

ISSN: 2322-455X



Scienceline Publication

Journal of World's Poultry Research

An international peer-reviewed journal which publishes in electronic format

Volume 7, Issue 3, September 2017

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Volume 7 (3); September 25, 2017

Review

Use of Mannan- Oligosaccharides (MOS) As a Feed Additive in Poultry Nutrition.

Saeed M, Ahmad F, Asif Arain M, Abd El-Hack M-E, Emam M and Ahmed Bhutto Z.

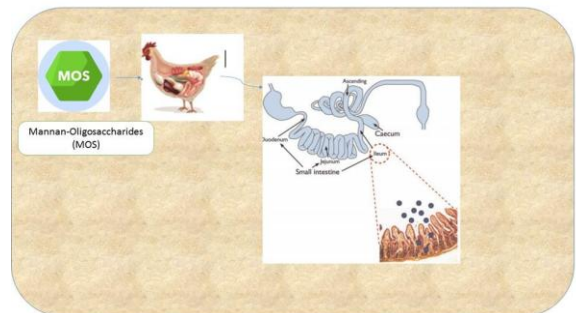
J. World Poult. Res. 7(3): 94-103; pii:

S2322455X1700012-7

ABSTRACT:

The European Union banned using all prophylactic antibiotics as growth promoters in poultry nutrition. As a result, the poultry nutritionist is now forced to look for growth promoting antibiotic alternatives, or at least considerably demote the amount of antibiotics used to sustain efficient broiler meat production and to be able to produce safe poultry egg and meat products. The Mannan-oligosaccharides (MOS), is a type of probiotics originated from the yeast cell wall (*Saccharomyces cerevisiae*) has gained more prominent attention, mainly due to its ability to bind the threadlike fimbriae on pathogenic bacteria preventing them from attaching to the gut wall, thereby averting their stabilization and the resulting colonization and multiplication, up to the disease level, so it had been showed to be a most capable solution for antibiotic-free diets, as well as furnishing effective support for digestion and immunity in poultry. Several investigations confirmed that using MOS as a feed supplement in poultry diets allowed birds to achieve a similar trend as when they were fed a diet enriched with antibiotic growth promoters. In addition, MOS has also shown to have a positive affection on bodyweight gain, feed conversion ratio, egg weight, egg production, fertility, and hatchability thus ameliorating well-being, energy levels and performance of avian species. Furthermore, it is also thought that it plays a role as an antioxidant, helping with mineral retention, improving bone mineralization and subsequently the overall improvement the performance of poultry birds. This review article has aimed to illuminate its sources, mode of action and beneficial applications of MOS in poultry diet for improving, production, immunity, safeguarding health among consumers and it ought to be used as a natural growth promoter on a commercial level in order to replace synthetic antibiotics in the poultry industry.

Key words: Antioxidant, Feed additive, Gastrointestinal health, Mannan-oligosaccharides (MOS), Performance, Poultry [Full text-[PDF](#)] [[XML](#)]



Research Paper

Effect of Ageing on Mitochondrial Gene Expression in Chicken Breast Muscle.

Tarai S, Thyagarajan D and Srinivasan G.

J. World Poult. Res. 7(3): 104-113; pii:

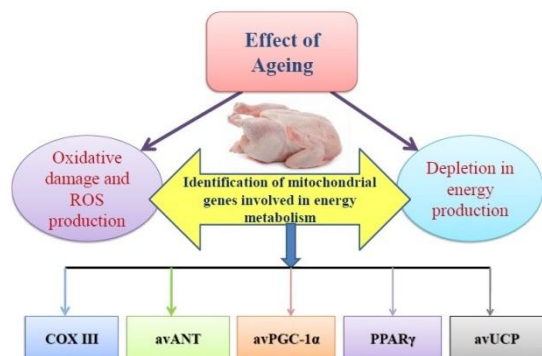
S2322455X1700013-7

ABSTRACT:

