is not only to provide enough nutrients to fulfill metabolic requirements of the body but also to modulate various functions of the body. Enzyme, prebiotics and probiotics are either beneficial microorganisms or substrates that facilitate the growth of microorganisms, which could be suitably harnessed by the food manufacturers and hold considerable promise for the health care industry. But among all, supplementations cocktail enzyme treatment improved the health status, intestine development in broiler chickensthus, appearing as an important feed additive for the poultry industry.

CONCLUSIONS

The overall results of the trial indicated that the birds from thetreatment group supplemented with multiple/cocktail enzyme recorded better productive performance with respect to the growth parameters also helped the birds in recording higher net profit per kg as compared to the birds from control group and other treatments.

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Effect of replacing fish meal by Soybean meal on the performance, metabolizability of nutrients and egg quality of Khaki Campbell laying Ducks

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ABSTRACT

Swain, B.K., Naik, P.K., Sahoo, S.K., Mishra, S.K., Kumar, D. and Beura, C.K. 2022. Effect of replacing fish meal by Soybean meal on the performance, metabolizability of nutrients and egg quality of Khaki Campbell laying Ducks. Indian Journal of Poultry Science. 57(02): 151-155.

An experiment was conducted to study the effect of replacing fish meal by soybean meal on the performance, nutrient utilization and egg quality of Khaki Campbell laying ducks. Khaki Campbell laying ducks (35 weeks) were divided into three groups (each having 3 replicates of eight laying ducks) and were randomly fed three experimental diets (T_1-Control diet soybean meal and fish meal, T_2- fish meal totally replaced by soybean meal, T_3-T_2+ supplementation of amino acids i.e. Lysine and Methionine for a period of 100 days. All the diets were made isonitrogenous and isocaloric. The egg production, duck day egg production percent and egg weight were significantly (P<0.05) higher for ducks fed diet containing fish meal. The feed conversion ratio was significantly better in ducks of fish meal group. The feed intake and cost of feed per dozen egg was significantly (P<0.05) reduced in fish meal fed ducks. The metabolizability of nutrients and nitrogen balance were similar for all the groups. However, the digestibility of organic matter was significantly better for the amino acid supplemented group (T_3). The egg quality characteristics viz., shape index, yolk index, albumen index and haugh unit score were significantly (P<0.05) higher for ducks fed diet where fish meal was completely replaced by soybean meal with addition of lysine and methionine. It may be concluded that replacement of fish meal by soybean reduced the performance of ducks in terms of egg production, egg weight, FCR and increased cost of egg production. However, the egg quality was improved due in soybean meal diet with addition of lysine and methionine.

Keywords: Amino acids, Egg quality, Fish meal, Khaki Campbell laying ducks, Metabolizability, Nutrients, Performance, Soybean meal

INTRODUCTION

India ranks second in world duck population, with a total duck population of around 33.5 millions according to the 20th livestock census of India, 2019 (BAHS,2019 and Naik *et al.*,2022)). Duck egg and meat are preferred by people next to chicken and has demand in market. Fish meal is valued by farmers and Nutritionists in developing countries because of its high content of digestible crude protein, essential amino acids, fats, vitamins and minerals (Blair, 2008 and Chadd, 2008). The nutritive value of fish meal resembles soybean meal in various aspects, but its protein quality and potency of some unidentified growth factors vary widely (Swain and Chakurkar, 2011). Previous studies indicated that protein from fish meal can be completely replaced by soybean meal protein with supplementation of 0.10 % methionine in broiler ration economically (Aziz *et al.*, 2001). Soybean meal supplemented with synthetic amino acids can replace fish meal as a main protein source of the diet for ducklings and broilers (Yuan,1989 and Okan and Ogun,1988). Fish meal is an excellent protein source for poultry feeding as it contains adequate quantities of all essential amino acids particularly lysine and methionine required by poultry (Scott *et al.*, 1982). It is a good source of unidentified factors (Bondi, 1987). However, limited availability in recent years, lack of uniformity and higher cost compared to plant protein sources has limited its inclusion in broiler diets (Blair,2008 and Chadd,2008). Soybean meal has the highest feeding value among plant protein sources due to its high protein and essential amino acid contents to meet the nutrient requirement of poultry (Yasothai, 2016).

Keeping in view of above facts, the present study was undertaken to examine the effect of replacing fish meal by soybean meal of the diet on the performance, nutrient utilization and egg quality of Khaki Campbell laying ducks.

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MATERIALS AND METHODS

Three experimental diets (Table 1) were formulated by replacing fish meal of the control diet with soybean meal and supplementation of lysine and methionine i.e. T1 (soybean meal 18 %+Fish meal 7%), T2 (Fish meal-0% only soybean) and T3 (Fish meal 0% + Lysine + Methionine). All the diets were made isonitrogenous and isocaloric. Khaki Campbell laying ducks (35 weeks) were divided into three groups (each group had three replicates with 8 laying ducks per replicate) and were randomly fed above three diets for an experimental period of 100 days. All the birds were reared on deep litter system with standard management practices. The weekly feed intake, daily egg production, and weekly egg weight were recorded and FCR was calculated as amount of feed consumed in kg to produce one dozen egg. The external egg quality parameters i.e. egg weight, length, width, shape index were recorded as per formula of Shultz (1953). The internal egg quality parameters i.e. height of the albumen and yolk, length and width of the albumen and yolk were recorded and albumen index, yolk index and haugh unit were calculated as per the formula of Heiman and Carver (1936), Sharp and Powell (1930) and Haugh (1937), respectively. The feed cost to produce dozen egg was calculated. Fish meal, soybean meal and standard layer diet were analyzed for proximate composition as perAOAC, 2005 (Table 1). At the end of the biological trial, a metabolic trial was conducted with 4 days collection period by keeping the laying ducks in individual metabolic cages. A known quantity of feed was offered daily and excreta voided over 24 hr period was collected quantitatively. The aliquots of excreta were collected daily after mixing it well for dry matter and nitrogen estimation. For dry matter estimation the excreta samples were kept in hot air oven at 70 ^{0}C for 72 hr (Sahoo *et al.*,2014). For faecal nitrogen estimations samples were preserved in 25 % sulphuric acid in duplicate (Pathak and Kamra,1999). The samples of feeds, residues and faeces were analyzed for proximate composition following standard procedures (AOAC, 2005). The metabolizability of the nutrients was calculated as the difference between nutrient intake and nutrient voided.

The data pertaining to various parameters were analysed (Snedecor and Cochran, 1989). The means were tested for significant differences by Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

The egg production, duck day egg production percent, egg weight and feed intake were significantly (P<0.05) higher in ducks fed diet with inclusion of fish meal (Table 2). The feed conversion ratio and cost of feed/dozen egg was significantly (P<0.05) better in laying ducks fed fish meal diet. Similar to present findings, Mundhem and Opstvedt (1981) observed that inclusion of fish meal in the diet of layers significantly increased the egg production with improvement in the efficiency of feed utilisation compared to soybean meal. In a previous study, significantly higher (P<0.05) egg production and better feed conversion ratio was reported in laying hens fed 3 % fish meal compared to control group fed only soybean meal (Rowghani *et al.*, 2007). In contrast, Yuan (1989) suggested that soybean meal supplemented with synthetic amino acids can replace fish meal as a main protein source for ducklings. Similarly, Thongwittaya and Tasaki (1996) concluded from a study that laying ducks produced eggs normally on a diet based

Table 1: Composition of Experimental Diets

Ingredients	T_1 (Control diet with Fish meal)	T_2 (Without Fish meal)	T_3 (Without FM and supplementation of Lysine & Meth.)	Fish Meal	Soybean Meal
Wheat	55	55	55		
Soybean meal	18	23	23		
Fish meal	07	-	-		
DORB	07	09	09		
Shell Grit	10	10	10		
DCP	02	02	02		
Calcite	01	01	01		
Trace Min.Mix.	100g	100g	100g		
Lysine	Nil	80g	230g		
DL-Meth.	100g	140g	240g		
AB2D3K	15g	15g	15g		
B-Complex	15g	15g	15g		
Toxin Binder	100g	100g	100g		
Choline Chloride	100g	100g	100g		
VitE Se	15g	15g	15g		
Chemical Composition, Analysed %					
Crude Protein	18.76	18.12	18.12	49.40	46.45
Ether Extract	3.13	3.17	3.14	4.78	1.65
Crude Fibre	4.45	4.36	4.32	3.52	6.88
Total Ash	10.47	9.22	9.10	20.45	9.39
NFE	63.19	65.18	65.32	21.85	35.63

on soybean meal (21%) with no fish meal, however, the egg production increased when diet was supplemented with methionine and lysine. In another study, RamaRao *et al*. (1998) demonstrated that replacement of fish meal with either different vegetable proteins or synthetic amino acids gave better results in efficiency of production and cost of production. The DM digestibility (76.64 to76.76, Table-3) was similar for all the treatments. Higher DM metabolizability (76.17 to 78.87) was reported in white pekin ducks (Naik *et al*., 2021) and (75.46 to 79.38) in Khaki Campbell ducks (Joshi *etal*.,2015). However, lower DM digestibility of 72.99 to 75.38 and 74.91 to 75.78 were reported in White Pekin and Khaki Campbell ducks, respectively (Sahoo *et al*., 2014). The OM digestibility was significantly (P<0.05) higher in T3 group but lower than the values reported by Sahoo *et al*. (2014) (77.29 to 80.78) and Joshi *et al*. (2015) (80.67 to 83.79). The metabolizability of CP (72.99 to 73.57) in this study was similar in all treatments but higher than the values reported by Naik *et al*. (2021) (67.40 to 70.09) and Sahoo *et al*. (2014) (67.26 to 70.73). The metabolizability of EE (76.82 to 77.17) in present study was similar in all treatment groups and higher than the values reported by earlier workers (Joshi *et al*., 2015). The CF metabolizability (67.06 to 67.34) was similar among all the groups and higher than the values (41.57 to 51.23 and 59.57 to 62.05) reported by earlier workers (Sahoo *et al*., 2014 and Naik *et al*., 2021). There was no significant (PÂ0.05) difference in the nitrogen balance (5.01 to 5.05) and nitrogen balance as % of N intake (72.99 to 73.57) among various treatment groups. Lower values of nitrogen balance (2.35 to 4.22) and nitrogen balance as % of N intake (67.40 to 70.09) were reported by earlier researchers (Naik *et al*., 2021). The difference in the values of nutrient metabolizability among the findings of various studies might be due to the difference in the feeding regime. The egg weight was significantly higher (P<0.05) in ducks fed fish meal diet (T_1) compared to those fed T_2 and T_3 diets. Similarly, higher (P<0.05) egg weight was reported in laying hens fed 3 % fish meal compared to those fed only soybean meal as a protein source (Rowghani *et al*., 2007). In contrast, significant (P<0.05) increase in egg weight was reported in egg laying ducks and Hy-Line laying hens as dietary lysine level was increased (Fouad *et al*., 2018 and Proschaska *et al*.,1996). The egg quality parameters i.e. shape index, albumen index,yolk index and haugh units were significantly (P<0.05) better in laying ducks fed diets with SBM (without fish meal diet) and addition of lysine and methionine (Table 4). However, earlier workers reported that dietary supplementation of lysine had no effects on egg shape index and haugh unit (Fouad *et al*.,

Table 2: Effect of replacement of fish meal by soybean meal on the egg production performance of Khaki Campbell laying ducks

Treatments/Attributes	**T_1**	**T_2**	**T_3**	**SEM**
Egg Production (Dozen)	5.58[a]	3.77[b]	3.39[c]	0.052
Duck Day Egg Production (%)	72.03[a]	46.65[b]	42.40[b]	4.64
Feed Intake, Kg	15.98[a]	15.18[b]	14.24[c]	0.103
FCR (Feed:EggProdn in dozen)	2.868[c]	4.027[b]	4.202[a]	0.033
Egg Weight (g)	66.68[a]	64.87[b]	63.97[b]	0.439
Cost of Feed/Dozen egg (Rs)	79.99[a]	110.94[b]	119.12[c]	6.009

[a,b]Means with different superscripts in a row differ significantly (P<0.05)

Table 3: Effect of replacement of fish meal by soybean meal on metabolizability of various nutrients and nitrogen balance in Khaki Campbell laying ducks

Parameters	Dietary Groups			SEM
	T_1	T_2	T_3	
Metabolizability (%) of Nutrients				
Dry matter	76.76	76.72	76.64	0.11
Organic matter	78.94[a]	78.56[ab]	78.01[b]	0.14
Crude protein	73.38	73.57	72.99	0.16
Ether extract	76.82	77.16	76.87	0.11
Crude fibre	67.06	67.34	67.12	0.20
Nitrogen Balance				
N intake (g/d)	5.01	5.05	5.05	0.04
Nitrogen outgo (g/d)	1.33	1.33	1.37	0.01
N Balance (g/d)	3.68	3.71	3.69	0.03
N balance as % of N intake	73.38	73.57	72.99	0.16

[a,b]Means with different superscripts in a row differ significantly (P<0.05)

Table 4: Effect of replacement of fish meal by soybean meal on the egg quality characteristics of Khaki Campbell laying ducks

Treatments/Attributes	T_1	T_2	T_3	SEM
Shape Index	71.95[a]	72.88[ab]	73.98[b]	0.350
Albumen index	0.114[a]	0.112[a]	0.125[b]	0.002
Yolk index	0.447[a]	0.457[a]	0.500[b]	0.008
Haugh Unit	87.17[a]	90.69[b]	92.77[b]	0.874
Shell thickness with membrane	0.540	0.547	0.542	0.006
Shell thickness without membrane	0.435	0.428	0.435	0.004
Egg Contents,%	86.56	86.72	86.92	0.134
Albumen,%	51.55[a]	50.66[ab]	49.75[b]	0.322
Yolk, %	38.52	38.17	38.41	0.152
Shell,%	11.43	11.77	11.85	0.085

[a,b] Means with different superscripts in a row differ significantly ($P<0.05$)

2018). In contrast, no improvement in haugh unit in Hy-Line Brown laying hens and Hy-Line W-36 laying hens was reported due to dietary supplementation of lysine (da Rocha *et al*., 2009 and Souza *et al*.,2014). The value of haugh unit ranged from 87.17-92.77 in present study. Similarly, a range value of 87.80 -90.45 was reported in Khaki Campbell laying ducks by earlier researchers (Swain *et al*., 2020).

It may be concluded that replacement of fish meal by soybean meal reduced the performance of Khaki Campbell laying ducks in terms of lower egg production and egg weight and poor FCR. However, the egg quality was improved due to replacement of fish meal by soybean meal with additional lysine and methionine.

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Effect of different stocking densities and flock size on welfare and behavior of White Leghorn layers in conventional California cages

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ABSTRACT

Roy, P., Kadam, M.M., Bhaisare, D.B. Bhanja, S.K. and Rokade, J.J. 2022. Effect of different stocking densities and flock size on welfare and behavior of White Leghorn layers in conventional California cages. Indian Journal of Poultry Science. 57(02): 157-163.

The present investigation was planned to study the welfare and behavior performance of laying hens at two different stocking densities and flock size in conventional California cages. A total of 180 commercial White Leghorn (strain BV 300) layers were divided into four groups with six replicates and reared under standard managemental practices. Interaction effect of two best stocking density (548 and 645cm^2/b) and flock size (06 and 09 birds) in conventional cages was conducted along with comparison of welfare and behavior of commercial laying hens in conventional California cages. Interaction effect of stocking density and flock size resulted in non-significant effect on all of the welfare parameters and total state and event behavioral parameters except significantly better sleeping, drinking and least feather pecking was observed in 548cm^2/b x 6B. Therefore, it could be concluded that rearing of commercial layer in conventional California cages is best suited for stocking density of 548cm^2/b or 85 sq. inch/b with flock size of 6 birds.

Keywords: Behaviour, California cages, Flock size, Layer, Stocking density, Welfare

INTRODUCTION

In India the poultry sector has undergone colossal transformation in structure and operation from a mere backyard activity into a major commercial agri-based industry over a period of time. The intent of poultry production is to provide safe and healthy animal protein to the ever-growing population, overcoming the challenges cropping up on its sustainability. Egg laying hens are kept traditionally in conventional cages (battery cage) for high intensity production so as to maximize egg production per unit area. In the process the hens have to live for the entire production cycle in a very limited space as the sole objective is to increase the productivity and profit (Asghar *et al*. 2012; Hester, 2016). This neglected the welfare state of caged hens which led to introduction of new minimum welfare standards. Hens housing environment and other general management practices are a matter of concern and determine the welfare of layers (Carmichael *et al*. 1999).

Since EU banned cage rearing of birds, the cage rearing become a global issue in poultry sector. Better stocking density, optimum flock size and raising birds in furnished cages are some of the attempts brought about in a response to the criticism of raising commercial laying hens in conventional battery cages for egg production (Weimer *et al*. 2019). In battery cages, stocking density of 300 to 650 cm^2 space provides per hen. Reportedly, providing Less than 465 cm^2 of space per hen causes stress (Widowski *et al*. 2013). In Indian context California cage space standards for rearing commercial laying hens must be tested. There is need to develop furnished cages with little modifications in existing California cages to maintain welfare friendly environment within the routinely used California cage system. Therefore, the present investigation was carried out at Poultry Research and Training Centre, Department of Poultry Science, Nagpur Veterinary College, Nagpur with an objective to study the effect of different stocking density and flock size in conventional California cages on welfare and behavior of commercial layers.

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MATERIALS AND METHODS

The experiment was carried out as per the approved protocol of Institute Animal Ethics Committee (IAEC) at Poultry Research and Training Centre, Department of Poultry Science, Nagpur Veterinary College, Nagpur, Maharashtra-440 006, India.

The research study was conducted for a period of 20 weeks on the 28 - 47 weeks old laying birds (BV 300) to note the effect of two different stocking densities of 548 cm^2 or 85 sq. inch per bird and 645cm^2 or 100 sq. inch per bird to test welfare and behaviour of laying hens at two flock sizes of 6 and 9 birds. A total of 180 birds were divided into four treatment groups A1, A2, B1 and B2. Each cage box represented one replicate and total six replicate were used to conduct the interaction effect of stocking density and flock size experiment to determine the best stocking density and flock size.

The 180 available commercial White leghorn layer (strain BV 300) birds of age 28 weeks at Poultry Research and Training Centre, Department of Poultry Science were employed to conduct the experiments. Before the initiation of an experiment the birds were dewormed and

Table 1: Experiment I Rearing of laying hens at two different stocking densities and flock sizes in conventional California cages

Treatments		Space per bird	Flock Size	Bird x cage Box	Flock Sizeeach box	Replicate	No. of birds
A	A1	85 sq. inch/bird (548 cm²/bird)	Double	3x2	06 birds	**6**	**36**
	A2		Triple	3x3	09 birds	**6**	**54**
B	B1	100 Sq. inch/bird (645 cm²/bird)	Double	3x2	06 birds	**6**	**36**
	B2		Triple	3x3	09 birds	**6**	**54**

*Restructured cage box specification of group A1 34" X 15"X16 " and A2 51"X15"X16 " (width x depth x height) Cage box specification of group B1 40"X15"X16"and B2 60"X15"X16" (width x depth x height)

vaccinated against New Castle Disease. As per experimental design, the birds were shifted three weeks before into the assigned cages so that the birds get acclimatize with change in housing environment. The birds were maintained on standard commercial layer feed. A commercial layer feed was procured from renowned poultry feed company M/s Megataj Agrovet Pvt Ltd, MIDC, Nagpur. As per recommendation (BV 300 manual) for commercial laying hen, the birds were offered with 110 gm feed/bird/day. The feed was offered twice a day in the proportion of 40g (Morning): 60g (Evening) for optimum production performance. Feed intake of the birds was calculated at weekly intervals. Throughout the experimental period, 16 hours of light was provided with ideal management conditions. The birds were given free access to fresh, clean and wholesome drinking water. The White leghorn layer birds were reared in high rise California (conventional) cage housing arranged in 3-tier system. The experimental birds were distributed in different replicates in such a way to nullify the row and tier effect of cage housing system. Throughout the experimental period 16 hours of light was provided with ideal management conditions. The specifications of BV 300 manual were followed for all management practices including medication and vaccination. Four CCTV cameras were installed in the experimental shed to cover all angles of the bird rearing to record the welfare and behaviour activities of the laying birds. The recording was stored in the digital video recorder (DVR) and retrieved time to time to study the different behavioural activities of the birds.

Parameters studied

Welfare of cage laying birds: The welfare of the laying birds was judged by physical, physiological and behavioural indicators at different stages of the experimental period [28th (start), 37th (mid) and 47th (end) weeks of age].

Physical Indicators

Feather score: Feather scores were recorded at different time period, considering the same bird for every time to measure the effect of stocking density and flock. Plumage condition was scored using a modified eight point scoring system (Abrahamsson *et al.,* 1996) for different areas of the body parts that is head, neck, back wings, tail, abdomen and breast.

Gait score: Similar to feather score method, gait score was recorded in the same birds used for feather score at different phases of the experimental period with modified 4-point scoring system (Kestin *et al.*, 1992). The gait scoring was performed by two persons, one gently driving and herding each bird with a light cane and the other observing from a crouched position. An understanding between the two assessors/examiners for each bird was maintained before the score was recorded.

Physiological Indicators

Heterophil to lymphocyte ratio was calculated as the method described by Gonzales *et al.* (2003) and Corticosterone has been analyzed by ELISA kit (Eiahcor 96-Invitrogen-Thermo Fisher Scientific). Six birds from each treatment were randomly selected for the blood collection at 28th (start of experiment), 37th (mid of experiment) and 47th (end of experiment) weeks of age. All blood samples were allowed to clot for 1 hour and serum was decanted in centrifuge tubes and stored at -20°C for further analysis.

Behavioural observations

Behavioural observations were assayed to quantify animal biological responses. Poultry behaviour is a reflex of their welfare status at a particular moment and is related to internal (physiological) and external (environmental) factors.

State behaviour: States are behaviour of longer duration, such as prolonged activities, body postures or proximity measures. The activities which were taken under state behaviour are standing, sitting, sleeping, walking, perching and investigation.

Event Behaviour: Relatively shorter duration of behavioural patterns such as discrete body movements or vocalization, which can be approximated as points in time. The activities undertaken are preening, feather pecking, feeding, drinking, scratching, wing flapping and fighting. All the state and event behavioural activity of the birds were video recorded in CCTV camera for 24 hrs during the entire experiment. Periodically the recording was extracted from hard disc of DVR on 28 (start of experiment), 37 (mid of experiment) and 47th (end of experiment) weeks of age of the bird to study the behaviour pattern of the laying birds at different stocking densities and flock sizes in conventional cages. Continuous samplings for three birds per treatment were individually compared for 24-hrs. The specific birds (03) from each treatment were marked with different colours (waterproofed, non-toxic dyes-black, red, blue, yellow, pink etc). From the specific 24 hrs recording, an activity chart or ethogram was prepared, an example shown for half an hr time period in Table 2. The time devoted (01 min) to each activity was expressed. The time budgets for feeding (1), drinking (2), standing (3), sleeping (4), sitting (6), walking (8), investigation (5), perching (7), feather pecking (11), wing flapping (13), scratching (10), fighting (12) and preening (9) on the perches were calculated as the mean proportion of birds performing each behaviour over the 24 hrs on 28th (start of experiment), 37th (mid of experiment) and 47th (end of experiment) weeks of age of the bird. As suggested by Li *et al*. (2016), the description for the various state and event behaviours is given in Table 3.

Statistical Analysis

Data emanated from different treatments were analyzed for statistical significance using completely randomized factorial designs (Snedecor and Cochran, 1989). All data were statistically analyzed using SPSS software package version 20.0. Variables having unequal observations were analyzed following least square design method and the Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

The results of the interaction effect of flock size and stocking density on the physical indicators of welfare parameter like feather score, gait score and physiological indicators like heterophil-lymphocyte ratio and serum corticosterone is presented in Table 4 and 5.

There was no interaction effect of flock size and stocking density observed for the various physical and physiological indicators like feather score, gait score, heterophil-lymphocyte ratio and corticosterone level. The mean feather score, gait score, heterophil-lymphocyte

Table 2: Activity chart format for half-hour time (12:00 to 12:30)

12-12:30pm Birds	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1																														
2																														
3																														
4																														
5																														
6																														

F= Feeding(1), D=Drinking(2), Sl= Sleeping(4), Si= Sitting(6), S=Standing(3), W= Walking(8), I=Investigating(5), F=Fighting, Ph=Perching(7), FP=Feather pecking(11), Sc=Scratching(10), P= Preening(9), WF-Wing Flapping(12).

Table 3: Behavioural categories and Definitions

Behavioural categories	Definition
Feeding	Hen directs its beak to feed trough and carries out pecking or eating,
Drinking	Hen directs its beak to nipple drinker and raises its head when getting water.
Standing	Both legs are straightened on the floor.
Sleeping	Hens' abdomen contacts with the floor and both legs are twisted under the body with eyes closed.
Investigation	Visual exploration and head movements while body is stationary.
Sitting	Hens' abdomen contacts with the floor and both legs are twisted under the body with eyes open.
Perching	Behaviors exhibited when the hens are on perches.
Walking	Hen raises one of its legs with the other leg standing on floor and moves forward.
Preening	Hen directs its beak to its own plumage of several body parts and carries on pecking, nibbling, combing or rotating movements, once or repeatedly.
Scratching	Hen directs its claw to the scratch-pad provided and rubbing it repeatedly.
Feather-Pecking	Hen pecking or pulling others' feather.
Fighting	Behaviors happened between two or more hens including pecking.
Wing Flapping	Bilateral movement of the wings including wing raising while standing.

ratio and corticosterone level did not differ significantly due to main effect of stocking density and various levels of flock size. Significant improvement in plumage condition ($P<0.05$) was observed by Davami *et al.*, (1987) due to interaction effect of flock size and stocking density. As the poorer plumage of densely populated cages results because of abrasion against cage wire and other hens. The plumage condition of hens has been reported to be related to feed conversion and in this instance FCR was better. This is in contrast to present experiment where feather score was non-significant among the various treatment groups probably because the best stocking density of 548 and 645 cm^2 space was provided, may be with higher stocking density and flock size on the feather and gait score issue arises. Craig and Milliken (1989) observed step wise adverse influence on feather score due to increase in stocking density, but there was no effect of flock size (four and eight birds) on the feather score and no interaction effect of stocking density and flock size.

Table 4: Interaction effect of stocking density and flock size on immune status, feather and gait score

Treatment	Feather Score	Gait score
548cm^2/b X 6B	6.89±0.22	8.00±0.00
548cm^2/b X 9B	6.67±0.00	8.00±0.00
645cm^2/b X 6B	7.00±0.62	8.00±0.00
645cm^2/b X 9B	6.67±0.52	8.00±0.00
Effect of stocking density		
548cm^2/b	6.78± 0.29	8.00± 0.00
645cm^2/b	6.83± 0.29	8.00± 0.00
Effect of flock size		
6 Birds	6.94±0.29	8.00±0.00
9 Birds	6.67±0.29	8.00±0.00
Pooled SEM	0.208	0.00
Statistical significance		
Treatment	NS	NS
Stocking density	NS	NS
Flock size	NS	NS

Non-significant difference in H/L ratio was observed by Mench *et al.*, (1986) when two group sizes of 1 and 2 birds were maintained in cages at same stocking density of 1394cm^2/bird also in comparison to 25 bird group size in pen at the same stocking density. Non-significant differences in H/L ratio for different flock sizes 2450 vs 4200 was also observed by Nicol *et al.*, (2001). In parallel present experiment also had non-significant differences in H/L ratio observed at different periods for the different flock sizes. Stress decreases the number of lymphocytes and consequently a rise in the H/L ratio, the non-significant difference is an indicative of no effect of interaction of stocking density and flock size on stress. Hens at same flock size of two birds per cage at different stocking density (1394 and 697 cm^2/bird) had significant difference ($P<0.05$) in corticosterone level, however different flock sizes -single and two at same stocking density (1394cm^2/bird) did not bring any change in the corticosterone level as reported by Mench *et al.* (1986). Craig *et al.* (1986) reported non-significant difference in plasma corticosterone level for different group size of one, four and six bird per cage at stocking density of 929, 464 and 310 cm^2/bird measured at 45 weeks after housing. Corticosteroid concentration in plasma appears to be so variable among hens treated alike that significance may be detectable only when a relatively large number of difference in the treatment and number of samples were collected. Nicol *et al.* (2001) did not report any significant differences in faecal corticosterone level at large different flock size of 2450 birds and 4200 birds at same stocking density of 12 bird/m^2. In contrast Cheng and Muir (2004) reported that laying hens showed significantly ($P<0.05$) lower plasma corticosterone levels in single bird cages (525 cm^2/bird) than in the 10 bird cages (419 cm^2/bird), indicating that social stressors could be a factor in higher production of corticosterone in hens.

In the present experiment non-significant results in corticosterone level due to interaction effect, main effect of flock size and stocking density was similar to the finding of few authors and was probably due to less stress at stocking density of 548 and 645 cm^2/bird in combination with flock size of six and nine birds. Significance may be detectable only when a relatively large number of difference in the flock size.

Behavioral Study

The various activities of the bird's behavior (standing, sitting, sleeping, investigating, walking, drinking, feeding, preening, feather pecking, scratching, wing flapping etc) were recorded by a CCTV camera for 24-hour period and an activity chart or ethogram from the video recordings were noted for behavior analysis. The time spent on each behavior was observed and expressed in minute for the specific 24-hour (1440 minutes) of the day. The specific 24 hour (1440 minute) behavioral observations collected at three different periods (28, 37 and 47 week) of experiment were averaged and presented in Table 6. The average of three different stages/weeks of each behavioral activity for the interaction effect and main effect of stocking density and flock size is discussed as below.

Interaction Effect of Stocking Density and Flock size

The averaged values of the interaction effect of stocking density and flock size were non- significant for behavioural activities observed at 28th, 37th and 48th week except for sleeping, drinking and feather pecking (Table 6). The birds with space allowance 548cm^2/b x 6B showed significantly ($P<0.01$) highest sleeping (305.83) behaviour compare to any other treatment groups.

Table 5: Interaction effect of stocking density and flock Size on corticosterone and heterophil: lymphocyte ratio

Treatment	H/L Ratio at 28th week	H/L Ratio at 37th week	H/L Ratio at 47th week	Corticosterone (ng/ml) at 28th week	Corticosterone (ng/ml) at 37th week	Corticosterone (ng/ml) at 47th week
548cm^2/b X 6B	0.26±0.02	0.31±0.03	0.23±0.01	1.202±0.01	0.933±0.09	1.022±0.06
548cm^2/b X 9B	0.30±0.04	0.34±0.02	0.23±0.01	1.158±0.07	0.915±0.09	0.857±0.08
645cm^2/b X 6B	0.31±0.04	0.29±0.02	0.25±0.02	1.078±0.07	1.010±0.04	1.005±0.06
645cm^2/b X 9B	0.27±0.05	0.29±0.04	0.23±0.02	1.233±0.06	0.870±0.09	0.893±0.16
Effect of stocking density						
548cm^2/b	0.28±0.03	0.33± 0.02	0.23± 0.01	1.180±0.04	0.924±0.06	0.939±0.07
645cm^2/b	0.29±0.03	0.29±0.02	0.24± 0.01	1.156±0.04	0.940±0.06	0.949±0.07
Effect of flock size						
6 Birds	0.28±0.03	0.30±0.02	0.24±0.01	1.140±0.04	0.972±0.06	1.013±0.07
9 Birds	0.28±0.03	0.32±0.02	0.23±0.01	1.196±0.04	0.893±0.06	0.875±0.07
Pooled SEM	0.019	0.014	0.007	0.029	0.041	0.049
Statistical significance						
Treatment	NS	NS	NS	NS	NS	NS
Stocking density	NS	NS	NS	NS	NS	NS
Flock size	NS	NS	NS	NS	NS	NS

Means bearing superscript within a column differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

Whereas, 645cm^2/b x 9B recorded significantly lowest sleeping (191.83) activity. The birds with 548 cm^2/b x 6B and 645 cm^2/b x 6B recorded significantly more sleeping behaviour than their counter parts i.e. 548 cm^2/b x 9B and 645 cm^2/b x 9B. Time spent on drinking was significantly higher (P<0.01) at both 548cm^2/b x 6B and 548cm^2/b x 9B treatments when compared to treatment groups of 645 cm^2/b x 6B and 645 cm^2/b x 9B, though resource allocation i.e availability of nipple drinkers were proportionately same. Feather pecking among the various treatment groups was significantly lower (P< 0.01) at 548cm^2/b x 6B when compared to 548cm^2/b x 9B and 645cm^2/b x 6B but remained non-significant with 645 cm^2/b x 9B.

Abrahamsson and Tauson (1997) reported no deteriorative effects on plumage condition or cannibalism/ feather pecking were detected between the different group sizes and suggested a group of 8 hens may be considered being below a critical group size, regarding outbreaks of feather pecking or cannibalism. The extent of damage on the feather as evident from feather score (Table 4) because of feather pecking behavior was non-significant, probably the feather pecks were non-aggressive, though gentle and aggressive feather pecks were not estimated in this trial. Feather pecking may be a type of social grooming with no injury intent and may be a form of recognition between birds.

It could be concluded that the interaction effect of stocking density and flock size brought a significant better sleeping and drinking activities and significant less time spent on the feather pecking for the group 548cm^2/b x 6B.

Main effect of Stocking density

The main effect of stocking density for the averaged values (28th, 37th and 47th week) of behavioural activities was observed significant for standing, sleeping and drinking (Table 6). The birds reared with lower stocking density 645cm^2/bird had more standing time (P<0.05) compared to the birds reared at 548cm^2/bird.

While behavioral patterns for sleeping and drinking was opposite with significantly (P<0.01) more time was spent by birds for sleeping and drinking in group 548cm^2/bird as compared to 645cm^2/bird for the main effects of stocking density. This was in contrast to higher drinking behavior for birds reared in 1016 cm^2/hen of low stocking density as reported by Albentosa *et al.* (2007) when compared to high stocking density of 762cm^2 and 609cm^2/bird.

Hughes and Black (1974) reported that the birds spent more time for standing when are at the higher stocking rate (P<0.001) which was confounding to the present result observed with main effects at stocking density 548cm^2/b which had the higher stocking density but significantly lower standing when compared to 645cm^2/b. Anderson *et al.* (2004) also indicated more movement acts (P<0.05) when birds were kept in low density (482cm^2/bird) than in high density (361cm^2/bird), in present main effects of stocking density on walking was though non-significant, numerical more movements observed in 645cm^2/bird as compared to 548cm^2/bird.

It was concluded that the significant higher drinking and sleeping in 548cm^2/bird for the main effect could be possible because of social dominance at lower stocking density of 645cm^2/bird and monopolization of the resources, leading to overall lower drinking time.

Main effect of Flock Size

Table 6: Interaction effect of Stocking Density(SD) and Flock Size (FS) on state and event behaviours of bird for 28, 37 and 47 week (average) of age

Parameters	Interaction Effect of Stocking density and Flock Size				Effect of Stocking Density (SD)		Effect of Flock Size (FS)		SEM	Statistical Significance		
	548cm^2/bX6B	548cm^2/bX9B	645cm^2/bX6B	645cm^2/bX9B	548cm^2/b	645cm^2/b	6 birds	9 birds		Tr.	SD	FS
Standing	217.50±14.49	175.67±9.11	232.00±13.24	231.83±25.90	196.58^{b}± 11.93	231.92^{a}±11.93	224.75±11.93	203.75±11.93	8.436	NS	*	NS
Sitting	187.17±0.48	227.50±12.17	197.83±13.47	197.00±14.20	207.33±8.15	197.42±8.15	192.50±8.15	212.25±8.15	5.764	NS	NS	NS
Walking	33.00±3.44	42.00±3.38	46.67±5.26	42.17±4.59	37.50±3.00	44.42±3.00	39.83±3.00	42.08±3.00	2.121	NS	NS	NS
Investigation	117.83±14.75	125.67±11.62	122.17±07.55	152.33±8.11	121.75±7.71	137.25±7.71	120.00±7.71	139.00±7.71	5.450	NS	NS	NS
Sleeping	305.83^{a}±5.31	238.67^{b}±03.28	216.50^{c}±04.24	191.83^{d} ±5.97	272.25^{a}± 3.40	204.17^{b}± 3.40	261.17^{a}±3.40	215.20^{b}±3.40	2.406	**	**	**
Total State	861.33±8.17	809.50±17.54	815.17±15.23	815.17±24.41	835.42±12.29	815.17±12.26	838.25±12.26	812.33±12.26	8.668	NS	NS	NS
Drinking	99.67^{a}±0.71	105.00^{a}±07.43	83.17^{b}±05.72	80.50^{b}±3.52	102.22^{a}± 3.55	81.83^{b}± 3.55	91.42±3.55	92.75±3.55	2.510	**	**	NS
Feeding	354.67±13.11	391.33±11.91	404.67±27.11	396.17±32.55	373.00±16.24	400.42±16.24	379.67±16.24	393.75±16.24	11.480	NS	NS	NS
Preening	108.67±8.00	113.33±02.49	115.33±05.78	129.83±10.04	111.00±5.06	122.58± 5.06	112.00±5.06	121.58±5.06	3.574	NS	NS	NS
Feather pecking	11.50^{b} ±0.22	15.83^{a}±01.72	16.50^{a}±01.09	14.50^{ab}±01.06	13.67±0.82	15.50±0.82	14.00±0.82	15.17±0.82	0.576	**	NS	NS
Wing Flapping	3.17±0.65	4.67±0.61	5.17±0.48	3.83±0.48	3.92±0.40	4.50±0.40	4.17±0.40	4.25±0.40	0.281	NS	NS	NS
Total events	578.67±8.17	630.50±17.54	624.83±15.23	624.83±24.41	604.58± 12.26	624.84±12.26	601.75±12.26	627.67±12.26	8.668	NS	NS	NS

Means bearing superscript within a row differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

The main effect of flock size for the averaged values (28th, 37th and 47th week) of behavioural activities observed significant only for sleeping behavior (Table 6). The treatment group with flock size of 6 birds had significantly higher (P<0.01) sleeping activity as compared to 9 bird flock size. In the present experiment the main effect of feather pecking for the averaged values were seen higher in the flock size with nine birds as compared to six though it were only numerical.

Bilcik and Keeling (1999) reported significant effect on feather pecking because of group size when birds were maintained in pens in group size of 15, 30, 60 and 120 birds. It was concluded that total number of observed gentle feather pecks (273) received was higher than number of severe pecks (137) received, 66·6% Vs 33·4% and only a small proportion of actual feather damage can be attributed to the gentle type of pecks. In contrast, severe feather pecks were found to be strongly related both to feather damage and skin injuries.

Anderson *et al.* (1989) reported that group size had significant (P< 0.05) effects on the amount of standing, crouching, preening, feeding and comfort movements during the first 5 days post-housing in layer cages. Hens housed four/cage spent significantly less time standing, more feeding and more time preening than hens housed six/cage which is similar to present experiment, though non-significant, but numerically more standing activity was observed for birds with flock size of six as compared to nine. Contrasting preening and feeding result was observed in present study with non-significant differences for flock size because the fact better feeding and cage space availability to all flock sizes in the present experiment could not be ignored.

CONCLUSION

The commercial laying hens (White Leghorn) reared in conventional California cages at 548cm^2/ b or 85 sq. inch/b and 645cm^2/ b or 100 sq. inch/b with six bird flock size performed best on the basis of welfare. It could be concluded that rearing of commercial laying hens (White Leghorn) in conventional California cages is best suited for minimum stocking density of 548cm^2/ b or 85 sq. inch/b with flock size of six birds with optimum expression of welfare and behavior of laying hens.

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Comparative efficacy of evaporative cooling pad and fogger system on behaviour of broiler chicken

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ABSTRACT

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High environmental temperature affects behaviour in poultry due to alteration in microclimate. The experiment was conducted for a period of six weeks to access the comparative efficacy of evaporative cooling pad and fogger system on behaviour of VenCobb broiler chicken (n=180) in semiarid region of Rajasthan. The birds were randomly divided into three treatment groups i.e. T_1, T_2 and T_3 comprising three replications of 20 birds each. The control group T_1 had pen without cooling device, while other treatment groups T_2 and T_3 had evaporative cooling pad and fogger systems, respectively. The behavioural status of broiler birds was recorded using handy cam video recorder. Instantaneous sampling technique was used for recording the behaviour of broiler chicks. The observations were taken for 15 minutes in each replicate between 01.00-03.00 pm, twice a week starting from the 3rd week and continued throughout the experiment. Feeding activities of broilers in evaporative cooling pad and fogger system were significantly ($p<0.01$) higher than in control group. Panting, drinking and resting activities were significantly ($p<0.05$) higher in control group as compared to evaporative cooling pad and fogger system groups but no significant difference was observed in resting behaviour. The leg stretching, scratching, preening and dust bathing activities were significantly ($p<0.05$) lower in control group than evaporative cooling pad and fogger system whereas, wing flapping was not significantly different among groups. It was concluded that the evaporative cooling pad and fogger system ensured significant improvement in the behavioural performance of broilers during heat stress period.