Efficient conversion of food into body mass has been associated with altered gene expression of some proteins of the electron transport chain. We evaluated the effect of age on the mRNA expression of Cytochrome oxidase III(COX III), avian adenine nucleotide translocator (avANT), avian PPAR-γ coactivator-1α (avPGC-1α), Peroxisome proliferator-activated receptor-γ (PPARγ) and avian uncoupling protein (avUCP) in chicken. A total of 90 male birds each from Nandanam B2, Rhode Island Red, Aseel and White Leghorn, were divided into three replicates containing 30 birds each and used for the study. Production parameters consist of body weight, body weight gain, cumulative feed consumption and cumulative feed efficiency at fourth and eighth weeks of age were recorded. Total RNA was extracted from the breast muscle tissue of male birds and reverse transcribed into cDNA. Real-time PCR analysis was performed using specific primers for the genes. The greatest reduction was observed when comparing fourth and eighth week old birds in COX III, avANT mRNA expression levels were then followed by avPGC-1α and increased mRNA expression levels were observed in PPARγ followed by avUCP at eighth week of age. The study revealed phenotypic differences in production traits as well as the difference in expression of mitochondrial gene like COX III, avANT, avPGC-1α, PPARγ and avUCP expression level change with age in chickens.

Key words: Ageing, Mitochondria, PPARγ, COX III, avANT, avPGC-1α, avUCP

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Research Paper

Growth Performance and Haemato-biochemical Parameters of Different Breeds of Rural Chickens.

Parveen A, Khan SH, Khawaja T, Iftikhar N and Khan S.

J. World Poult. Res. 7(3): 114-122; pii: S2322455X1700014-7

ABSTRACT:

A total of 2000 un-sexed day-old-chicks of each Desi, Fayoumi and Rhode Island Red (RIR) breeds were reared and maintained on deep litter system for a period of 20 weeks. In floor pens, each breed was reared separately in a single pen until 7 weeks of age when the 2000 birds had been randomly distributed between pens, with 21 to 24 birds of the same breed per pen (2.00 to 2.50 ft²/bird). The results had revealed that the average day old weight was the highest in RIR, intermediate in Desi and lowest in Fayoumi. The RIR breed had consumed more feed and therefore, gained the highest weight gain than as compared to those of Desi and Fayoumi breeds at all ages of growing phase. The feed conversion was best in RIR and lowest in Desi breed. Desi and Fayoumi chicks had a lower mortality than the RIR breed chicks. The meat composition was found to be insignificant amongst the three breeds. There was no significant difference in blood glucose, triglyceride, cholesterol, calcium, protein, uric acid and alkaline phosphatase values amongst the three breeds. There was also no significant difference in hematological values among all breeds. The total erythrocyte count, hemoglobin and packed cell volume increased with the advancement of age. However, erythrocyte sedimentation rate, mean corpuscular volume and mean corpuscular hemoglobin values decreased gradually with the advancement of age. It may be concluded that overall, RIR chickens had performed better than Fayoumi and Desi chickens. However, a lower mortality rate had been observed in Desi chickens.

Key words: Rural chicken, Body weight, Feed intake, Meat composition, Biochemical parameter
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Desi

Fayoumi

Rhode Island Red

Research Paper

Performance, Serum Biochemical Parameters and Immunity in Broiler Chicks Fed Dietary *Echinacea purpurea* and *Thymus vulgaris* Extracts.

Habibi H and Firouzi S.