Keywords: Broiler, Behaviour, Evaporative cooling pad, Fogger system

INTRODUCTION

Poultry production, particularly broiler production is the quickest way to increase the availability of high-quality protein for human consumption (Karnani *et al*., 2019). The broiler industry faces the challenge of heat stress (HS), which increases production cost and severely damages the meat quality due to poultry's susceptibility to heat because of their higher metabolic rate and growth (Nawaz *et al*., 2021). Poultry appears to be particularly vulnerable to environmental problems related to temperature, notably heat stress. High environmental temperature and temperature humidity index value above the critical level lead to decrease feed intake, lower body weight, and lower feed conversion efficiency (Sohail *et al.*, 2012). The metabolic heat along with high ambient temperature reduce the body weight and feed intake by 23 per cent and 15 per cent respectively, in broiler birds (Yalcin *et al*., 1997). The effect of heat stress is leading to a sequence of behavioural, physiological and biochemical adaptive changes (Chib, 2015) culminating in to serious production and economic losses (Mack *et al*., 2013) in broilers. Environmental controlled poultry houses are yet uncommon in India and such facilities may increase the cost of production, making poultry production uneconomical. To reduce heat and its consequences in broiler houses, other effective alternatives include evaporative pads, fogger pads, and fogger nozzles (Weaver, 2002). Further, use of these cooling systems becomes impractical during monsoon season, when both temperature and humidity remains high (Gupta *et al*., 2013). In order to decrease the interior temperatures, evaporative cooling systems are utilized to mitigate heat stress. Therefore, the present study was planned to access comparative efficacy of evaporative cooling pad and fogger system on behaviour of broiler chicken during heat stress period.

MATERIALS AND METHODS

The proposed study was conducted on 180 day old broiler chicks (Ven Cobb strain) procured from commercial hatchery. The experiment was conducted for a period of 6 weeks in May-July 2019. The shed, consisted of pens with 10x10 square feet area. Out of the total three (03) of such pens, two (02) were modified for installation of two (02) different cooling systems and the remaining one without any cooling system was considered as control. Further, each pen was partitioned into three identical compartments of 20 square feet each by using wire nettings. One square feet floor space per bird was provided in these sheds.

Design of Evaporative cooling pad system

The evaporative cooling pad system consisted of

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cellulose pads which were made up of cellulose paper hedges in aluminium casing with a water distributor through a Galvanized Iron header. The complicatedly woven cellulose pads were enough to provide required amount of water to fulfill maximum cooling of air coming in contact. The evaporative cooling pads were placed at one end of the shed and exhaust fan of the opposite end. The water was pumped to the pads through a pump and the pads were kept wet.

Fogger system

Fine foggers (2 in number) each having diameter of 0.2 mm capable of producing mist with a pressure of 30 psi were installed at the height of 6 ft from ground, which were connected with water tank through a high pressure pipe. The filtered water was pumped into the foggers. The foggers were set up for the controlled on/off–timing with the help of a timer. This cycles for 30 second on and 60 seconds off time of the foggers were repeated whole day. When switched on, foggers generated a fine mist to cool the shed. Exhaust fan was running whole day irrespective of foggers. Temperature (ºC) and humidity (per cent) of shed of T_1, T_2 and T_3 groups were 38.36 and 48.00, 28.28 and 71.57, 32.43 and 64.43, respectively.

Broilers were maintained under standard management practices regarding brooding, feeding, watering and health care throughout the trial period. The cooling systems were operated from start of 3rd week to the end of 6th week of age as brooding of chicks was carried out for first 2 weeks. The behavioural status of the birds was recorded using handy cam video recorder. Instantaneous sampling technique was used for recording the behaviour of broiler chicks, the observations were taken for 15 minutes in each replicate between 01.00-03.00 pm, twice a week starting from the 3rd week and continued throughout the experiment. The behaviour activities like preening, scratching, panting, wing flapping, feeding, drinking, dust bathing, leg stretching and resting of broiler in response to microclimate of shed was examined on nominal and ordinal scale. In nominal scale, data was recorded to determine the percentage of total birds expressed a particular behavioural activity in response to microclimate at a point of time. However, in ordinal scale average time spent by broilers in a particular activity was assessed. The experimental data were subjected to statistical analysis using one way analysis of variance as described by Snedecor and Cochran (1994) to test for significant variation between treatment groups. Comparison of mean values was carried out by Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Per cent duration of time spent in different behavioural activities by broilers

Percent duration of time spent in showing different behavioural activities by birds under different treatment groups are presented in Table 1.

Feeding behaviour: The percent duration of feeding throughout the experimental period were significantly higher ($p<0.01$) in T_2 and T_3 groups than T_1 group. However, no significant difference was observed between T_2 and T_3 groups.

Drinking behaviour: The per cent duration of drinking time spent during 4th and 5th weeks in T_2 group was significantly lower ($p<0.05$) than T_1 and T_3 treatment groups and no significant difference was found between T_1 and T_3 groups. While at the 3rd week T_3 group was intermediately equivalent to T_2 group. During 6th week average drinking time spent in T_2 and T_3 groups were significantly lower ($p<0.05$) than T_1 group but no significant difference was found in drinking time spent of birds in T_2 and T_3 groups.

Panting behaviour: The observation of percent duration of panting time spent indicated that none of the birds showed panting activity in T_2 group at 3rd week of age, while highly significant difference ($p<0.01$) was found between T_1 and T_3 groups. During 4th, 5th and 6th weeks results showed highly significant difference ($p<0.01$) among treatment groups. Spent panting time was highest in T_1 group followed by T_3 and T_2 groups.

Leg stretching behaviour: The observations of percent duration of leg stretching activity was highly significantly ($p<0.01$) in T_2 and T_3 groups than T_1 group at the 3rd week of age and at the 4th and 6th weeks significant difference ($p<0.05$) was found between T_1 and T_2 groups but T_3 group was intermediately equivalent to T_1 group. While at the 5th week of age no significant difference was found among different treatment groups.

Resting behaviour: The observations of percent duration of resting time spent during entire experimental period in T_1 group was highly significantly ($p<0.01$) at 3rd, 5th and 6thweeks of age and significant ($p<0.05$) at 4th week. There was no significant difference in resting duration of birds in T_2 and T_3 groups at 3rd, 5th and 6th weeks. While at the 4th week T_1 and T_3 group did not differ significantly to each other but T_1 group was intermediately equivalent to T_2 group.

Scratching behaviour: The observations of percent duration of scratching time spent at the 3rd week was highly significantly ($p<0.01$) and at 4th week was significant ($p<0.05$) among various treatment groups. It was highest in T_3 group followed by T_2 and T_1group. No significant difference was found among various treatment groups on 5th and 6th weeks.

Dust bathing: The observations of percent duration of dust bathing time spent at the 3rd, 4th and 5thweek was significantly differ ($p<0.01$) among treatment groups. It was highest in T_3 group followed by T_2 and T_1 group. At 4th week T_2 and T_3groups were significantly higher ($p<0.01$) than the control (T_1) group while T_2 and T_3 group did not differ significantly. At the 5th week of age

Table 1: Per cent duration of time spent in showing different behavioural activities by birds under different treatment groups

Behaviour	Treatment groups	Age in weeks			
		3rd	4th	5th	6th
Feeding	T_1	3.50^b±0.40	4.26^b±0.16	5.30^b±1.30	4.00^b±1.00
	T_2	7.40^a±0.30	8.12^a±0.12	10.55^a±0.45	9.00^a±1.00
	T_3	6.50^a±0.20	7.48^a±0.48	9.60^a±0.60	8.00^a±1.00
	Sig. level	**	**	**	**
Drinking	T_1	4.50^a±0.50	5.60^a±0.40	7.87^a±0.87	9.00^a±1.00
	T_2	2.50^b±0.30	2.40^b±0.40	4.56^b±0.44	5.20^b±0.80
	T_3	3.50^{ab}±0.20	4.42^a±0.42	6.40^a±0.20	6.18^b±1.18
	Sig. level	*	*	*	*
Panting	T_1	10.55^a±1.00	20.93^a±0.93	66.40^a±0.80	67.20^a±1.00
	T_2	0.00	4.33^c±0.22	15.40^c±0.80	17.33^c±0.67
	T_3	4.52^b±0.32	9.76^b±0.76	40.00^b±0.50	51.50^b±1.50
	Sig. level	**	**	**	**
Leg stretching	T_1	3.60^b±0.20	3.40^b±0.30	4.60±0.60	5.00^b±0.50
	T_2	7.00^a±0.20	6.58^a±0.58	6.55±0.25	7.64^a±0..36
	T_3	6.55^a±0.25	5.78^{ab}±0.78	5.59±0.41	6.46^{ab}±0.54
	Sig. level	**	*	NS	*
Resting	T_1	28.00^a±2.00	15.00^{ab}±1.00	28.00^a±1.00	26.00^a±0.50
	T_2	11.00^b±2.00	11.00^b±1.00	20.14^b±0.86	16.22^b±0.78
	T_3	15.00^b±1.00	16.03^a±1.03	18.08^b±0.92	14.33^b±0.67
	Sig. level	**	*	**	**
Scratching	T_1	9.50^b±0.20	11.13^b±1.13	10.60±0.40	7.48±0.48
	T_2	12.60^a±0.40	14.56^a±0.56	12.44±0.44	8.40±1.40
	T_3	13.30^a±0.20	15.80^a±0.80	12.20±1.80	9.10±0.90
	Sig. level	**	*	NS	NS
Dust bathing	T_1	5.20^c±0.30	6.05^b±0.05	5.00^b±1.00	6.10±1.00
	T_2	7.40^b±0.20	8.65^a±0.65	4.50^b±1.00	8.12±1.00
	T_3	9.30^a±0.30	10.45^a±0.45	10.40^a±0.60	10.20±1.00
	Sig. level	**	**	**	NS
Preening	T_1	6.40^c±0.40	7.85±0.85	5.20±1.00	5.00^b±0.50
	T_2	10.60^a±0.20	12.50±0.50	8.13±1.03	8.20^a±0.20
	T_3	8.20^b±0.30	10.50±1.50	7.20±1.00	7.00^a±0.50
	Sig. level	**	NS	NS	*
Wing flapping	T_1	8.90±0.20	9.24±0.24	6.45±0.35	6.25±0.75
	T_2	9.30±0.30	10.02±0.08	8.60±0.60	8.10±1.00
	T_3	9.10±0.20	10.12±1.12	7.33±1.33	7.00±0.50
	Sig. level	NS	NS	NS	NS

**Highly Significant ($p<0.01$), *Significant ($p<0.05$), NS = Non-significant. Values having different superscripts in a row vary significantly from one another

T_3 group was significantly higher ($p<0.01$) than the T_1 and T_2 treatment groups but no significant difference was found between T_2 and T_1 groups. While at the 6th week there was no significant difference among different treatment groups.

Preening behaviour: The activities of preening per cent duration time spent at 3rd week was significantly higher ($p<0.01$) in T_2 group than T_3 and T_1 groups. While at the 4th and 5th week of age no significant difference was found among different treatment groups. At 6th week preening behaviour in broilers of T_2 and T_3 were observed to be significantly higher ($p<0.05$) than T_1 group.

Wing flapping behaviour: The observations of per cent duration of wing flapping in broilers at the 3rd, 4th, 5th and 6th week showed no significant difference among different treatment groups.

Per cent incidence of different behavioural activities by broilers

Per cent incidence in showing different behavioural activities by birds under different treatment groups are presented in Table 2.

Feeding behaviour: The percent incidence in feeding behavior during entire experimental period was found to be highly significant ($p<0.01$). At the 3[rd] and 4[th] week percent incidence of feeding by broilers of T_1 group was significantly lower ($p<0.01$) than those of T_2 and T_3 groups but no significant difference was found between T_2 and T_3 groups. Whereas, at the 5[th] and 6[th] weeks per cent incidence of feeding was significantly higher ($p<0.01$) in broilers of T_2 group followed by broilers of T_3 and T_1 treatment groups.

Drinking behaviour: The observations of per cent incidence of drinking by broilers throughout the entire experimental period was significantly higher ($p<0.01$) in

Table 2: Per cent incidence in showing different behavioural activities by birds under different treatment groups

Behaviour	**Treatment groups**	**Age in weeks**			
		3rd	**4th**	**5th**	**6th**
Feeding	T_1	$36.25^b±1.00$	$33.05^b±0.95$	$24.20^c±1.00$	$16.40^c±1.40$
	T_2	$68.60^a±0.60$	$62.00^a±1.00$	$52.33^a±0.67$	$42.40^a±1.00$
	T_3	$67.40^a±1.10$	$59.50^a±1.00$	$43.42^b±0.42$	$34.50^b±1.00$
	Sig. level	**	**	**	**
Drinking	T_1	$36.00^a±1.00$	$39.50^a±1.00$	$45.05^a±0.95$	$46.40^a±0.60$
	T_2	$20.00^b±0.50$	$22.50^b±1.00$	$25.00^b±0.50$	$26.33^c±1.00$
	T_3	$21.60^b±0.60$	$23.05^b±0.50$	$27.00^b±0.50$	$30.25^b±0.75$
	Sig. level	**	**	**	**
Panting	T_1	$62.00^a±1.50$	$68.00^a±1.00$	$76.50^a±2.00$	$87.50^a±1.00$
	T_2	0.00	$5.00^c±1.00$	$28.00^b±1.00$	$39.42^b±0.58$
	T_3	$9.00^b±0.50$	$15.00^b±1.50$	$33.40^b±1.00$	$42.50^b±1.00$
	Sig. level	**	**	**	**
Leg stretching	T_1	$12.20^b±1.00$	$13.33^b±1.33$	$12.90^b±0.90$	$13.40^b±0.60$
	T_2	$22.30^a±0.10$	$21.60^a±0.60$	$22.41^a±1.59$	$20.05^a±0.95$
	T_3	$20.34^a±1.34$	$19.00^a±1.50$	$20.60^a±1.40$	$19.05^a±1.05$
	Sig. level	**	*	*	*
Resting	T_1	$83.50^a±1.00$	$81.00^a±1.00$	$84.05^a±0.95$	$86.00^a±0.50$
	T_2	$61.50^b±1.00$	$63.50^b±1.00$	$65.00^b±1.50$	$68.50^b±1.00$
	T_3	$60.00^b±5.00$	$65.50^b±1.00$	$64.00^b±1.50$	$70.32^b±0.68$
	Sig. level	**	**	**	**
Scratching	T_1	$9.40^b±0.60$	8.00±1.50	$7.00^b±0.50$	$6.22^b±0.12$
	T_2	$15.20^a±1.20$	12.33±0.33	$11.00^a±0.50$	$5.00^b±0.20$
	T_3	$16.44^a±0.56$	11.50±1.00	$12.90^a±1.10$	$11.22^a±1.22$
	Sig. level	**	NS	*	**
Dust bathing	T_1	$5.90^c±0.10$	$8.50^c±1.00$	$9.22^c±1.00$	$10.55^b±1.45$
	T_2	$13.05^b±0.95$	$15.00^b±1.00$	$15.20^b±0.80$	$17.60^a±1.40$
	T_3	$18.40^a±0.20$	$21.00^a±1.00$	$22.00^a±1.00$	$23.60^a±1.40$
	Significance level	**	**	**	**
Preening	T_1	$9.05^b±0.95$	$26.00^b±2.00$	$33.50^b±1.00$	43.44±6.44
	T_2	$20.20^a±1.00$	$47.00^a±2.00$	$53.00^a±1.00$	59.60±0.60
	T_3	$18.60^a±0.40$	$41.50^a±1.00$	$48.00^a±2.00$	55.64±1.36
	Sig. level	**	**	**	NS
Wing flapping	T_1	6.10±0.10	7.00±1.00	8.00±1.50	9.50±1.00
	T_2	10.00±2.00	11.00±1.00	12.20±1.00	15.50±1.00
	T_3	8.20±0.20	9.50±1.00	10.00±1.50	13.50±2.00
	Sig. level	NS	NS	NS	NS

**Highly Significant ($p<0.01$), *Significant ($p<0.05$), NS =Non-significant, Values having different superscripts in a row vary significantly from one another

T_1 group than T_2 and T_3. At 3rd, 4th and 5th week no significant difference was found between T_2 and T_3 groups. However, during 6th week per cent incidence in drinking behaviour of birds differ significantly ($p<0.01$) among treatment groups. It was highest in T_1 followed by T_3 and T_2 groups.

Panting behaviour: The observations of per cent incidence in panting behavioural activity in entire experimental period in T_1 group was significantly higher ($p<0.01$) than T_2 and T_3 groups. At the 3rd week none of the birds of T_2 group showed panting activity while T_1 group showed highly significant value than T_2 group. During 4th week per cent incidence in panting behaviour of birds differ significantly ($p<0.01$) among various treatment groups. T_1 group showed highest activity followed by T_3 and T_2 treatment groups. During 5th and 6th weeks panting behaviour in T_1 group was significantly higher ($p<0.01$) than T_2 and T_3 treatment groups. No significant difference was found between T_2 andT_3 groups.

Leg stretching behaviour: The observations of leg stretching activity per cent incidence of behavioural activity in entire experimental period was highly significant ($p<0.01$) at in 3rd, 4th, 5th and 6th week in T_2 and T_3 than T_1 group. No significant difference was found between T_2 and T_3 groups.

Resting behaviour: The observations of per cent incidence of resting during entire experimental period in T_1 was significantly higher ($p<0.01$) than T_2 and T_3. There was no significant difference ($p<0.05$) in resting incidence of birds between T_2 and T_3 groups.

Scratching behaviour: The observations of per cent incidence of scratching activity at the 3rd week showed highly significant ($p<0.01$). T_1 group showed significantly lower value than T_2 and T_3 groups. While at the 4th week no significant difference was found among different treatment groups. During the 5th week per cent incidence of scratching activity was significantly lower ($p<0.05$) in T_1 than T_2 and T_3 groups but there was no significant difference ($p<0.05$) between T_2 and T_3 group. At 6th week T_3 group showed significantly higher ($p<0.01$) stretching activity than T_1 and T_2. No significant difference was found between T_1 and T_2 groups.

Dust bathing: The observations of per cent incidence of dust bathing activity in control (T_1) was significantly lower ($p<0.01$) than T_3 and T_2 groups, during the 3rd, 4th and 5th week per cent incidence of dust bathing activity in T_3 group was significantly higher ($p<0.01$) than T_2 group, T_2 and T_3 group was significantly higher ($p<0.01$) than T_1. While at the 6th week evaporative cooling pad (T_2) and fogger system (T_3) treatment group did not differ significantly ($p<0.05$) to each other but significantly higher ($p<0.01$) than the T_1 group.

Preening behaviour: The activities of preening per cent incidence of behavioural activity at the 3rd, 4th and 5th week was significantly higher ($p<0.01$) in T_2 and T_3 groups than T_1 group but T_2 and T_3 did not differ significantly ($p<0.05$) to each other. While at the 6th week no significant difference ($p<0.05$) was found among various treatment groups.

Wing flapping behaviour: The observations of wing flapping per cent incidence behavioural activity during entire experimental period was non-significant among various treatment groups.

Thus, in present study, T_1 experienced more heat stress, thereby consumed less feed in an attempt to reduce metabolic heat production, showed panting to increase rate of evaporative cooling and wing flapping to increase convective heat loss. Similar to these results Spinu *et al.* (2003) and Maria *et al.* (2004) also reported significant decrease in locomotory activity in heat stressed birds. Whereas, lower per cent of panting in birds of T_2 and T_3 groups indicated cooling in the shed might have led to reduced heat production or body temperature of birds thus lowered evaporative heat loss through panting. Similar results were also reported by Gupta *et al.* (2017) who found that birds in cooling pad and fogger system treatment group spent more time ($p<0.05$) in dust bathing, scratching, body preening, wing flapping, leg stretching, preening to wing or uropygial gland and eating in comparison to control group. Panting behavior was significantly higher ($p<0.01$) in control group than both the treatment groups.

CONCLUSION

Broiler chickens are sensitive to heat stress, high ambient temperature and relative humidity are major environmental stressors that influence performance of broilers while the ideal environmental temperature enhanced production. Evaporative cooling pad system and fogger system improved the behavioural performance of the broiler chicks during summer and thus took care of animal welfare during the period of heat stress. Since poultry industry is rapidly growing in Rajasthan, the farmers may be advocated to use these devices during the peak summer months to sustain the industry.

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Optimization of cooking time of chicken meat loaf prepared with microwave cooking

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ABSTRACT

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The present study was carried out to evaluate the effect of microwave cooking at 540 MHz for 15 minutes (MC1), 20 minutes (MC2) and 25 minutes (MC3) on various physico-chemical properties, texture profile analysis, colour parameter, mineral content as well as sensorial properties of chicken meat loaf. The MC1 group had significantly (P<0.05) higher cooking yield, moisture content, water activity and moisture retention values than other two treatments. However, the protein, fat, carbohydrate, fat retention and cholesterol content were increased with the increase in time of microwave cooking. Hardness, chewiness and resilience values were increased significantly (P<0.05) with increase of cooking time but; springiness, cohesiveness and gumminess values were remained changed amongst the treatments. Lightness and yellowness values were decreased (P<0.05); while redness value was increased significantly (P<0.05) with increased time of microwave cooking. Zinc content increased significantly (P<0.05) with increase time of microwave cooking. Potassium, calcium, copper, sodium, manganese, iron and phosphorous content were increased significantly (P<0.05). The scores of sensory parameters decreased significantly (P<0.05) with increase of cooking time. Therefore, it was concluded that well acceptable chicken meat loaf can be prepared by microwave cooking at 540 MHz for 15 minutes.

Keywords: Chicken meat loaf, microwave cooking, Texture, mineral content, cooking time.

INTRODUCTION

Meat is a very good source of animal protein that consists of essential amino acid, minerals, vitamins and essential fatty acids (Lawrie, 1991). Meat provides calories from fat, proteins and limited quantities of carbohydrate (Judge *et al*., 1990). Lean meat contains from 15 to 20% of protein, which varies inversely with percentage of fat. As per DAHD (2019), total meat production in India was 8.11 million tons in 2018-19, with contribution of buffalo, cattle, sheep, goat, pig and poultry as 19.05%, 4.02%, 8.36%, 13.53%, 4.98% and 50.06%, respectively. Poultry is the most prolific meat producer, accounting for almost half (DAHD, 2019) of total meat production in India. When foods are prepared to be eaten, there are significant changes in the flavor as well as in the nutritional composition of the food. As meat is usually cooked before consumption it is important to understand the physico-chemical and sensorial characteristics of meat products that were cooked before consumption. Cooking of meat is essential to achieve a palatable and safe product (Tornberg, 2005) as it enhances flavour and tenderness, inactivates pathogenic microorganisms (Broncano *et al*., 2009; Rodriguez-Estrada *et al*., 1997), denature proteins and increases the digestibility and bioavailability of nutrients (Meade *et al*., 2005). Time plays an important role in the characteristics of cooked muscle-based food products (Sobral *et al*., 2018). Cooked meat flavor is influenced by water-soluble components that contribute to taste. The volatile compounds formed during cooking contribute the characteristic flavors of meat. Based on sensory evaluation, eight general odor qualities (buttery, caramel, burnt, green, fragrant, oily/fatty, nutty and meaty) have been used to describe cooked meat odor (Mottram, 1998). Therefore, meat composition combined with a specific cooking methodology (time and temperature) is one of the factors that mostly affect the final quality of meat products (Chiavaro *et al*., 2009). Generally, dry and moist cooking methods have been used for processed meat products. Microwave cooking is also being used frequently in processed meat products preparation. Some workers have observed that microwave oven cooked meat products had lower moisture content than conventional oven cooking (Hoda *et al*., 2002); but Nath *et al*. (1996) reported no moisture difference in microwave oven and conventional oven cooked chicken patties. Therefore, the present study was conducted to evaluate the effect of microwave cooking on physico-chemical properties, textural parameters, colour parameters, mineral components and sensory properties of chicken meat loaf.

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MATERIALS AND METHODS

The experiments were conducted in Department of Livestock Products Technology, College of Veterinary Sciences and Animal Husbandry, U.P. Pt. Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan, Mathura, 281001 (UP), India.

Source of Raw Material

Live birds were procured from Department of Poultry Science, DUVASU Mathura and slaughtered in Meat Processing Laboratory of Department of Livestock Products Technology as per standard procedure following Halal method. The hot carcass was kept in refrigerator at 4±2^{0}C for 4-6 hours. The meat was deboned, trimmed-off separable fat and connective tissue. The samples were kept for conditioning in a refrigerator at 4±2^{o}C for 6–8 h and then frozen at -18^{o}C till further use. Other ingredients such as refined wheat flour, condiments, food grade refined oil, salt, spice mix were purchased from local market of Mathura. Low density Polyethylene (LDPE) bags of 250 gauges were sourced from local market and sterilized by exposing to U.V. light for 30 minutes before use. For preparation of spice mix, different ingredients were procured from the local market in the desired ratio, dried at 45±2°C for 2 hours followed by grinding and sieving through the mesh of 0.5 mm. The spice mix was packed in pre-sterilized low-density polyethylene bags and freshly prepared spice mix was used. The formulation of standardized spice mix is given in footnote of Table 1. All chemicals used in the study were of analytical grade and procured from Hi Media Laboratories (P) Ltd, Mumbai.

Table 1: Composition of spice mix for preparation of chicken meat loaf

S. No.	Spices	Percentage (%)
1.	Black cardamom (Badi elaichi)	05
2.	Cinnamon (Dalchini)	05
3.	Caraway seed (Ajwain)	07
4.	Clove (Loang)	05
5.	Red chilli	08
6.	Coriander (Dhania)	18
7.	Cumin (zeera)	16
8.	Black pepper (Kalimirch)	10
9.	Fennel seed (Soanf)	07
10.	Dried ginger powder (Soanth)	08
11.	Mace (Javitri)	03
12.	Nutmeg (Jaifal)	02
13.	Green cardamom (Choti elaichi)	02
14.	Star anise	02
15.	White pepper	02
	Total	**100**

Preparation of chicken meat loaf

Chicken meat loaf was prepared as per method followed by Devatkal *et al*. (2004) with slight modifications. Frozen deboned meat was thawed at refrigeration temperature overnight. Thawed lean meat was cut into smaller chunks and minced in electrical meat mincer (Sirmen mincer, MOD-TC 32 R10U.P. INOX, Marsango, Italy) with 6mm plate followed by 4 mm plate. The common salt, vegetable refined oil, refined wheat flour (maida), sodium tripolyphosphate, spice mix and condiments were weighed accurately as per the formulation. Meat emulsion was prepared in Sirman Bowl Chopper (MOD C 15 2.8G 4.0 HP, Marsango, Italy). The minced meat was blended with salt, sodium tri polyphosphate for 1.5 minute. Water in the form of crushed ice was added and blending continued for 1 minute. This was followed by addition of refined vegetable oil and blended for another 1 to 2 minutes. Then spice mixture, condiments and other ingredients were added and again mixed for 1.5 to 2 minutes to get the desired emulsion. Adequate care was taken to maintain temperature below 18^{o}C by preparing the emulsion in cool hours of morning, by addition of meat and other ingredients in chilled/partially thawed form and by addition of crushed ice. About 300 g of emulsion was filled into the glass molds. The height and width of the chicken meat loaf was determined by Vernier Callipers. The following abbreviations were used for present experiment: MC1- chicken meat loaf prepared by microwave cooking at 540MHz for 15 minutes, MC2- chicken meat loaf prepared by microwave cooking at 540MHz for 20 minutes, MC3- chicken meat loaf prepared by microwave cooking at 540MHz for 25 minutes. The chicken meat loaf was cooled at ambient temperature, packed in pre sterilized low-density polyethylene pouches and stored at refrigerated temperature (4±2ºC). The formulation used for preparation of chicken meat loaf is given in table 2.

Table 2: Formulation for preparation of chicken meat loaf

Ingredient	MC1	MC2	MC3
Chicken (%)	74.2	74.2	74.2
Refined vegetable oil (%)	8	8	8
Ice flakes (%)	8	8	8
Salt (%)	1.5	1.5	1.5
Dry spices mix (%)	2.0	2.0	2.0
Condiments (%)	3.0	3.0	3.0
Refined wheat flour (%)	3.0	3.0	3.0
STPP (%)	0.3	0.3	0.3
Total	100	100	100

Chicken meat loaf prepared by microwave cooking at 540MHz for 15 minutes (MC1), 540MHz for 20 minutes (MC2), 540MHz for 25 minutes (MC3)

Analytical procedure

Developed chicken meat loaf was evaluated for various Physico-chemical properties– pH (Trout *et al.*, 1992), cooking yield (Murphy *et al*., 1975), proximate analysis (AOAC, 1995), fat retention (Murphy *et al*., 1975), water activity by Aqua LAB 3 TE8220, Inc. Pullman, WA water activity meter, moisture retention by El-Magoli *et al*. (1996), total cholesterol estimation (Zaltkis *et al*., 1953), texture profile analysis (Bourne, 1978), Instrumental colour analysis (Hunter and Harold, 1987) and mineral profile (Horowitz, 1965). Sensory evaluation was evaluated by using 8 point hedonic scale with 8 point as extremely desirable and 1 as extremely poor (Keeton, 1983).

Statistical analysis

The data obtained in the study on various parameters were analyzed as per standard methods of Snedecor and Cochran (1994). Duplicate samples were drawn for each parameter and the experiment was replicated thrice (n=6). Sensory evaluation was performed by a panel of seven member judges three times, so total observations being 21 (n=21). Data were subjected to one way analysis of variance, homogeneity test and Duncan's Multiple Range Test (DMRT) for comparing the means to find the effects between samples.

RESULTS AND DISCUSSION

Physico-chemical properties

The effect of microwave cooking on physico-chemical properties of chicken meat loaf is presented in table 3. There was no significant difference in pH values among the treatments. Cooking yield, moisture content, moisture retention and water activity values decreased significantly (P<0.05) with increase time of microwave cooking at 540 MHz, which might be due to direct contact with heat during microwave cooking, resulting in increased loss of moisture from the product. Ruyack and Paul (1972) also reported more cooking loss during heating of beef by microwave cooking than other conventional methods due to higher moisture loss. Brugiapaglia and Destefanis (2012) reported that increasing the temperature during the cooking process significantly (P<0.05) decreased water content in meat products. They also reported that denaturation of the proteins during the cooking resulted in evaporation of water molecules and thereby less water content in the structures of the proteins. Protein, fat, carbohydrate, fat retention as well as cholesterol content increased significantly (P<0.05) with increase time of microwave cooking due to more water evaporation during microwave cooking resulting in a quantitative increase in protein and fat content. Cholan *et al*. (2011) observed significantly higher (P<0.05) protein content in microwave cooked chicken patties in comparison to steam and oven cooked chicken patties. Higher cholesterol content with increase time of microwave cooking might be related to quantitative increase in fat content. Ash content of MC3 was significantly (P<0.05) higher than MC1 and MC2, however there was no significant difference between MC1 and MC2. The findings of Pathera *et al*. (2017) in chicken meat nuggets are in agreement with the results of the present study.

Table 3: Effect of microwave cooking on physico-chemical properties of chicken meat loaf

Ingredient	MC1	MC2	MC3
pH	6.10±0.05	6.14±0.04	6.12±0.04
Cooking yield (%)	87.23^a±0.08	85.55^b±0.06	78.39^c±0.06
Moisture (%)	60.10^a±0.06	58.01^b±0.05	53.36^c±0.04
Protein (%)	16.85^c±0.05	16.87^b±0.05	16.95^a±0.08
Fat (%)	9.53^c±0.05	9.73^b±0.06	9.94^a±0.05
Carbohydrate (%)	12.32^c±0.05	14.10^b±0.08	18.25^a±0.04
Ash (%)	1.20^b±0.06	1.29^b±0.05	1.50^a±0.04
Fat retention (%)	85.64^c±0.08	85.85^b±0.09	86.05^a±0.07
Water activity (a_w)	0.954^a±0.01	0.878^b±0.01	0.625^c±0.01
Moisture retention (%)	52.42^a±0.09	49.63^b±0.04	41.83^c±0.05
Cholesterol (mg/100g)	83.11^c±0.35	84.59^b±0.41	86.66^a±0.51

Chicken meat loaf prepared by microwave cooking at 540MHz for 15 minutes (MC1), 540MHz for 20 minutes (MC2), 540MHz for 25 minutes (MC3)
Overall means bearing different superscripts in a row (a,b,c,d.......) differ significantly (P<0.05), n= 6 for each treatment

Textural parameters

The effect of microwave cooking on textural properties of chicken meat loaf is presented in table 4. Hardness, chewiness and resilience values increased significantly (P<0.05) due to more moisture loss with increased time of microwave cooking. Briones Labarca *et al*. (2012) also reported that chewiness values increased significantly (P<0.05) with increase time of microwave cooking which might be due to increase in gumminess and springiness in red abalone (*Haliotis rufecens*). There was no significant difference in springiness, cohesiveness and gumminess values among the treatments. Parang *et al*. (2011) also observed similar findings in microwave cooking of *Longissimus dorsi* of veal meat.

Table 4: Effect of microwave cooking on textural parameters (Mean±SE) of chicken meat loaf

Parameters	MC1	MC2	MC3
Hardness (N/cm^2)	13.53^c±0.05	14.49^b±0.07	16.63^a±0.07
Springiness (mm)	29.03±0.23	29.14±0.09	29.20±0.06
Cohesiveness (Ratio)	0.82±0.04	0.85±0.04	0.88±0.04
Gumminess(N/cm^2)	7.87±0.04	7.95±0.07	7.99±0.07
Chewiness (N/cm)	153.59^c±0.05	178.79^b±0.07	194.60^a±0.08
Resilience (Ratio)	0.62^c±0.05	0.67^b±0.06	0.79^a±0.07

Chicken meat loaf prepared by microwave cooking at 540MHz for 15 minutes (MC1), 540MHz for 20 minutes (MC2), 540MHz for 25 minutes (MC3)
Overall means bearing different superscripts in a row (a,b,c,d ....) differ significantly (P<0.05), n= 6 for each treatment

Colour parameters

The effect of microwave cooking on colour properties of chicken meat loaf is presented in table 5. Lightness and yellowness values decreased significantly (P<0.05) while redness values increased significantly (P<0.05) with increased time of microwave cooking, which might be due to cooking and non enzymatic browning reaction of chicken meat loaf. Higher redness and lower lightness values with the increased of time during microwave cooking might also be explained by pigment oxidization (heme group) after cooking having less brightness (Garcia *et al*., 2007). Katherine (1999) reported that the increase in red color in irradiated meats could be due to the production of carboxymyglobin, which was possibly same mechanism in meat during microwave cooking. Yang and Chen (1993) observed significant (P<0.05) decrease in lightness and yellowness while significantly (P<0.05) increased redness values of chicken meat in microwave cooking.

Table 5: Effect of microwave cooking on colour parameters (Mean±SE) of chicken meat loaf

Parameters	MC1	MC2	MC3
Lightness (*L**)	43.30^{a}±0.06	40.21^{b}±0.05	37.04^{c}±0.05
Redness (*a**)	11.68^{c}±0.06	12.61^{b}±0.07	13.86^{a}±0.08
Yellowness (*b**)	8.56^{a}±0.07	8.23^{b}±0.07	8.00^{c}±0.07

Chicken meat loaf prepared by microwave cooking at 540MHz for 15 minutes (MC1), 540MHz for 20 minutes (MC2), 540MHz for 25 minutes (MC3)
Overall means bearing different superscripts in a row (a,b,c,d.......) differ significantly (P<0.05), n= 6 for each treatment

Mineral content

The effect of microwave cooking on mineral content of chicken meat loaf is presented in table 6. Zinc content increased significantly (P<0.05) with increase time of microwave cooking; however there was no significant difference between MC1 and MC2. There was no significant difference in chromium and magnesium content among the treatments. Potassium, copper, sodium, manganese, iron and phosphorous content increased significantly (P<0.05) with increase in time during microwave cooking. The change in mineral content noted in the study might be explained by the nature of the minerals, which are present in meat in water-soluble form and, thus, they leach to the broth during thermal treatment. Monika *et al*. (2019) also observed significant increase (P<0.001) in Ca, Zn and Cu contents and a decrease in K and Na contents in sous vide and steam meat as compared to the raw meat. MC3 had significantly (P<0.05) higher calcium content than MC1; however calcium content of MC2 was comparable to both MC3 and MC1. Similar findings were also observed by Ersoy and Özeren (2009) in African catfish and Gokoglu *et al*. (2004) in microwave cooked rainbow trout fish (*Oncorhynchus mykiss*).

Table 6: Effect of microwave cooking on mineral content (Mean±SE) of chicken meat loaf

Mineral (ppm)	MC1	MC2	MC3
Zinc	24.16^{b}±1.81	26.16^{b}±2.18	32.16^{a}±1.51
Potassium	4532.20^{c}±2.30	4648.70^{b}±2.48	4890.80^{a}±2.00
Calcium	109.17^{b}±1.35	114.83^{ab}±2.21	117.33^{a}±2.26
Copper	5.16^{c}±0.06	5.52^{b}±0.03	6.78^{a}±0.07
Sodium	1220.00^{c}±4.76	1235.10^{b}±4.61	1246.80^{a}±4.30
Manganese	12.67^{c}±0.03	13.48^{b}±0.05	13.71^{a}±0.03
Iron	20.30^{c}±0.03	20.90^{b}±0.02	21.50^{a}±0.02
Chromium	0.66±0.03	0.70±0.05	0.61±0.04
Phosphorus	1810.20^{c}±3.04	1833.30^{b}±2.74	1844.80^{a}±3.40
Magnesium	229.17±2.71	231.50±2.04	234.33±1.70

Chicken meat loaf prepared by microwave cooking at 540MHz for 15 minutes (MC1), 540MHz for 20 minutes (MC2), 540MHz for 25 minutes (MC3)
Overall means bearing different superscripts in a row (a,b,c,d.......) differ significantly (P<0.05, n= 6 for each treatment

Sensory evaluation

The effect of microwave cooking on sensory scores of chicken meat loaf is presented in table 7. Colour and appearance, flavour, mouth coating, meat flavor intensity and overall acceptability scores decreased significantly (P<0.05) with increased time of microwave cooking, which might be due to long cooking time resulting in increased muscle toughness in cooked product. Texture, juiciness and saltiness scores decreased significantly (P<0.05) with increased time of microwave cooking; however, there was no significant difference between MC2 and MC3. MC2 and MC3 were not much liked by sensory panelists due to toughness, burnt flavour and over cooking of product. Similar findings were observed by Korschgen *et al*. (1976) in microwave cooked beef roast. Khanam (2017) also reported that chicken spread prepared by microwave cooking at 540 MHz for 7 minutes was found optimum for sensory attributes. Oltrogge and Prusa (1987) found that cooking

Table 7: Effect of microwave cooking on sensory scores (Mean±SE) of chicken meat loaf

Attributes	MC1	MC2	MC3
Colour and appearance	7.22^{a}±0.08	6.88^{b}±0.08	6.60^{c}±0.05
Flavour	7.21^{a}±0.08	6.82^{b}±0.06	6.58^{c}±0.04
Texture	7.16^{a}±0.07	6.64^{b}±0.07	6.57^{b}±0.09
Juiciness	7.16^{a}±0.07	6.64^{b}±0.07	6.57^{b}±0.09
Saltiness	7.14^{a}±0.07	6.61^{b}±0.06	6.47^{b}±0.07
Mouth coating	7.15^{a}±0.07	6.68^{b}±0.07	6.40^{c}±0.05
Meat flavour intensity	7.22^{a}±0.07	6.88^{b}±0.08	6.60^{c}±0.05
Overall acceptability	7.15^{a}±0.07	6.68^{b}±0.07	6.42^{c}±0.05

Chicken meat loaf prepared by microwave cooking at 540MHz for 15 minutes (MC1), 540MHz for 20 minutes (MC2), 540MHz for 25 minutes (MC3)
Overall means bearing different superscripts in a row (a,b,c,d.......) differ significantly (P<0.05), n= 21 for each treatment

of hen breast at lower microwave power levels and short cooking time was associated with more tender meat product when compared with cooking at high microwave power levels with long cooing time. Therefore, MC1- chicken meat loaf prepared by microwave cooking at 540 MHz for 15 minutes was selected as the best treatment.