J. World Poult. Res. 7(3): 123-128; pii: S2322455X1700015-7

ABSTRACT:

The objective of this study was to evaluate the effect of administrating herbal extracts of *Echinacea purpurea* and *Thymus vulgaris* into broilers drinking water on performance, immune response and serum biochemical and Phyto hemagglutinin. 270 day-old Ross chicks were assigned to nine dietary treatments in a randomized manner. Each treatment was given to two replicates of 15 birds. The variables of *T. vulgaris* extract were 1% and 2% and variables of *E. purpurea* extract were 0%, 1% and 2% in drinking water. Body Weight (BW), Feed Intake (FI) and Feed Conversion Ratio (FCR) were recorded at the end of the experiment. Antibody responses against Newcastle disease viruses were measured after blood sampling at 42 days of age. The plant extracts did not affect BW, FI and FCR ($P > 0.05$). Antibody titers against NDV were significantly affected by the administration of *E. purpurea* ($P < 0.05$). The highest elevation was for the birds that were administrated with 2% *E. purpurea* from 1 to 42 days ($P < 0.05$). Administration of thyme extracts had improved serum biochemical parameters as compared with *Echinacea* and control group. It was concluded that under these research conditions, high levels of *E. purpurea* extracts had increased the broiler chickens' immunity.

Key words: Broiler, *Echinacea purpurea* extract, Immunity, Performance, *Thymus vulgaris* extract.
[Full text-[PDF](#)] [[XML](#)]



Research Paper

Effect of Plumage Color and Body Weight on the Semen Quality of Naked Neck Chicken.

Abbass W, Jabbar A, Riaz A, Akram M and Allah Ditta Y.

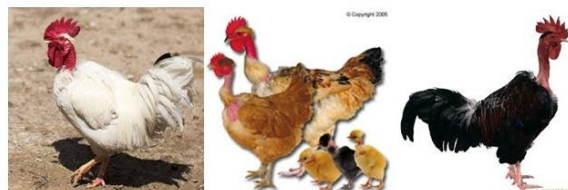
J. World Poult. Res. 7(3): 129-133; pii: S2322455X1700016-7

ABSTRACT:

The low fertility of local chicken breeds is a major issue in backyard poultry system. The fertility rate varies among different males due to their difference in semen quality. The objective of the present study was to evaluate the effect of plumage color and body weight on the semen quality of Naked Neck chicken. The Naked Neck males (n=18) vary in three plumage colors (black=6, brown=6, white=6) and each color contains two body weight sub groups (heavy= >1600gm n=3 and light= 1600 gm) contained significantly more semen volume (0.21±0.02 ml) and sperm concentration

J. World Poult. Res. 7(3): 129-133; pii: S2322455X1700016-7

Naked Neck chicken three varieties black, white and brown were divided into three groups on the basis of body weight to evaluate semen volume, sperm concentration, livability and morphological defects. The semen volume and sperm concentration were influenced by body weight while other parameters were not affected. Body weight and plumage color have effect on semen quality and physiological semen traits of naked neck chicken. Naked Neck chicken with heavy weight and black color plumage can be used for breeding purpose to get better fertility rates.



(1.88±0.06×10⁹ ml) than the lightweight group (light = < 1600 gm). So, black plumage color roosters of can be use to enhance fertility rate of naked neck chickens.

Key words: Body weight, Naked neck chicken, Plumage color, Semen quality

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Research Paper

Potential of Commercial Spice Mixes to Enhance the Quality and to Extend the Shelf Life of Raw Chicken Breasts.

Subbarayan S, Ruggoo A and Neetoo H.

J. World Poult. Res. 7(3): 134-144; pii:

S2322455X1700017-7

ABSTRACT:

Raw chicken harbors spoilage microorganisms such as the Mesophilic Aerobic Bacteria (MAB), Lactic Acid Bacteria (LAB), Spoilage Yeasts (SY) and Pseudomonas, which limit product shelf life. This study compared the potential of three spice mixes ("Tandoori", "Kalia" and "Massala") to extend the shelf life of raw chicken. Chicken breasts were marinated with each of the spice mixes (3% w/w), and subsequently refrigerated for up to 15 days. Marinated and un-marinated samples were withdrawn at three-day intervals and analyzed for enumeration of MAB, LAB, SY and Pseudomonas. After three days, chicken marinated with "Tandoori" and "Kalia" spices had a significantly (P 0.05) observed in the final MAB counts between treated samples (7.51-7.88 log cfu/g) and untreated controls (7.88 log cfu/g). There were also no significant (P > 0.05) differences in the counts of Pseudomonas (2.65-3.64 log cfu/g), LAB (2.56-4.20 log cfu/g) and SY (2.60-4.15 log cfu/g) over the 15-day storage. Since the onset of microbial spoilage is marked by MAB reaching 7 log cfu/g, the microbiological shelf-life of marinated and un-marinated chicken breasts were estimated at 12 and 6 days respectively. However, based on the sensorial attributes, both marinated and un-marinated chicken received poor acceptability scores after six and three days respectively. Commercial spice mixes can thus extend the refrigerated shelf-life of raw chicken by three days to a maximum of six days.

Keywords: Breast, Chicken, Quality, Shelf-life, Spice

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Research Paper

Efficacy of Combined Vaccine against Salmonellosis and Infectious Coryza in Poultry.

Ibrahim HM, Abd El-Aziz WR, El Sawy H, Sayed RH and Mohammed GM.

J. World Poult. Res. 7(3): 145-153; pii:

S2322455X1700018-7

ABSTRACT:

In the present study, efficacy of two prepared combined vaccines against salmonellosis and infectious coryza in poultry has been studied. Two vaccines were prepared using *Salmonella* Typhimurium and Enteritidis combined with *Avibacterium paragallinarum* serovars A, B, and C. one vaccine was adjuvanted with aluminium hydroxide gel and the other adjuvanted with montanide ISA71. The two vaccines were assayed in six weeks old Specific Pathogen Free (SPF) white Lohman layer chickens by injecting two doses of each vaccine 3 weeks apart. These chickens were challenged with either *Salmonella* virulent strains or *Avibacterium paragallinarum* different serovars 3 weeks post second dose. Antibody titers in sera of chickens against different antigens were higher in groups vaccinated with montanide oil vaccine than those vaccinated with aluminium hydroxide gel vaccine as detected by different serological tests; ELISA, micro-agglutination test and haem-agglutination inhibition test. Protection rate against challenge test were 80% and 85% for *Salmonella* and (80%; 90%, and 70%) and (90%; 100%, and 90%) to *Avibacterium paragallinarum* serovars A, B, and C respectively for combined vaccine adjuvanted by aluminum hydroxide gel and montanide ISA71. The protection rate was 15% against *Salmonella* Typhimurium and Enteritidis and 0% against infectious coryza among the unvaccinated chicken group. it could be concluded that producing a vaccine from locally isolated *Salmonella* and *Avibacterium* (Haemophilus) *paragallinarum* strains adjuvanted with montanide ISA71 is recommended to aid in controlling avian salmonellosis and Infectious coryza at the same time.

Key words: Aluminum hydroxide gel, Chicken, Infectious coryza, Salmonellosis, Vaccine.

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Efficacy of Combined Vaccine against Salmonellosis and Infectious Coryza in Poultry

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Journal of World's Poultry Research



ISSN: 2322-455X

Frequency: Quarterly

Current Issue: 2017, Vol: 7, Issue: 3 (25 September)

Publisher: [SCIENCELINE](http://www.science-line.com)

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Use of Mannan- Oligosaccharides (MOS) As a Feed Additive in Poultry Nutrition

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Received: 09 Jul 2017
Accepted: 14 Aug 2017