CONCLUSION

From the results it can be concluded that the microwave cooking had a significant effect on the physicochemical and sensory quality of cooked chicken meat loaf. The well acceptable chicken meat loaf can be prepared by microwave cooking at 540MHz for 15 minutes without any adverse effect on physic-chemical and sensory properties.

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Evolutionary Analysis of Hemagglutinin-Neuraminidase (HN) Protein of Newcastle Disease Virus

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ABSTRACT

Singh, P. and Mukhopadhyay, C.S. 2022. Evolutionary Analysis of Hemagglutinin-Neuraminidase (HN) Protein of Newcastle Disease Virus. Indian Journal of Poultry Science. 57(02):177-179.

The economy of countries like India mainly depends upon agriculture-based industries. The poultry sector is one of the most important domains in the Indian agricultural diaspora, as the country is the fifth largest egg producer and 18th largest producer of meat. Newcastle disease is a fatal disease in poultry caused by the Newcastle disease virus (NDV), which expresses the Hemagglutinin-Neuraminidase (HN) protein on its surface. The HN protein is considered as an integral part of the virus infection process. The present study focuses on the evolutionary analysis of the HN) protein of the NDV. The available, 26 amino acid sequences of HN protein were retrieved from NCBI and the sequences were subjected to the multiple sequence alignment (MSA) using the online Clustal omega tool. The MSA results were visualized in the Jalview to observe the conserved regions among the different sequences. MEGA-7 software was used to evaluate the phylogenetic relationship using the Maximum likelihood method with a bootstrap value of 1000. Jones-Taylor-Thornton with Gamma substitution (JTT+G) was found to be the best evolutionary model with the lowest Bayesian information criterion (BIC) and Akaike's information criterion (corrected) (AICc) scores. The pairwise distance calculation revealed the close relationship of NDV R2B HN protein (Acc. No. AFX98110.1) with H strain (Acc. No. CAA76656) and JS-09 strain. The phylogenetic tree construction predicted HN protein of NDV R2B is closely related to H- strain 9 (China) and the Victoria strain of Australia.

Keywords: Chicken, Docking, Hemagglutinin Neuraminidase (HN) protein, Newcastle Disease Virus, Phylogenetic tree.

INTRODUCTION

India is a multi-culturally rich country and the economy of this country mainly depends on agriculture. The agricultural sector contributed to 18% of the total gross domestic product (GDP) of the Indian economy employing approximately 50% people of the country (Madhusudan, 2015). The poultry sector is one of the most important versatile fields of agriculture because India is the 5th largest egg producer and 18th largest producer of meat. During the financial year 2020-21, India exported 2,55,686.92 metric tons of poultry products for USD 58.70 million to other countries (http://apeda.gov.in/apedawebsite/SubHead_Products/Poultry_Products.htm,https://mofpi.nic.in/sites/default/files/OpportunityinMeat%26PoultrysectorinIndia.pdf). Although the poultry sector has shown exponential growth, there are some diseases like Avian Influenza, Marek's disease, Newcastle disease, etc. which create a negative impact on the growth of this sector (Sultana *et al*., 2021; Patidar *et al*., 2021).

Newcastle disease is a fatal disease caused by the Newcastle disease virus (NDV), which is a negative sense, single-stranded RNA virus belonging to *Paramyxoviridae* family and *Avulavirus* genus of the order *Mononegavirales*. It is spread through contaminated water, feed, and feces, and from infected birds to healthy birds. Sometimes, workers also got a mild infection while working with these infected birds (Kannaki *et al*., 2021). The symptoms in humans include conjunctivitis and flu-like symptoms and these symptoms can be treated with antibiotics. The hemagglutinin-neuraminidase (HN) protein is a functional glycoprotein present on the surface of the NDV. It is a multifunctional protein that helps in both recognition of receptors and the activities of neuraminidase (NA) integrated with the virus. It initiates the activity of fusion protein via recognition of sialic acid-containing receptors on the host cell surface and thus promotes the entry of the virus into the cell. It also avoids self-agglutination of adjacent viral particles by acting as neuraminidase which helps in the removal of sialic acid receptors from those viral particles. Therefore, the HN protein is an integral part of the virus infection process (Huang *et al*., 2004). Reports on the evolutionary perspective of the NDV concerning the R2B vaccine strain are very limited. So, the present study focuses on the evolutionary analysis of the hemagglutinin-neuraminidase (HN) protein of the Newcastle disease virus, with special emphasis on the R2B strain/vaccine strain.

MATERIAL AND METHODS

This experiment was carried out at the College of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana.. No animal was used in this biocomputation research work

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Sequence download

A total of 26 amino acid sequences of NDV HN protein were retrieved from NCBI-Protein, with the HN protein of the NDV R2B strain (Accession No. AFX98110) serving as the reference sequence.

Multiple sequence alignment

Multiple sequence alignment (MSA) was performed on the obtained sequences using EMBL's online Clustal omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) programme (Iquebal *et al*., 2015). The result files were downloaded in the jalview format (.jvl) for visualization.

Visualization of MSA results

The MSA findings were shown using the Jalview tool to find out the conserved regions among the NDV HN protein sequences (Singh *et al*., 2015).

Pairwise distance

MEGA7 software was used to determine the pairwise distance (Kumar *et al*., 2016).

Construction of phylogenetic tree

The maximum likelihood method was used to do the phylogenetic analysis, with a bootstrap value of 1000.

RESULTS AND DISCUSSION

Multiple sequence alignment

The findings of multiple sequence alignment (MSA) of HN protein amino acid sequences demonstrate that the majority of the sections of all sequences have conserved regions (blue colour), while some mismatches and missing sequence parts are depicted in white and grey colour, respectively (Fig. 1).

Best evolutionary model selection

The MEGA7 software was also used to choose the optimum evolutionary model. The Bayesian Information Criterion's lowest scores are used to make the selection (BIC). Other metrics addressed include the maximum likelihood value (InL), Akaike information criterion, corrected (AICc) values. The best evolutionary model was JTT+G, which had the lowest BIC and AICc values of 6173. 987946 and 5794.05837, respectively (Fig. 2).

Pairwise distance

MEGA7 software was used to determine the pairwise distance. The distance between NDV R2B HN protein and H strain (Accession No. CAA76656) and JS-09 strain (Accession No. ABS87861) of China was determined using pairwise distance analysis, whereas the distance between HN protein of NDV strain of China and MW-01 isolate of Victoria (Australia) was determined using pairwise distance analysis (Fig. 3).

Phylogenetic analysis

Phylogenetic analysis is a method of determining the relationship between two or more. NDV R2B (Acc. No. AFX98110.1) is closely linked to H-strain 9 (China) and the Victoria strain of Australia, according to a phylogenetic study of HN protein. Texas (Acc. No.P12553) and Rokain (Acc. No. AAQ54640) isolates are grouped since both strains originated in the United States (Fig. 4).

The HN protein sequences of PIV-2 showed definite amino acid sequence relatedness with those of Simian virus 5 (SV5), Sendai virus (SV, parainfluenza virus type 1), Human parainfluenza virus type 3 (PIV-3), type 4 (PIV-4), Bovine parainfluenza virus type 3 (BPIV-3), Mumps virus (MuV), and Newcastle disease virus (NDV), indicating a common ancestor. Furthermore, statistical analysis of the protein sequences revealed that the paramyxoviruses may be related evolutionarily. This is the first time a phylogenetic tree has been created for all human-infectious parainfluenza viruses and mumps viruses (Kawano *et al*., 1990).

Fig. 1: Overview window of multiple sequence alignment of amino acid sequences of Hemagglutinin-neuraminidase (HN) protein of Newcastle disease virus

Model	#Param	BIC	AICc	InL	Invariant	Gamma
JTT+G	50	6173.987946	5794.05837	-2846.85683	n/a	0.350504374
JTT+G+I	51	6182.510059	5794.98881	-2846.315143	0.410640086	1.078678411
JTT+I	50	6191.823138	5811.893561	-2855.774425	0.398423818	n/a
JTT	49	6217.602621	5845.26499	-2873.46691	n/a	n/a

Fig. 2: Selection of Best evolutionary model of HN protein of NDV by using MEGA7 software

AFX98110|HN_Av_OAV1		0.008	0.009	0.009	0.009	0.010	0.010	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.012	0.012	0.011	0.012	0.012	0.012	0.012	0.012	0.012	0.012
CAA76656|HN|NDV_Strain_H	0.039		0.005	0.004	0.005	0.006	0.010	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.012	0.012	0.014	0.014	0.011	0.012
ABS87861|HN_Av_OAV1	0.043	0.012		0.003	0.004	0.005	0.010	0.010	0.011	0.011	0.010	0.010	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.014	0.014	0.011	0.012
AOW42562|HN|Av_OAV1	0.043	0.011	0.005		0.002	0.004	0.010	0.010	0.011	0.011	0.010	0.010	0.011	0.010	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.014	0.014	0.011	0.011
ASL70005|HN|Av_OAV1	0.047	0.014	0.009	0.004		0.004	0.010	0.010	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.012	0.011	0.014	0.014	0.011	0.012
ACJ53753|HN|Av_OAV1	0.052	0.019	0.014	0.009	0.011		0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.012	0.012	0.015	0.015	0.012	0.012
QGF19406|HN|Av_OAV1	0.060	0.060	0.058	0.056	0.060	0.063		0.003	0.004	0.004	0.003	0.004	0.004	0.005	0.004	0.004	0.005	0.005	0.004	0.005	0.005	0.005	0.010	0.010	0.007	0.006
ADI70524|HN|Av_OAV1	0.063	0.063	0.061	0.056	0.060	0.063	0.007		0.005	0.003	0.004	0.004	0.005	0.003	0.004	0.005	0.005	0.004	0.005	0.005	0.006	0.005	0.010	0.010	0.007	0.006
AOG62363||HN|Av_OAV1	0.065	0.067	0.065	0.063	0.067	0.071	0.011	0.014		0.005	0.005	0.006	0.005	0.006	0.004	0.006	0.005	0.006	0.006	0.006	0.006	0.006	0.011	0.011	0.008	0.007
AFS50118|HN|Av_OAV1	0.065	0.065	0.063	0.061	0.065	0.069	0.009	0.005	0.016		0.005	0.005	0.005	0.003	0.005	0.005	0.006	0.005	0.005	0.006	0.006	0.005	0.011	0.011	0.007	0.007
QBK56564|HN|Av_OAV1	0.067	0.065	0.063	0.061	0.065	0.067	0.007	0.011	0.014	0.012		0.005	0.004	0.005	0.004	0.005	0.005	0.005	0.004	0.006	0.006	0.006	0.011	0.011	0.007	0.007
P12555|NDV_D26/76	0.067	0.067	0.065	0.063	0.067	0.071	0.011	0.012	0.019	0.014	0.016		0.006	0.005	0.005	0.006	0.006	0.005	0.006	0.005	0.004	0.005	0.010	0.010	0.006	0.006
AHJ60623|HN_Av_OAV1	0.067	0.069	0.067	0.065	0.069	0.073	0.009	0.012	0.012	0.014	0.012	0.018		0.006	0.004	0.005	0.005	0.005	0.005	0.006	0.006	0.006	0.011	0.011	0.008	0.007
AGO50491|HN|Av_OAV1	0.069	0.067	0.067	0.061	0.065	0.069	0.012	0.005	0.019	0.007	0.016	0.018	0.018		0.005	0.006	0.006	0.005	0.006	0.005	0.006	0.006	0.011	0.011	0.008	0.007
QBB84909|HN|Av_OAV1	0.069	0.065	0.063	0.061	0.065	0.069	0.009	0.012	0.012	0.014	0.012	0.018	0.011	0.018		0.005	0.005	0.006	0.005	0.006	0.006	0.006	0.011	0.011	0.008	0.007
AFQ37803|HN|Av_OAV1	0.069	0.073	0.071	0.067	0.071	0.075	0.012	0.014	0.019	0.018	0.016	0.019	0.014	0.019	0.014		0.005	0.006	0.006	0.006	0.007	0.007	0.011	0.011	0.008	0.007
AFQ37809|HN|Av_OAV1	0.071	0.075	0.073	0.067	0.071	0.075	0.014	0.014	0.018	0.019	0.018	0.023	0.012	0.019	0.016	0.014		0.005	0.006	0.006	0.007	0.006	0.011	0.011	0.008	0.007
P12558|NDV|Ulster67	0.071	0.071	0.065	0.063	0.067	0.071	0.014	0.009	0.019	0.014	0.019	0.014	0.018	0.014	0.021	0.023	0.019		0.006	0.005	0.006	0.003	0.010	0.010	0.007	0.006
QJX65268|HN|Av_OAV1	0.073	0.071	0.067	0.067	0.071	0.075	0.012	0.016	0.019	0.018	0.012	0.021	0.018	0.021	0.018	0.021	0.023	0.023		0.006	0.007	0.006	0.011	0.011	0.007	0.007
P13850|NDV|Queensland/66	0.073	0.073	0.071	0.065	0.069	0.073	0.016	0.014	0.025	0.019	0.021	0.016	0.023	0.019	0.023	0.025	0.025	0.016	0.027		0.006	0.006	0.011	0.011	0.007	0.004
AAA46672|HN||ORF-5_Av_OAV1	0.075	0.075	0.073	0.071	0.075	0.078	0.018	0.019	0.025	0.021	0.023	0.011	0.025	0.025	0.025	0.028	0.030	0.021	0.028	0.023		0.006	0.011	0.011	0.007	0.007
AAA46677|HN|Glycoprot|Av_OAV1	0.075	0.075	0.069	0.071	0.075	0.078	0.018	0.016	0.023	0.018	0.023	0.018	0.021	0.021	0.025	0.028	0.027	0.007	0.027	0.023	0.025		0.011	0.011	0.007	0.007
AAQ54640|HN|Av_OAV1	0.076	0.111	0.107	0.111	0.111	0.119	0.061	0.063	0.069	0.065	0.067	0.061	0.067	0.069	0.069	0.069	0.071	0.061	0.069	0.067	0.069	0.065		0.002	0.012	0.012
P12553|NDV_TEXAS-GB48	0.076	0.111	0.107	0.111	0.111	0.119	0.061	0.063	0.069	0.065	0.067	0.061	0.067	0.069	0.069	0.069	0.071	0.061	0.069	0.067	0.069	0.065	0.004		0.012	0.012
QDY92317|HN|protein_Av_OAV1	0.078	0.073	0.069	0.069	0.073	0.076	0.027	0.028	0.036	0.030	0.032	0.023	0.034	0.034	0.034	0.037	0.039	0.028	0.030	0.028	0.030	0.032	0.075	0.075		0.008
AKJ85759|HN|Av_OAV1	0.080	0.080	0.078	0.073	0.076	0.080	0.023	0.021	0.032	0.027	0.028	0.023	0.030	0.027	0.030	0.032	0.032	0.023	0.034	0.011	0.030	0.030	0.075	0.075	0.036	

Fig. 3: Pairwise distance calculation of HN protein of NDV using MEGA7 software

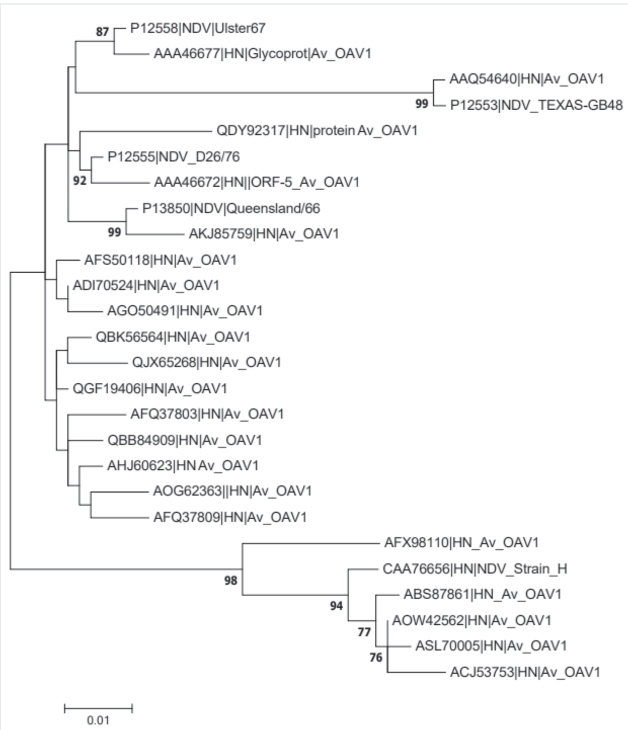

Fig. 4: Phylogenetic tree of HN protein of different strains of Newcastle disease virus. 1000 Bootstrap value was used in the analysis

CONCLUSION

The overall conclusions suggested that the HN protein is conserved in almost all the strains isolated from different geographical locations and the role of this protein in pathogenesis will require further investigation in future studies. The relationship of the divergent NDV strains to the R2B strain which is a mesogenic strain from India that has been used for secondary vaccination in chicken

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Molecular detection and differentiation of *Mycoplasma gallisepticum* field isolates of poultry in Andhra Pradesh

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ABSTRACT

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A total of 228 pooled Cloacal swabs, 228 pooled nasal swabs, 228 pooled tracheal swabs samples from the ailing birds and 152 pooled tracheal tissues, 152 pooled lung tissues and 76 pooled oviduct samples from the dead birds were collected from the 19 suspected poultry farms in A.P. and were labelled farm wise and specimen wise. The DNA was extracted from the samples by using Trizol method. The PCR test was standardized by targeting *mgc2* gene. The Sequence similarity and variations of newly sequenced isolates of *Mycoplasma gallisepticum* (MG) i.e. MG VLP, MG7G, MG9G, MG12G and vaccine isolates were compared with the available *mgc2* for MG from Gene Bank (NCBI) using multiple sequence alignment software MEGA 10 and Bio edit programs. Analysis of MG field isolates showed 42.71 to 100 per cent similarity. The MG field isolates showed variations between 41st to 757th nucleotide positions and insertions at -610T, -611C, -622G, -631A, -632C, -633A and -734A positions and these variations reflected the variations in amino acids between 22nd to 257th positions. The Phylogenetic analysis revealed that the vaccine strain (MG5G) and two field strains MG9G, MG7G formed one cluster and these isolates were closely related. The MG5G vaccine strain and two field isolates (MG9G and MG7G) were closely related with Telangana isolates. The MGVLP and MG12G formed one cluster and they are closely related with the UFMG2/Brazil strain and S6Mgc2/Australian strain.
Keywords: MG, mgc2gene, PCR, sequencing, phylogenetic analysis

INTRODUCTION

Growth in poultry sector is being challenged by increased incidence and re-emergence of diseases caused due to evolution of several pathogens and use of live vaccines. The continuous changing of the pathogenicity of the respiratory pathogens may cause increased virulence. Avian mycoplasmosis is caused by several pathogenic mycoplasmas. Among them, *Mycoplasma gallisepticum* and *Mycoplasma synoviae* are the most impactful to the poultry industry. *Mycoplasma gallisepticum* is one of the respiratory pathogens affecting chicken and causes Chronic Respiratory Disease (CRD). The *Mycoplasma gallisepticum (*MG*)* pathogens, have the ability to modify their surface antigens, may help in persistency in their hosts. Persistent infections lead to entry of other pathogens like *E. coli*, as *E. coli* alone cannot invade the air sacs. Though much research work has been reported from all over the states in India, limited work was done in Andhra Pradesh. In spite of regular vaccinations, still outbreaks were recorded in poultry, to control the disease the confirmatory diagnosis and variations of new isolates is essential. Hence this study was undertaken with the objectives, to detect and differentiate the *Mycoplasma gallisepticum* by application of advanced molecular techniques and to compare the field isolates and vaccine strains that are regularly being used in the field.

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MATERIALS AND METHODS

The *Mycoplasma gallisepticum*, live, freeze dried vaccine (Nobilis MG 6/85) (Intervet) was used as positive control for MG infections.

Samples

A total of 19 suspected poultry farms, from each few live birds, few dead birds and pooled samples of 12 cloacal swabs, 12 tracheal swabs, 8 tracheal tissues, 8 lung tissues and 4 oviducts are collected and were labelled separately as per farm.

DNA extraction

The *Mycoplasma gallisepticum*, live, freeze dried vaccine (Nobilis MG 6/85) (Intervet) was used as positive control for MG infections.MG-DNA was extracted from the triturated tissue samples using TRIsol (TRIsoln of Genei, Bengaluru) reagent (Baert *et al.*, 2007).