ABSTRACT

The European Union banned using all prophylactic antibiotics as growth promoters in poultry nutrition. As a result, the poultry nutritionist is now forced to look for growth promoting antibiotic alternatives, or at least considerably demote the amount of antibiotics used to sustain efficient broiler meat production and to be able to produce safe poultry egg and meat products. The Mannan-oligosaccharides (MOS), is a type of probiotics originated from the yeast cell wall (*Saccharomyces cerevisiae*) has gained more prominent attention, mainly due to its ability to bind the threadlike fimbriae on pathogenic bacteria preventing them from attaching to the gut wall, thereby averting their stabilization and the resulting colonization and multiplication, up to the disease level, so it had been showed to be a most capable solution for antibiotic-free diets, as well as furnishing effective support for digestion and immunity in poultry. Several investigations confirmed that using MOS as a feed supplement in poultry diets allowed birds to achieve a similar trend as when they were fed a diet enriched with antibiotic growth promoters. In addition, MOS has also shown to have a positive affection on bodyweight gain, feed conversion ratio, egg weight, egg production, fertility, and hatchability thus ameliorating well-being, energy levels and performance of avian species. Furthermore, it is also thought that it plays a role as an antioxidant, helping with mineral retention, improving bone mineralization and subsequently the overall improvement the performance of poultry birds. This review article has aimed to illuminate its sources, mode of action and beneficial applications of MOS in poultry diet for improving, production, immunity, safeguarding health among consumers and it ought to be used as a natural growth promoter on a commercial level in order to replace synthetic antibiotics in the poultry industry.

Key words: Antioxidant, Feed additive, Gastrointestinal health, Mannan-oligosaccharides (MOS), Performance, Poultry

INTRODUCTION

In the past decades, a variety of feed accretive had been employed in poultry diet. These feed accretive led to an improved rendition and effective utilization of feed in poultry birds (Chand et al., 2016a; Shah et al., 2016; King et al., 2017; Saeed et al., 2017a, b). Routinely being

utilized in accretive of feed as: emulsifiers, antimicrobials, antioxidants, biological products, herbs, pH control agents binders and enzymes as well (Vahdatpour and Babazadeh, 2016; Siyal et al., 2017; Tareen et al., 2017; Saeed et al., 2017c, d, e).

Growth promoting is not the only use of feed additives but they have used also for stabilizing the

beneficial gut microflora by forestalling beneficial microorganisms (Hashemi and Dawoodi, 2011; Abudabos et al., 2017). In the last decades, antibiotics that are used as growth promoters in animal feed have been under severe attention, since they pose a potential threat to consumers by generating resistance in the host against the bacteria (Sultan et al., 2015). Conclusively, the European Union had banned the supplementation of growth promoting antibiotics in the animal diet since 2006 (Khan et al., 2016). Now, it is most important for the poultry researcher to find alternatives to antibiotic growth promoters to boost the health and production performance of poultry birds (Janardhana et al., 2009; Babazadeh et al., 2011; Vahdatpour et al., 2011). Feed additives of plant origin have gained a great interest in the poultry industry as they are safer, with wide dose range and so rare adverse effects (Alzawqari et al., 2016; Abudabos et al., 2016). Recently, many experiments had shown a number of significant effects on growth parameters, immune response and gut health status in birds fed diets contain phytochemicals (Tanweer et al., 2014; Saeed et al., 2015; El-Hack et al., 2016, Saeed et al., 2017f, g, h). These studies have shown that the small intestine with the main role in the absorption of nutrients; it then proves that, both the proper structure and the proper function of the intestine is efficient in improving poultry performance and health (Sultan et al., 2014). It has been suggested that intestinal digestion and absorption of the nutrients is higher if the surface area of the villi is increased (Chand et al., 2016b). The beneficial microflora of young birds gut's are counted to be somewhat irregular and can easily be disturbed by several external factors. The subclinical infection is one of these external factors which posed by the pathogenic challenge. So, the ability to preserve an optimal or normal level of beneficial microflora in the gut becomes one of the main factors in the determination of the ultimate health status and consequently the genetic growth expression of poultry. At commercial basis, available mannan-oligosaccharide has exhibited to enhance the bird growth parameters including feed intake and feed utilization (Hooge, 2004a; Rosen, 2007a, b; Nikpiran et al., 2013). The beneficial impacts of MOS on the development gut microflora were also revealed by Kocher et al. (2005) and Yang et al. (2008). The addition of MOS constantly elevates the caecal beneficial populations like *Bifidobacterium* and *Lactobacillus* spp. (Sadeghi et al., 2013). Decreasing the pathogenic bacteria and the increasing the beneficial bacteria could be belonged to the receptor sites competition and producing volatile fatty acids by bacteriocins along with IgA antibodies by the host immune system (Kim et al., 2009).