Typing of various strains of MG was done by using mgc2 gene

The following *mgc2* gene primers were used (Ferguson *et al*., 2005) Forward**:** 5'-GCTTTGTGTTT CTCGGGTGCTA-3', Reverse 5'-CGGTGGAAAACCA GCTCTTG-3'. Optimized PCR mixture composition was Target DNA 2 µL, forward primer1 µL**,** reverse primer 1µL**,** Emerald Amp GT PCR Master Mix 12.5 µL and NFW 8.5 µL and the optimized conditions were initial denaturation at 94^{o}C for 5 min, followed by 35 cycles of denaturation at 94^{o}C for 30 seconds, annealing at 56^{o}C for 30 seconds and extension at 72^{o}C for 30 seconds and final extension step was at 72^{o}C for 10 mins.

Sequencing and analysis of mgc2 gene

Sequencing was done in both directions using *mgc2* F and *mgc2* R primers to generate a continues sequence using automated DNA sequencer (ABI Perkin Elmer). The sequence analysis was done after converting into FASTA format on internet (website: http://www.ncbi.nim.nih.gov), multiple alignments of nucleotide and deducted amino acid sequence were done using CLUSTALW software available on internet .both percent nucleotide identity and amino acid residue substitutions were studied on comparison with those of different published isolates and isolates of MG from various geographical regions (Table 1).

Phylogenetic analysis

Phylogenetic analysis was performed using CLUSTALW (1.6) software (available on internet: http://www.ch.embnet.org./software/clustalw), an unrooted neighbors joining tree was constructed using the partial nucleotide and deducted amino acid sequences of the MG isolates and other MG isolates from different parts of the world.

RESULTS AND DISCUSSION

In Andhra Pradesh, respiratory infections are very common in poultry flocks in spite of regular vaccinations against the diseases. All the respiratory pathogens manifest similar type of symptoms, hence accurate and rapid diagnosis is very much essential to control the respiratory infections. The present study was aimed, to detect and differentiate *Mycoplasma gallisepticum* field isolates in poultry by application of advance molecular techniques and to compare the field isolates with that of the vaccine strains that are regularly being used in the field. In the present study, the samples were collected from 19 farms where respiratory infections were suspected from different districts of Andhra Pradesh. The DNA extraction was carried out from the suspected samples by Trizol method, PCR test was standardized using MG vaccine for studying the variations of MG strains by targeting *mgc2* gene. A 35 cycle PCR with annealing temperature of 56^{o}C for 30 seconds was found to be optimum for the amplification of 824 bp product in positive DNA sample (MG vaccine). The negative control kept in the reaction did not produce any amplification. The 824bp PCR products of *mgc2* gene were sequenced (Bioserve Biotechnologies, Hyderabad) and multiple sequence analysis of the field isolates was carried out with that of the vaccine and other reference MG strains from Genbank (Table 1). The multiple nucleotide sequence alignment of MG vaccine (Nobilis MG 6/85 strain, (MG5G)), field isolates (MG7G, MGVLP, MG9G, MG12G) with that of nucleotide sequences of strains from different countries revealed nucleotide substitutions, and insertions between 41st to 757th nucleotide positions (Fig. 1a,1b,1c and 1d). Similarly, Couto *et al.* (2016) also observed the nucleotide variations between 494 to739 positions of nucleotide sequences in *mgc2* gene of different Brazilian strains and other published sequences and concluded high variations in field strains when compared to vaccines and hence vaccination may not guarantee cross protection against different strains. The variations in nucleotides may reflect in the amino acid substitutions and these changes may influence the pathogenesis. Ferguson *et al.* (2017) studied the variations in MG field isolates and vaccine by targeting the *mgc2* gene and found the presence of different nucleotide insertions and deletions in the sequences between 196th to 780th positions. The *mgc2* gene sequence of MG7G field isolate did not show many changes except in one position G141C when compared with vaccine MG5G strain. Similarly, Ricketts *et al.* (2017) also noticed no major genomic changes among the ts-11vaccine, avirulent and virulent isolates but there were numerous SNPs. The field strain MG7G showed lesser

Table 1: List of various strains of MG –*mgc2* gene from GenBank and their accession numbers used in sequencing and phylogenetic analysis

S. No	Strain	Origin	Year of Isolation	Gene Bank ID
1.	MGclone1030cytadhesin	IRAN	2009	GQ436786
2.	K6112B-8	USA	2016	K577608
3.	EiS10-17	EGYPT	2017	KY421065
4.	S6*Mgc2*	AUSTRALIA	2013	JQ770177
5.	UFMG2	BRAZIL	2013	KJ019177
6.	NC08_2008.031-4-3P	USA	2012	CP003513
7.	MGS3B	INDIA	2014	KP279741
8.	MGS9B	INDIA	2015	KP279742
9.	MGS927	INDIA	2014	KP261894
10.	Eidmg8-1-108	EGYPT	2018	MH102389
11.	MGS1345	INDIA	2014	KP300762
12.	MGS1167	INDIA	2014	KP300758
13.	MGS1210	INDIA	2014	KP300761

Figure 1(a)

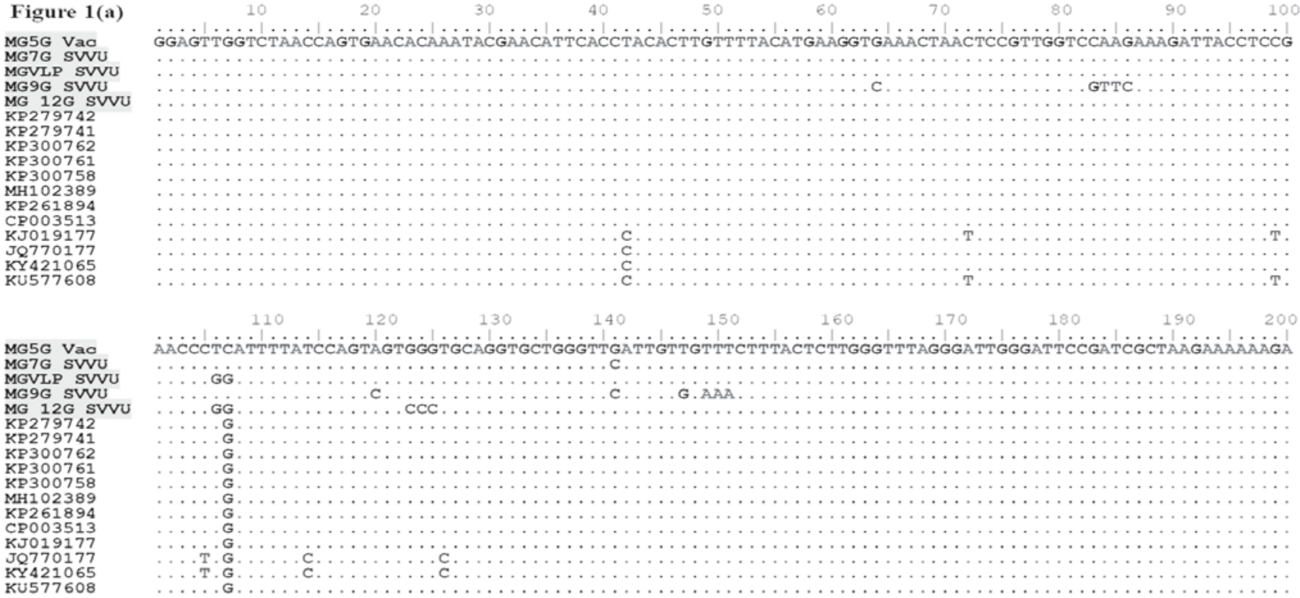


Figure 1(b)

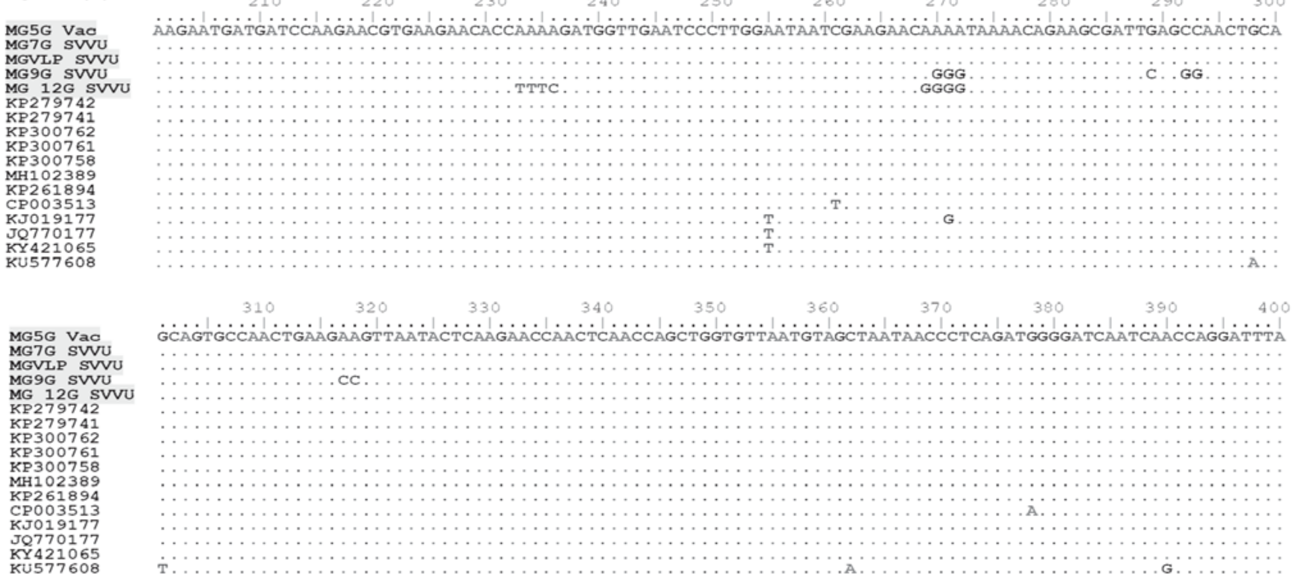
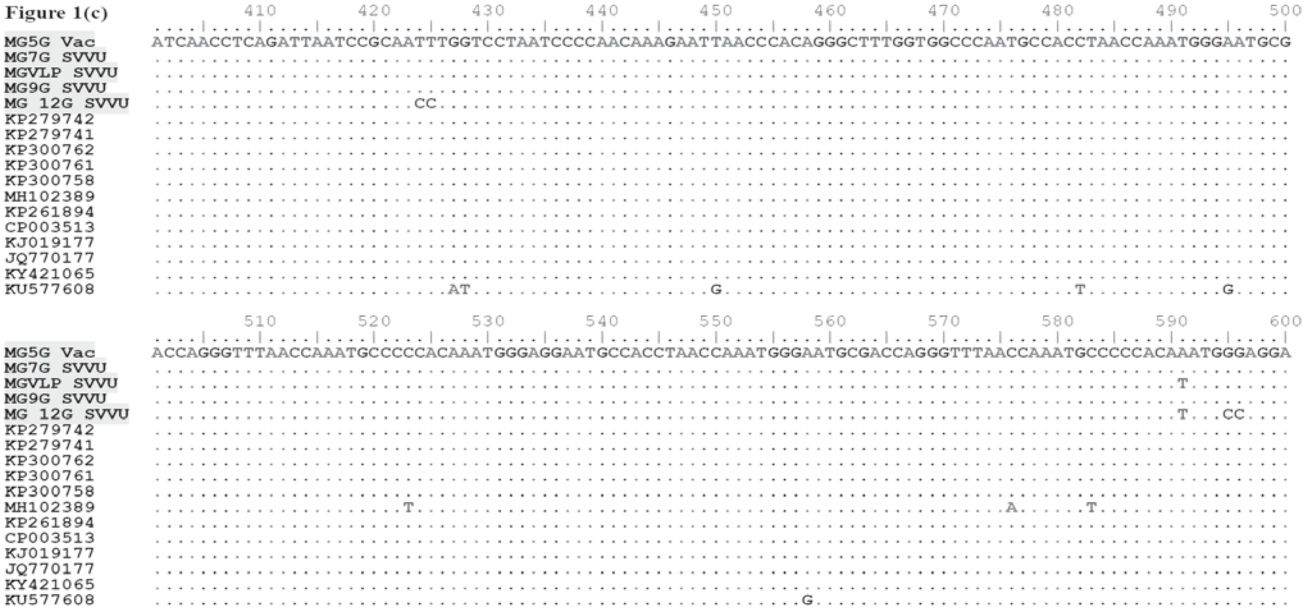


Figure 1(c)



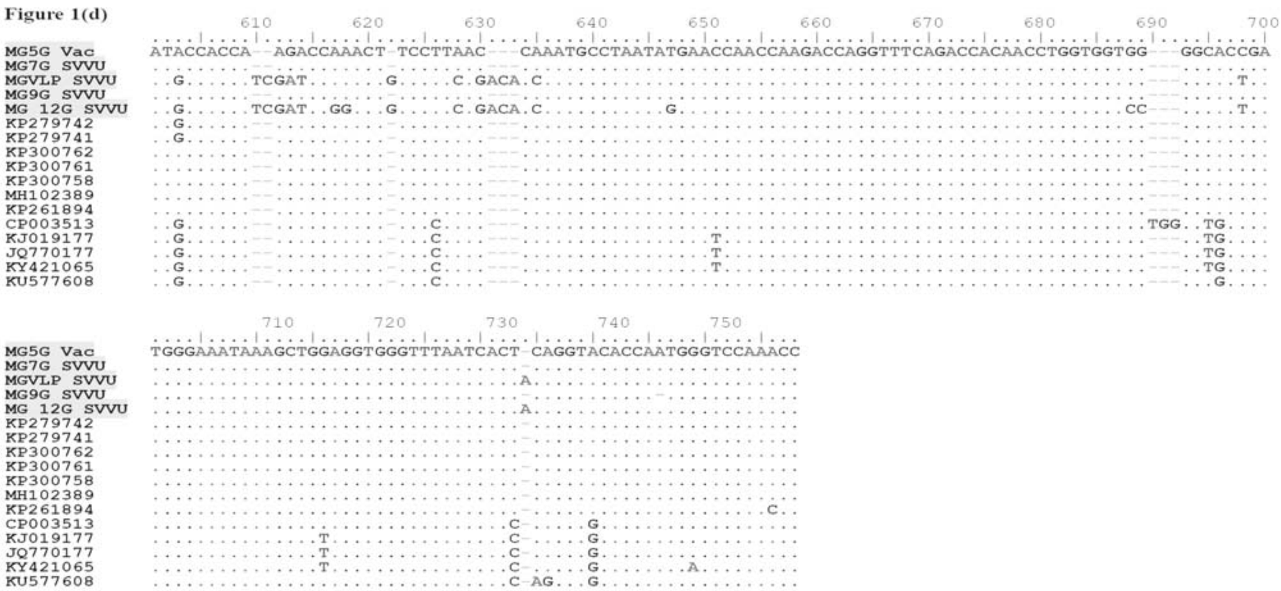
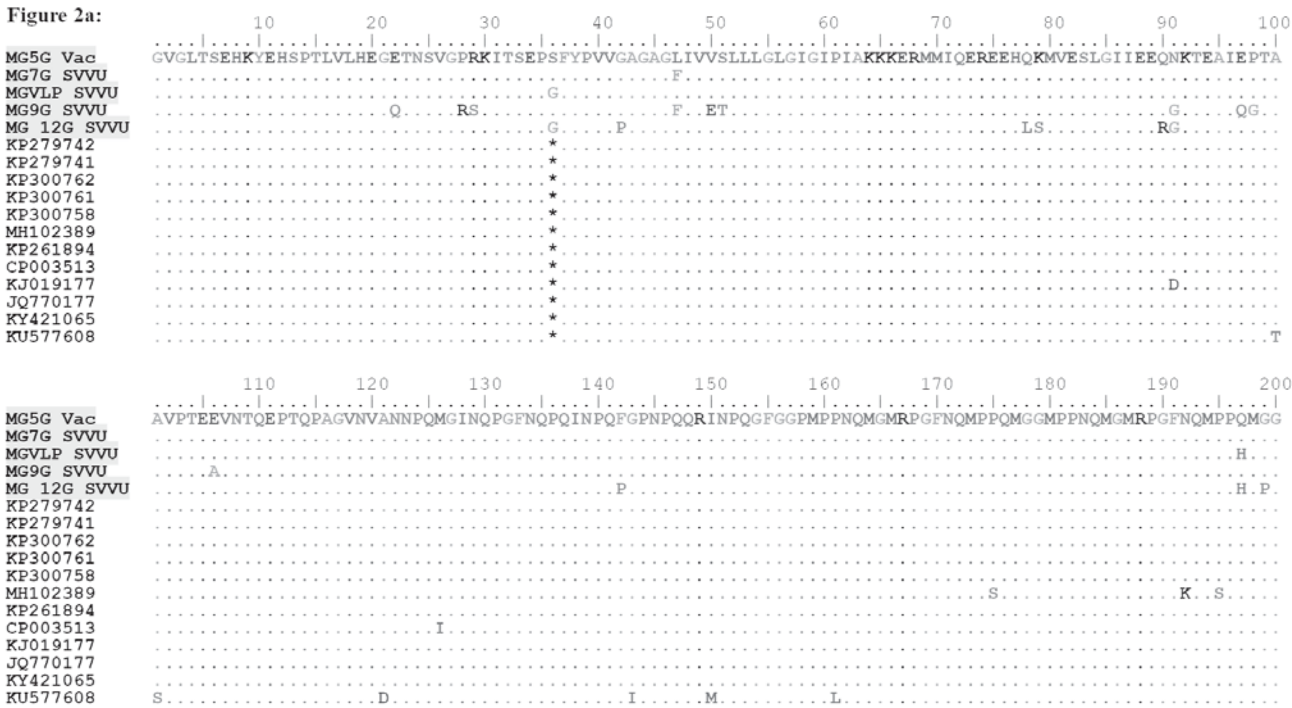


Fig. 1a, 1b, 1c and 1d: Multiple nucleotides sequence analysis of *mgc2* gene of vaccine strain, field isolates and reference strains



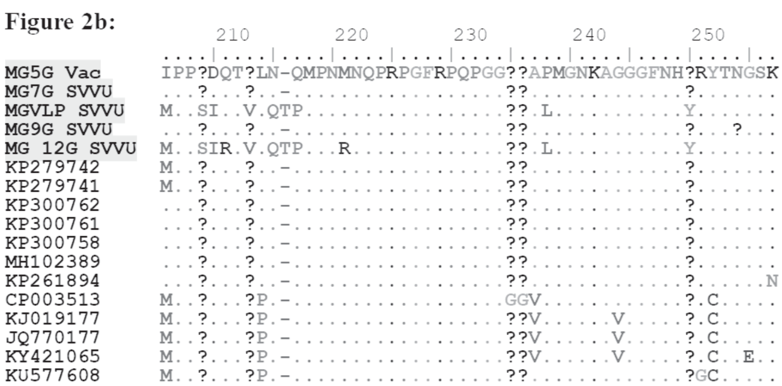


Fig. 2a and 2b: Multiple amino acids sequence analysis of mgc2 gene of vaccine strain, field isolates and reference strains

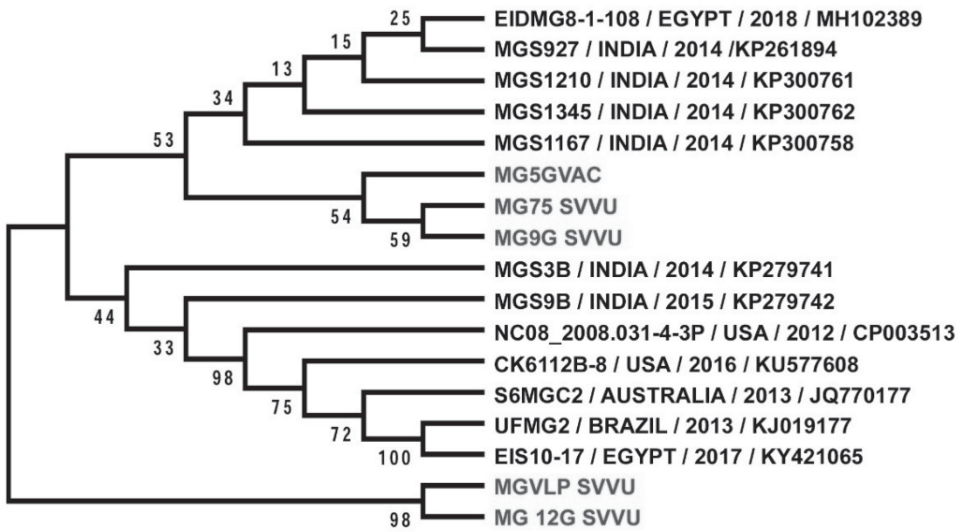


Fig. 3: Phylogenetic analysis of *mgc2* sequences of MG field, vaccine and GenBank strains. The phylogenetic tree was constructed using the MEGA version 10.0 by Neighbor–joining method with 1000 bootstrap replicates using p-distance model (Tamura *et al.*, 2007).

variations which might be due to horizontal and vertical transmission of the vaccine strain. These observations almost coincided with findings of Couto *et al.* (2016). In the present study field isolates showed lesser changes when compared with Telangana strains KP300761, KP300762, KP300758, MGS3B, MGS9B and MGS927.

In the present study the multiple sequence alignment of amino acids of *mgc2* gene of vaccine, field and reference strains was studied for variations and revealed that amino acid substitutions and insertions were observed at different positions (Fig. 2a & 2b). The amino acid substitutions were noticed in *mgc2* sequences at different positions in field isolates when compared with vaccine sequence. The amino acid substitution in case of MG7G at L47F indicates that Leucine was replaced by Phenylalanine because of substitution of nucleotide at G141C. The amino acid substitutions in case of MGVLP were noticed because of substitution and insertion of nucleotides at T106G, C107G, A591T, A603G, A612G, G613A, A614T, A628C, C630G, A635C and C698T and -610T, -611C, -622G, -631A, -632C, -633A and -734A reflected the change of amino acids at 36th position serine to glycine, 197th position glutamine to histidine, 206th position isoleucine to methionine, 210th position aspartic acid to isoleucine, 215th position asparagine to glutamine, 217th position glutamine to proline, 218th position proline to leucine and insertions at 209th serine, 216th threonine, 250th tyrosine.

The amino acid substitutions in case of MG9G isolate, might be due to nucleotide substitutions at G64C, C83G, A84T, A85T, G86C, A120C, G141C, T147G, T149A, T150A, T151A, A270G, A271G, A272G, G289C, C292G, C293G, A317C and A318C reflected the change of amino acids at 22nd glutamic acid to glutamine, 28th proline to arginine, 29th arginine to serine, 47th leucine to phenylalanine, 50th valine to glutamic acid, 51st serine to threonine, 91st asparagine to glycine, 97th glutamic acid to glutamine, 98th proline to glycine and 106th glutamic acid to alanine.

The amino acid substitutions in case of MG12G isolate, might be due to nucleotide substitutions at T106G, C107G, G123C, G124C, G125C, A233T, A234T, A235T, A236C, A269G, A270G, A271G, A272G, T424C, T425C, A591T, G595C, G596C, A603G, A612G, G613A, A614T, A617G, A618G, A628C, C630G, A635C, T647G, G688C, G689C and C698T and insertion of nucleotides also observed at different positions *i.e* -610T,-611C, -622G,-631A,-632C, -633A and -734A reflected the change of amino acids at 36th serine to glycine, 42nd glycine to proline, 78th glutamine to leucine, 79th lysine to serine, 90th glutamine to arginine, 91st asparagine to glycine, 142nd phenylalanine to proline, 197th glutamine to proline, 206th isoleucine to methionine, 210th aspartic acid to isoleucine, 211th glutamine to arginine, 215th asparagine to glutamine, 217th glutamine to proline, 221st methionine to arginine and 238th proline to leucine and insertion of amino acids at 209th serine,213th valine, 216th threonine and tyrosine. Rasoulinezhad *et al.* (2017) also observed high sequence similarity between some Iranian isolates with Indian and Pakistani MG isolates by sequencing, identified the amino acid substitutions in MG field turkey isolates in Iran by comparing the *mgc2* gene sequences with each other and found the position 10 was changed from Methionine to Isoleucine and position 21 the leucine substituted by proline in seven isolates. Moreover, we also found change of polar methionine to nonpolar isoleucine and proline to leucine in five isolates in positions 62 and 90 respectively.

In the present study, the phylogenetic tree was constructed with 13 gene bank isolates, one vaccine strain (MG5G) and four field isolates (MG7G, MG9G, MG12G and MGVLP) of AP. The phylogenetic analysis revealed that the vaccine strain (MG5G) and two field strains formed one cluster and the two field isolates (MG9G, MG7G) were closely related with vaccine strain MG5G (Fig. 3). Loolmani *et al*. (2014) also observed high homogenicity between Tehran province origin MG turkey isolates based on the *mgc2* gene sequences. These vaccine strain and two field isolates (MG9G and MG7G) were closely related with Telangana isolates (KP300761, KP300762 andKP300758).

The field strains MGVLP and MG12G formed one cluster and they are closely related. In a similar study, Marouf *et al*. (2020) reported that the field *MGC2*-EGY1 isolate was in one cluster close to HQ591357 MG-EIS6-T-10, which was related to an Egyptian strain isolated from a local turkey breed in 2011 and *MGC2*-EGY2, was in another cluster close to KP279742 MG-MGS-9B, which is related to an Indian strain isolated from chickens in 2014.

In this study, the vaccine isolate showed closer relationship with Indian strain i.e MGS927 strain isolated in 2014 from Telangana state of India. Similarly, Rasoulinezhad *et al.* (2017) observed RH1376.58 and RH1373.61 Iranian turkey field isolates were quite similar to Indian strains and RH1376.60 and RH1376.62. Iranian, turkey field isolates were grouped with Pakistani strains showing more similarity and also RH1376.5, RH1376.49 and RH1376.74 arranged in one group with 99 per cent homogeneity in comparison to ts-vaccinal strain.

CONCLUSION

This study is useful for detection and differentiation of field isolates of MG for variations in gene sequences due to insertions and deletions of nucleotides it may leads to changes in virulence of pathogens.

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Typing of field isolates of *Infectious bronchitis virus* from Kerala using reverse transcriptase polymerase chain reaction-restriction fragment length polymorphism analysis

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ABSTRACT

Sruthy, C.B., Sankar, S., Mini, M., Priya, P.M. and Dhanush, K.B. 2022. Typing of field isolates of Infectious bronchitis virus *from Kerala using reverse transcriptase polymerase chain reaction-restriction fragment length polymorphism analysis. Indian Journal of Poultry Science. 57(02): 187-191.*

Poultry farming is one of the booming industries in India. But the sector is still the victim of menace caused by various viruses. Infectious bronchitis is one such disease, which cause economic loss to the industry worldwide. In Kerala, vaccination against the disease is not strictly followed and there are frequent reports of the disease outbreaks from different parts of the state. Hence, monitoring of Infectious bronchitis virus strains circulating in the state is necessary. Pooled tissue samples were collected from ailing/ recently dead birds with symptoms/ lesions suggestive of infectious bronchitis, from various parts of the state. Reverse transcriptase polymerase chain reaction targeting hypervariable region 3 was done to detect the virus from tissue samples followed by virus inoculation in embryonated chicken egg. The ribonucleic acid extracted from allantoic fluid was used for reverse transcriptase polymerase chain reaction targeting S1 subunit of S gene. The representative amplicons were then subjected to nucleotide sequencing and restriction fragment length polymorphism analysis. Thirty-two samples were found to be positive by RT-PCR. Restriction fragment length polymorphism analysis of S1 subunit of S gene generated restriction patterns similar to vaccine strain, Massachusetts type.

Keywords: Infectious bronchitis, *Infectious bronchitis virus,* S1 subunit of S gene, restriction fragment length polymorphism

INTRODUCTION

Infectious bronchitis virus (IBV) is the prototype of the family *Coronaviridae* which causes infectious bronchitis (IB), an acute and contagious disease of poultry which often shatters the economy of global poultry industry. It affects chicken of all ages, characterized by upper respiratory symptoms and urinary and reproductive system failures (Lambrechts *et al*., 1993; Balestrin *et al*., 2014; Xu *et al*., 2018; Patidar *et al*., 2021). It is a round to pleomorphic enveloped virus (Panda and Kurkure, 2020) having a diameter of approximately 120 nm with club shaped surface projections/ spikes with a length of 20 nm, which conferred a crown like appearance to the virus (Cavanagh, 2007). The IBV consists of a single stranded positive sense RNA genome of size 27.6 kb. It encodes polyproteins 1a and 1ab, four major structural proteins, and various accessory proteins. The structural proteins are spike (S), envelope (E), membrane (M) and nucleocapsid (N) (Cavanagh and Gelb Jr, 2008). Mutations and recombination in the genome, especially variations in the S1 protein contribute to the emergence of new IBV variants. Thus, the virus has many genotypic, serotypic, and pathotypic variants, some of which are distributed globally, while others are restricted to local areas (Cook *et al*., 2012).

In Kerala, vaccination against the disease is not strictly followed and there are frequent reports of IB outbreaks from different parts of the state. Hence, monitoring of IBV strains circulating in the state is necessary. In this scenario, the present study focuses on the detection of IBV isolates from Kerala followed by genotyping based on nucleotide sequencing and reverse transcriptase polymerase chain reaction- restriction fragment length polymorphism (RT-PCR-RFLP) analysis of S1 subunit of S gene.

MATERIALS AND METHODS

Collection of clinical samples

Pooled tissue samples were collected from 103 ailing/ recently dead chicken with symptoms/ lesions suggestive of IB, brought to the departments of Veterinary Microbiology and Veterinary Pathology, College of Veterinary and Animal Sciences, Mannuthy and from different poultry farms in various parts of the state during September 2019 to September 2021. The suspected birds had symptoms of respiratory distress, weakness and droopiness and the gross lesions included air-sacculitis, exudates and hemorrhages in trachea, pulmonary congestion, salpingitis and enlargement of kidneys.

Direct detection of IBV from tissue samples by RT-PCR

The tissue samples collected in RNA later were used for the extraction of viral RNA by TRIzol method (Ahmad *et al*., 2007). The RNA pellets were dissolved in 20-50 µL sterile nuclease free water and those with an optical density ratio between 1.8 and 2 at 260/280 nm and 260/230 nm were chosen for further studies. The

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extracted RNA was stored at -80°C until further use. Samples with RNA concentration in the range 100 fg to 1 ìg were selected for complementary deoxyribonucleic acid (cDNA) synthesis. Reverse transcription was carried out using iScript™ cDNA synthesis kit (Bio-Rad, USA), according to the manufacturer's instructions. Reverse transcriptase polymerase chain reaction (RT-PCR) was done by targeting hypervariable region 3 of S1 subunit of S gene using the primers described by Shyma *et al*. (2018) (Table 1). The reaction was carried out in 0.2 mL PCR tubes with 3 µL of template cDNA, 1 µL each of forward and reverse primer (10 p*M*/ìL), 6.25 µL of Emerald Amp GT PCR master mix and remaining nuclease free water to obtain a total reaction volume of 12.5 µL. The amplification was done at an initial denaturation temperature of 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 30 sec., annealing at 44.4°C for 45 sec. and denaturation at 72°C for 1 min. This was followed by final extension at 72°C for 7 min. The amplified products were detected by electrophoresis in one per cent agarose gel in TBE buffer (1X).

Virus isolation and confirmation by RT-PCR

The tissue homogenate from all the RT-PCR positive samples were treated with antibiotic-antimycotic solution, filtered through 0.22 µm syringe filter and 0.1 mL of each was inoculated into nine to eleven day-old embryonated chicken eggs (ECE) *by* allantoic route. Control eggs were inoculated with sterile PBS. The eggs were incubated at 37ºC and those which were dead before 24 h were discarded. The eggs with live embryos after 48 h of incubation were terminated by chilling in refrigerator. Allantoic fluid (AF) was harvested separately in sterile vials and stored at -20ºC until taken for RNA extraction. The total RNA from harvested AF was extracted and the presence of the virus in AF was confirmed by RT-PCR. Complementary DNA from avian infectious bronchitis vaccine, live, Massachusetts strain (Ventri Biologicals) was used as positive control. The amplified products were detected by electrophoresis in one per cent agarose gel in TBE buffer (1X).

RT-PCR targeting S1 subunit of S gene

Prior to restriction fragment length polymorphism analysis, RT-PCR targeting the whole S1 subunit of S gene was performed. The total RNA extracted from harvested AF was subjected to reverse transcription using Protoscript First Strand cDNA synthesis kit (New England Biolabs (NEB), USA), according to manufacturer's instructions. The RT-PCR was conducted using the primers described by Okino *et al*. (2005) (Table 2). The PCR mix was subjected to 30 cycles of denaturation at 98°C for 10 sec., annealing at 55°C for 15 sec. and extension at 68°C for 10 sec. The amplified products were detected by electrophoresis in 0.8 per cent agarose gel in TBE buffer (1X) (Fig. 1). The representative amplicons from northern, central and southern Kerala were subsequently sequenced.

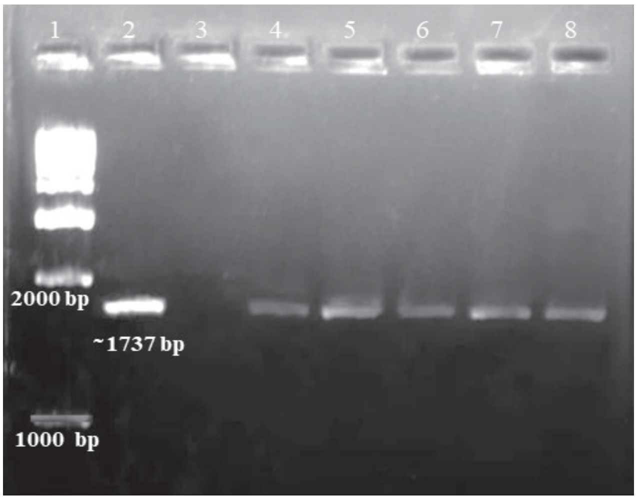

Fig. 1: Agarose gel electrophoresis of PCR amplified products of the whole S1 subunit of S gene (1737 bp), Lane 1: 1000 bp ladder, Lane 2: positive control, Lane 3: negative control, Lane 4 to 8: positive samples

Nucleotide sequencing and amino acid profile analysis of S1 subunit of S gene

The representative amplicons from different zones of Kerala (North, Central and South), obtained after RT-PCR targeting S1 subunit of S gene were gel eluted and sequenced by Sanger's dideoxy chain termination method at Sci genome, Angamaly, Cochin. The sequences were queried in the nucleotide database and blasted in order to confirm that the BLASTn hits were of spike gene. The sequences were compared with that of reference strain (H120) and other IBV sequences from India available in

Table 1: Sequences of primers used for RT-PCR targeting hypervariable region 3 of S1 subunit of S gene and its product size

Primer	Primer sequence (5'-3')	Product size (bp)	Reference
IBH3 F	*AATCCTAGTGGTGTYCAG*	257	Shyma *et al*. (2018)
IBH3 R	*GTTGCTCTACCACTAAAG*		

Table 2: Sequences of primers used for RT-PCR targeting the whole S1 subunit of S gene and its product size

Primer	Primer sequence (5'-3')	Product size (bp)	Reference
SYU+	TAYTAYTACCARAGYGCYTT	1737	Okino *et al*. (2005)
VBIS	GGACCTTATCCATACGC		

GenBank database. Expasy translate was used for sequence translation for deducting amino acid sequence.

Restriction fragment length polymorphism

Representative samples were subjected to restriction fragment length polymorphism (RFLP). The restriction enzymes (REs) such as *Hae*III, *BstY*I (Thermo Fischer Scientific, Lithuania) and *Xcm*I (New England Biolabs (NEB), USA) were used to digest the RT-PCR products targeting S1 subunit of S gene of IBV, according to the instructions of manufacturer. The restriction patterns were then analysed by electrophoresis on two per cent agarose gel.

RESULTS AND DISCUSSION

Polymerase chain reaction has the advantage of rapid detection with high sensitivity and specificity. Furthermore, an optimised PCR allows the detection of the virus directly from tissue samples even when the virus concentration is low (Sylvester *et al*., 2006). Even though the more conserved nature of N and M genes make them the targets for designing universal primers for IBV, the presence of conserved as well as varying sequences in S1 make it suitable targets for designing universal as well as strain specific RT-PCR primers (Okino *et al*., 2005). The S1 subunit is reported to have three hypervariable regions and the variability of this region is supposed to be the inculminating factor for the emergence of new genotypes and serotypes of IBV (de Wit *et al*., 2011). Many researchers (Shyma *et al*., 2018; Al-Jallad *et al*., 2020; Al-Mubarak and Al-Kubati, 2020), have successfully used the hypervariable region 3 for the amplification of viral RNA. In the present study also, primers targeting hypervariable region 3 was used for the direct detection of IBV from tissue samples, which yielded an amplicon size of 257 bp from 32 samples. The gradient PCR using cDNA from vaccine showed better annealing at a temperature of 44.4ºC.

Infectious bronchitis virus replicates so well in embryonated chicken eggs regardless of the route of inoculation. Nevertheless, allantoic route is preferred, because the chorioallantoic membrane (CAM) epithelium supports extensive replication of the virus and shed high titres in AF (Jordan and Nassar, 1973). As per the studies of Purchase *et al.* (1966), by about one to two days post inoculation (PI), the virus titre in the AF reached maximum. Hence in this study, tissue homogenate from RT-PCR positive samples were processed and inoculated into 9–11-day old ECE by allantoic route and incubated for 48 h. Embryos that were dead before 24 h were discarded suspecting contamination, toxicity of inoculum or injury. This is in accordance with procedure formulated by Guy, 2008. According to Handberg *et al.* (1999), the presence of IBV in harvested allantoic fluid can be identified by RT-PCR. The isolation of IBV by egg inoculation is a tedious and time-consuming process and thus researchers have effectively replaced it by another technique in which a shortened isolation step followed by RT-PCR was performed for the multiplication of IBV and detection of antigen, respectively (de Wit, 2000). Pertinent to this statement, in the present study, the AF after first passage was subjected to RNA extraction. All the samples which showed positive results in RT-PCR with direct tissue samples again produced positive results with RNA from harvested AF. Similar study was conducted by Chen and Wang (2010), in which RNA extracted from AF harvested after 48-72 h PI was subjected to multiplex RT-PCR targeting the hypervariable region 1 and 2 of S1 subunit of S gene.

Previous studies have showed that even if majority of the genome of IBV remain unchanged, few mutations in the nucleotide sequence of S1 subunit can generate new variants (Lai and Cavanagh, 1997), thus in the present study, S1 subunit of S gene was selected for sequencing and RT-PCR-RFLP analysis. The S1 subunit of S gene contains both conserved as well as variable regions, which enable the researchers to develop universal as well as strain specific primers. In the present study, SYU+ and VBIS- primers were used for RT-PCR, which was complementary to the beginning of S1 subunit and S2 subunit, respectively. A similar approach has been reported by Okino *et al*. (2005) wherein; the authors used these primers as universal primers followed by amplification of a small region of S1 subunit while performing semi-nested PCR. Jain *et al*. (2016) and Al-Jallad *et al*. (2020) have also done RT-PCR with primers targeting S1 subunit, but annealing sites were different. In the present study, gradient PCR showed better annealing at a temperature of 55 ºC. All the 32 samples as well as the positive control revealed an amplicon size of 1737 bp on RT-PCR that targeted S1 subunit of S gene. Agarose gel electrophoresis results of the same are depicted in fig. 1. On sequencing, the isolates from different zones of Kerala (North, Central and South) showed more than 99 per cent similarity in S1 subunit of S gene with H120 vaccine strain and the Indian isolates from various regions. On amino acid profile analysis of S1 subunit of S gene, non-synonymous mutations have been detected in 720 and 1277 positions in the open reading frame. Similar results were obtained in a study by Van Santen and Toro (2008), wherein, majority of amino acid variations occurred in the S1 subunit and also all the mutations except one was non-synonymous.

The representative amplicons of S1 subunit of S gene, including those with nucleotide variations, were subjected to RFLP with REs such as *Hae*III, *Xcm*I and *BstY*I. Kwon *et al.* (1993), Sumi *et al.* (2012) and Rafique *et al*. (2018) also used these REs to type the IBV isolates. In the current study, while using *Hae*III, restriction patterns of all the representative samples were similar to the positive control, which was Massachusetts type

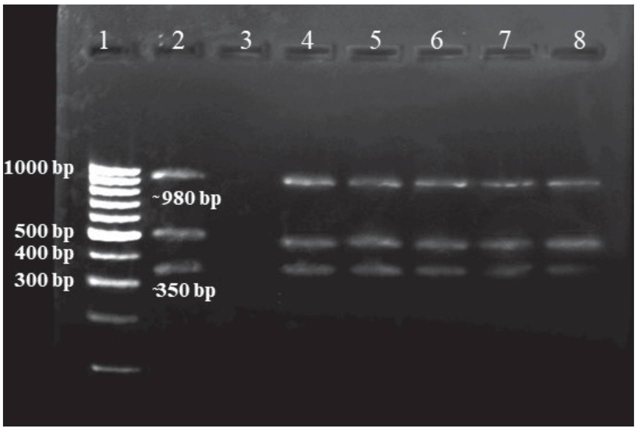

Fig. 2: RFLP patterns of PCR amplified S1 subunit of S gene from representative positive samples digested with *Hae* 111; Lane 1: 100 bp ladder, Lane 2: positive control, Lane 3: negative control, Lane 4 to 8: positive samples

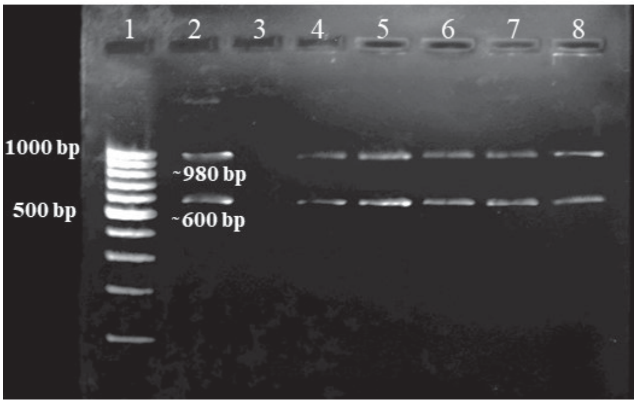

Fig. 3: RFLP patterns of PCR amplified S1 subunit of S gene from representative positive samples digested with *Xcm*I; Lane 1: 100 bp ladder, Lane 2: positive control, Lane 3: negative control, Lane 4 to 8: positive samples

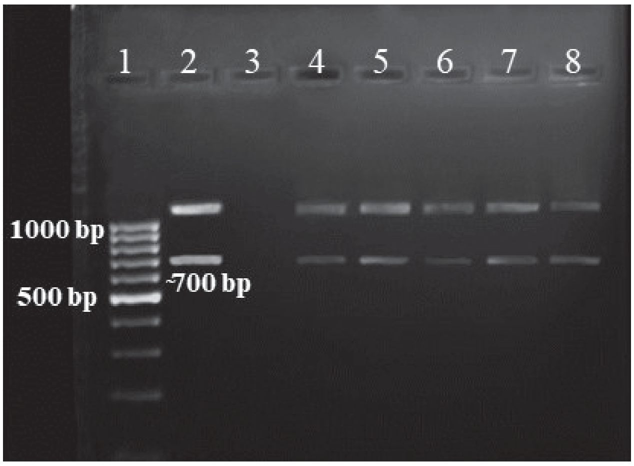

Fig. 4: RFLP patterns of PCR amplified S1 subunit of S gene from representative positive samples digested with *BstY*I; Lane 1: 100 bp ladder, Lane 2: positive control, Lane 3: negative control, Lane 4 to 8: positive samples

vaccine strain. Beaudette/ Massachusetts strain could be distinguished from Connecticut or Florida by *Xcm*I and *BstY*I could distinguish between the latter two. Therefore, to further rule out Connecticut and Florida strains, *Xcm*I and *BstY*I restriction enzymes were used, which also resulted in restriction patterns similar to the positive control, suggesting that all the representative samples selected were of Mass type (Fig. 2, 3, and 4, respectively). Wang and Khan (2000) also reported similar results, which were confirmed subsequently with virus neutralisation test.

The technique may result in change in cleavage pattern even if there is a mutation that does not affect the antigenicity or biological function of the virus. On the contrary, it may also sometimes miss the variant, if the mutation occurred in between the cleavage site, resulting in unaltered cleavage sites (de Wit, 2000). Nevertheless, the method could effectively use to detect new variants and genotype of the isolate.

CONCLUSION

In the current study, out of the 103 samples collected from chicken with suspected symptoms/ lesions of IB, 32 were found to be positive in RT-PCR targeting the hypervariable region 3 of S1 subunit of S gene. The positive samples on embryonated chicken egg inoculation also produced similar results. This was followed by sequencing and RT-PCR-RFLP analysis of S1 subunit of gene, which generated restriction patterns similar to vaccine strain, Massachusetts type.

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Application of latex agglutination test in the diagnosis of *Riemerellosis* in ducks

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ABSTRACT

Greeshma, R., Priya, P.M., Mini, M., Sreeja, R.N. and Vinodkumar, K. 2022. Application of latex agglutination test in the diagnosis of Riemerellosis *in ducks. Indian Journal of Poultry Science. 57(02): 193-197.*

Riemerellosis, caused by *Riemerella anatipestifer (R. anatipestifer)* is a septicemic infection, mainly affecting ducks. Its occurrence is common and outbreaks are frequently reported with heavy mortality and morbidity. Currently, diagnosis of riemerellosis is mainly dependent on conventional methods like isolation and identification and molecular techniques like polymerase chain reaction (PCR). The available techniques demand time and require expensive equipment and reagents, making them impractical to be used under field conditions. In the present study, a latex agglutination test (LAT) was developed to detect antibodies against riemerellosis using the crude outer membrane protein (Omp) of *R. anatipestifer* as antigen. The developed LAT was easy to perform, economic, gave results rapidly and capable of testing large number of samples simultaneously. The comparative efficacy of the LAT was assessed using indirect enzyme linked immunosorbent assay (I-ELISA).

Keywords: *Duck, Riemerellosis, Latex agglutination test, indirect ELISA*

INTRODUCTION

Riemerellosis is one the most important bacterial diseases affecting ducks and is caused by *R. anatipestifer*, a Gram-negative bacteria belonging to the family *Flavobacteriaceae*. There are two clinical forms of the disease, an acute septicaemic form affecting ducks below eight weeks of age and a chronic localized form affecting older ducks (Subramaniam *et al*., 2000). The disease is mainly transmitted through respiratory route and skin wounds (Ruiz and Sandhu, 2013). An outbreak of riemerellosis poses several economic concerns like loss of valuable poultry stock and low production rates due to weight loss and reproductive affections. As Kerala is a state where duck population is comparatively high and since no vaccination against the disease is practiced so far, an outbreak can take a tremendous toll on small-scale duck farmers. Therefore, rapid diagnosis and timely treatment of the affected flock is of utmost importance. Currently, diagnosis of riemerellosis is achieved by isolation and identification, though it is a gold standard method, it is time-consuming, or by molecular techniques like PCR which requires expensive equipment and reagents. All these factors make the utility of both the techniques unfeasible under field conditions. As of now, there are 21 serotypes of *R. anatipestifer* (Tsai *et al*., 2005) providing little to no cross-protection. But in Kerala only one serotype has been detected, giving scope for serological diagnosis of infection. Thus, the present study was conducted to develop a LAT to make the diagnosis rapid and easy. Sumitha and Rajasekar (2014) opined that LAT could replace molecular techniques like PCR, since it is very costly, and other serological tests like AGPT, which is time consuming and requires skilled personnel. The main advantage with LAT is that the results can be visually read and does not require any equipment or professional expertise.

The objective of the present study was to develop a latex agglutination test (LAT) for detecting antibodies against riemerellosis using the crude outer membrane protein (Omp) of *R. anatipestifer* as antigen.

MATERIALS AND METHODS

Bacterial isolate

The *R. anatipestifer* (RA1), representative isolate of the circulating serotype, maintained in the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, as lyophilised culture was utilised in the present study. The culture was revived using 5-10 per cent bovine blood agar (BA) and incubated at 37ºC in microaerophilic condition for 24 to 48 h.

Identification of the organism

The obtained colonies were stained by Gram's method and the tests for identification were performed according to Surya (2011). The morphology, cultural characteristics, haemolysis on BA, reaction for catalase and oxidase and growth on MacConkey agar (MCA) were noted. In addition, tests recommended by OIE (2015) were also performed like indole, ornithine decarboxylase utilisation, gelatin liquefaction and oxidation-fermentation test. The genomic DNA extracted (heat lysis method) from the isolate was subjected to *R. anatipestifer* species specific PCR developed by Kardos *et al*. (2007). On confirmation of the identity of RA1, its Omp was extracted to standardise LAT.

Outer membrane protein extraction

The Omp of *R. anatipestifer* was extracted using

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sarcosine detergent according to Davies and Donachie (1996) with some modifications. The organism was grown on BA and incubated at 37° C for 24 h. The well grown colonies were scrapped from BA plates into sterile centrifuge tubes containing sterile phosphate buffered saline (PBS) and subjected to centrifugation at 8000 x g for 30 min. The pellet obtained was washed with PBS, re-suspended in 10 *mM* HEPES buffer and incubated at room temperature for 20 min. This suspension was then kept on ice and a titanium probe with 12 mm diameter was placed 1-1.5 cm deep into the suspension. Then, cells were disrupted by sonication for 30 sec, 10 times at 250V with a brief pause of 30 sec between each burst. The sonicated suspension was kept on ice for 15 min. and centrifuged at 4000 x g for 20 min. at 4° C. The supernatant was carefully transferred into polyallomer ultracentrifuge tubes and centrifuged at 100000 x g for one hour at 4° C in a Beckman ultracentrifuge. The pellet was decanted and mixed with 7 mL of 0.5 per cent sodium lauryl sarcosine detergent, and incubated at 25°C for 20 min., with gentle rotation. The sarcosyl insoluble fraction containing the Omp was centrifuged at 100000 x g for one hour at 4°C in a Beckman ultracentrifuge and the pellet obtained was resuspended in 0.5 mL Tris-HCl (20 m*M*) and stored at -20°C till further use. Protein concentration was estimated using Nanodrop 2000.

Latex agglutination test

The test was performed according to Mitui *et al* (1981), with some modifications. The latex beads were washed in carbonate-bicarbonate buffer twice by centrifugation at 6800 x g for two minutes each and made into a two per cent suspension with carbonate-bicarbonate buffer. This suspension was mixed with equal volume of Omp (50 ìg/mL, 100 ìg/mL and 150 ìg/mL) and incubated at 37°C for six hours with constant shaking at 250 x g. The sensitised beads were then centrifuged at 6800 x g for three minutes and the pellet was resuspended as two per cent suspension in PBS containing 5 mg/mL of bovine serum albumin (BSA). This suspension was kept at 37°C in a water bath overnight and centrifuged at 6800 x g for three minutes. The pellet was then resuspended in PBS containing 0.5 mg/mL BSA and 0.1 per cent sodium azide as 0.5 per cent suspension. The sensitised beads were stored at 4°C until use. The LAT was performed on a glass slide by mixing equal volumes (20 ìL) of sensitised latex beads and hyperimmune serum raised in rabbits. The slide was rocked gently for two to five minutes. Phosphate buffered saline and normal rabbit serum were used as negative controls. The developed LAT was tested with 58 numbers of serum samples of ducks vaccinated with inactivated vaccine. The test was also done using 12 known negative serum samples stored in the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy.

Indirect ELISA

Indirect ELISA was carried out as per the method described by Shancy (2015) with slight modifications. Checker board analysis was employed to estimate the optimum concentration of Omp as antigen for ELISA. By titrating the negative sera and using the formula Mean OD + 3 standard deviation, cut-off values were determined.

Carbonate-bicarbonate buffer (0.05 *M*) having pH 9.6 was used in the ratio 1:100 for diluting the crude Omp. One hundred microlitres of diluted antigen was used for coating the wells of the two ELISA plates and incubated overnight at 4°C. Phosphate buffered saline containing 0.5 per cent Tween-20 (PBS-T) was used to wash the wells of the plates five times. Five per cent skim milk in PBS-T was used to block the wells which were then subjected to incubation for one hour at 37°C and again washed five times with PBS-T. One hundred microliters of 58 serum samples from vaccinated ducks, diluted in the ratio of 1:50 were loaded into the wells in duplicates and incubated for one hour at 37°C. Following incubation, the plates were again washed five times using PBS-T. The wells of ELISA plates were loaded with 100 µL of goat anti-chicken HRP conjugate (Invitrogen) in 1:4000 dilution and incubated for one hour at 37°C. Then, the wells were again subjected to five washes with PBS-T, followed by loading the wells of each plate with 100 µL of TMB/H_2O_2 (20X) diluted in distilled water in the ratio of 1:19. For colour development, these plates were incubated in dark for 10 min. at room temperature. Finally, 50 µL of sulfuric acid was added to terminate the reaction and the absorbance was measured in an ELISA reader (Bio-Rad) at 450 nm. The I-ELISA was also done on the 12 numbers of known negative serum samples.

RESULTS AND DISCUSSION

Riemerellosis has been reported around the globe, wherever there is large duck population. Kerala, a state where duck rearing is practiced widely, has encountered numerous outbreaks of riemerellosis over the past two decades. The worldwide occurrence of riemerellosis, its ability to cause huge morality and thereby causing great economic loss signifies the need for timely diagnosis and proper treatment. Therefore, diagnosis at field level may be attempted with rapid tests like LAT that can provide fast results, are economic and also may not require sophisticated equipment or costly reagents.

Bacterial Isolate

The colonies obtained on BA after incubation were convex and translucent with butyrous nature (Fig. 1). Priya *et al*. (2008) and Surya *et al*. (2016) also used bovine blood agar to isolate *R. anatipestifer from clinical samples.* On Gram's staining, they were Gram- negative organisms with morphology varying from coccobacilli,

Fig. 1: Translucent, butyrous colonies of RA1

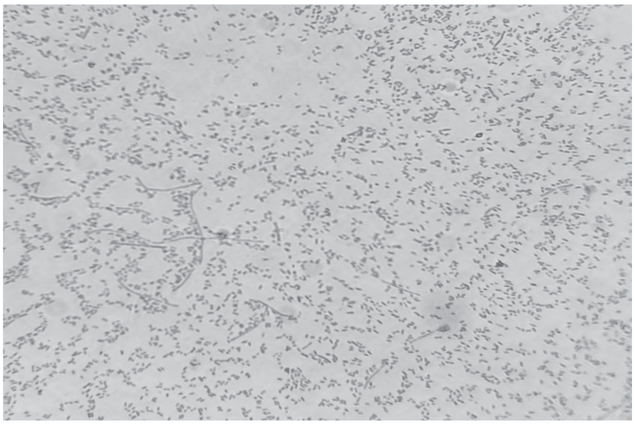

Fig. 2: Gram-negative coccobacilli to short bacilli with filaments

short rods to filamentous forms (Fig. 2). The results of further identification tests performed are summarised in table 1 and they were in accordance Surya (2011).

Table 1: Biochemical reactions of *R. anatipestifer*

Test	Result
Growth on MCA	–
Haemolysis on BA	–
Catalase	+
Oxidase	+
Indole production	–
Ornithine decarboxylase activity	–
Gelatin liquefaction	+
O/F test	Unreactive

The genomic DNA, isolated from pure culture by heat lysis method, on *R. anatipestifer* species specific PCR, revealed an amplified product of 546 bp on submarine agar gel electrophoresis with one per cent agarose (Fig. 3). The result was similar to that obtained by Soman *et al*. (2014). This confirmed the isolate as *RA1*

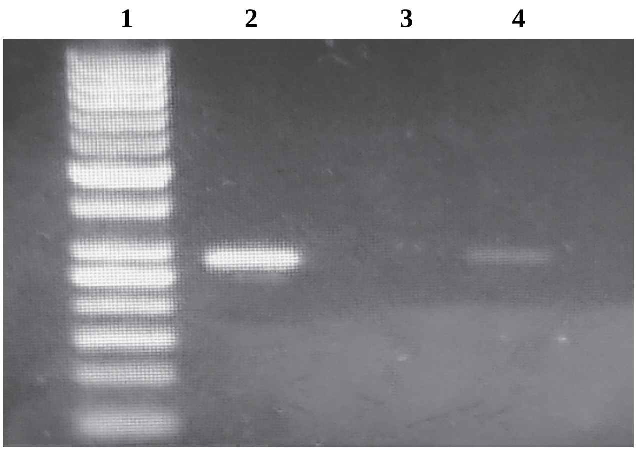

Fig. 3: *R. anatipestifer species specific PCR*
Lane 1: 100 bp ladder
Lane 2: R. anatipestifer positive control
Lane 3: Negative control
Lane 4: RA1

Outer membrane protein extraction

The Omp of *RA1 was extracted and protein concentration was estimated to be 1.32 mg/mL using Nanodrop 2000.* An Omp concentration of 1.31 mg/mL was obtained by Ahmad (2017) for RA1 with the same procedure.

Latex agglutination test

The LAT is a qualitative test assessed by the clumping activity of latex beads. Latex beads are inert, uniform and stable particles on to which antigen or antibody can be easily adsorbed and can react to its specific counterparts to produce agglutination. Natalia (2001) developed an antibody detecting LAT for the field diagnosis of haemorrhagic septicaemia and the test was described to be simple, easy and specific which could support the clinical signs and necropsy findings to confirm the disease.

The LAT developed in this study utilises crude Omp of RA1 as antigen and appreciable clumping of latex particles was observed with an Omp concentration of 100 ìg and no agglutination was seen in the negative controls. Agglutination of latex beads within 5 to 10 min. was considered as positive and absence of agglutination was considered as negative. Out of 58 serum samples of vaccinated birds tested, 42 were positive and 16 were negative. All the 12 negative sera tested did not give any agglutination. The results are summarised in table 2. Agglutination of positive samples and non-agglutination of negative samples are shown in figure 4. A B

Fig. 4: Latex agglutination test
A: Agglutination of positive serum
B: Non-agglutination of negative serum

Table 2: Sera screened by LAT

Serum samples	LAT		Total
	Positive	Negative	
Vaccinated birds	42	16	58
Known negative	0	12	12
Total	70		

Indirect ELISA

The ELISA is a popular assay in the serodiagnosis of infections. Unlike LAT, I-ELISA is a quantitative assay and is highly sensitive. It can detect antibodies even at lower levels and require only minimum concentration of antigen to do so. An ELISA developed by Huang *et al*. (2002) required only 50 ng of antigen to detect antibodies specific to *R. anatipestifer*. However, the test requires special equipment for reading results and is not directly interpreted. Also, it generates more waste products. All these factors limit the use of I-ELISA under field conditions.

In the present study, optimum working concentration of Omp, primary antibody and HRP conjugate, determined by checker board analysis were 4.1 µg/100 µL, 1:50 and 1:4000, respectively. Using positive and negative serum samples, the cut-off value was determined to be 0.068. The serum samples showing an OD value more than 0.068 were considered positive. All the 58 serum samples tested with I-ELISA were positive for antibodies against *R. anatipestifer* and the 12 known negative serum samples tested gave negative result. The test results are summarised in table 3

Table 3: Sera screened by indirect ELISA

Serum samples	Indirect ELISA		Total
	Positive	Negative	
Vaccinated birds	58	0	58
Known negative	0	12	12
Total			70

Statistical analysis

Comparison of antibody detection by LAT with I-ELISA was done by using McNemar's test. On analysis, the P-value was less than 0.001 indicating there was significant difference in the antibody detection by LAT when compared to I-ELISA. Kappa statistics was worked out for testing the agreement in the detection of LAT compared to I-ELISA and it was greater than zero with a P-value less than 0.001. So, there is some agreement in the detection of antibody by LAT compared to I-ELISA. The relative sensitivity, specificity and accuracy of the LAT in comparison to I-ELISA were 72.41 per cent, 100 per cent and 77.14 per cent respectively.

On comparison of results, I-ELISA was found to be more effective in detecting antibodies against *R. anatipestifer*. But the developed LAT had antibody detection capability and the test can be correlated with clinical signs and gross lesions to arrive at a diagnosis.

CONCLUSION

The LAT is a simple, rapid and economic technique. The results can be read visually without the need of sophisticated equipment and professional expertise. However, LAT as the sole means of diagnosis may not be effective as it is not very sensitive. Nonetheless, it can be used to support clinical signs and necropsy findings to arrive at a diagnosis. Since LAT is portable and produce minimum biomedical waste, it is convenient for field use and also can handle large number of clinical samples.

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