Owing to these changes in the beneficial microflora, the goblet cells number and intestinal villi length increase as well, which ultimately promotes functions and health of the host GIT (Bonos et al., 2010). The diet supplemented with MOS has been reported to have a positive effect regarding body weight, feed efficiency, egg yield, fertility, egg mass and egg hatchability in various poultry species (Guclu, 2011; El-Samee et al., 2012). In another study by Iqbal et al. (2017) who had fed birds with MOS that had significant effects on body and egg mass, egg weight, and egg number and it has shown that feeding MOS as a substitute for antibiotics, as growth enhancer, can positively impact productive traits as well as health aspects in breeders of quail. This can also improve the manifest utilization of energy in feed and improve the birds feed efficiency that could partially belong to the modulatory impacts of mannan-oligosaccharide on the GIT microflora in broilers (Yang et al., 2008). The current review article discusses the potential aspects of using MOS; including its sources, mode of action and beneficial applications of MOS and its practical uses in the nutrition and production of poultry industry for improving, production, immunity and safeguarding health, among consumers and to prioritize this natural growth promoter as opposed to synthetic antibiotics to cope the medicinal cost in poultry.

Chemical traits and source of mannan-oligosaccharides

Mannan-oligosaccharides originated from the mannose blocks that exist in the yeast cell wall as it is mostly non-digestible carbohydrates (*Saccharomyces cerevisiae*). The cell wall consists of up to 25–30% of cell dry weight. The *Saccharomyces cerevisiae* is known yeast in the brewery and bakery industries. The MOS product which is a derivative of the yeast is used in animal nutrition. *Saccharomyces cerevisiae* cell wall involves both α -glucans and mannan-proteins. The essential building block for yeast cell wall are polymers of mannan with α (1-2) and α (1-6) bonds and to a less extent α (1-3) bounded side chains (Kogan and Kocher, 2007). The host enzymes or the intestinal bacteria enzymes cannot break these bonds apart and as a result carbohydrates (MOS) have no direct nutritive value, but it has benefits in keeping the gut health. It can be theorized from the several scientific research work that although mannan as a derivative from yeast (*Saccharomyces cerevisiae*) is attributed to production and processing technologies, it might have different chemical formation and biological efficiency as reported by Spring (1999).

Mode of action

The beneficial microbiota development and the sustainment of eubiosis act an important role in the mechanisms of defense in the body and health of gut as well. There is elevating evidence confirming that the composition of microflora in the gastro intestinal tract in an adult healthy host remains statistically stable as theorized by Williams et al. (2001). Results of current studies suggesting that the supplementation of MOS to poultry diets can minimize the count of hind gut pathogenic bacteria during the high exposure to the pathogen (White et al., 2002; Castillo et al., 2008). The MOS supplementation was indeed accompanied with increasing beneficial flora, especially *lactobacilli* (Rekiel et al., 2007). Another experiment has also confirmed the

beneficial impact of MOS, however, it has been also found to decline animal gut concentration of ammonia (Juskiewicz et al., 2003). Literature documented data indicated that dietary MOS fed diets can greatly lower the number of pathogens. In some studies on poultry, proves found that if the dietis supplemented with MOS a considerable positive effect on gut histological structure in broilers chicken (Iji et al., 2001a). Similarly, it is reported that dietary supplementation of mannan products had the effect of increases the ratio of villous height/crypt depth in young broilers (Iji et al., 2001a; Yan et al., 2008) and in turkeys as well (Ferket, 2002) (Figure 1). Nochta et al. (2010) found that the addition of mannan as feed supplement remarkably enhanced the nutrients apparent digestibility.

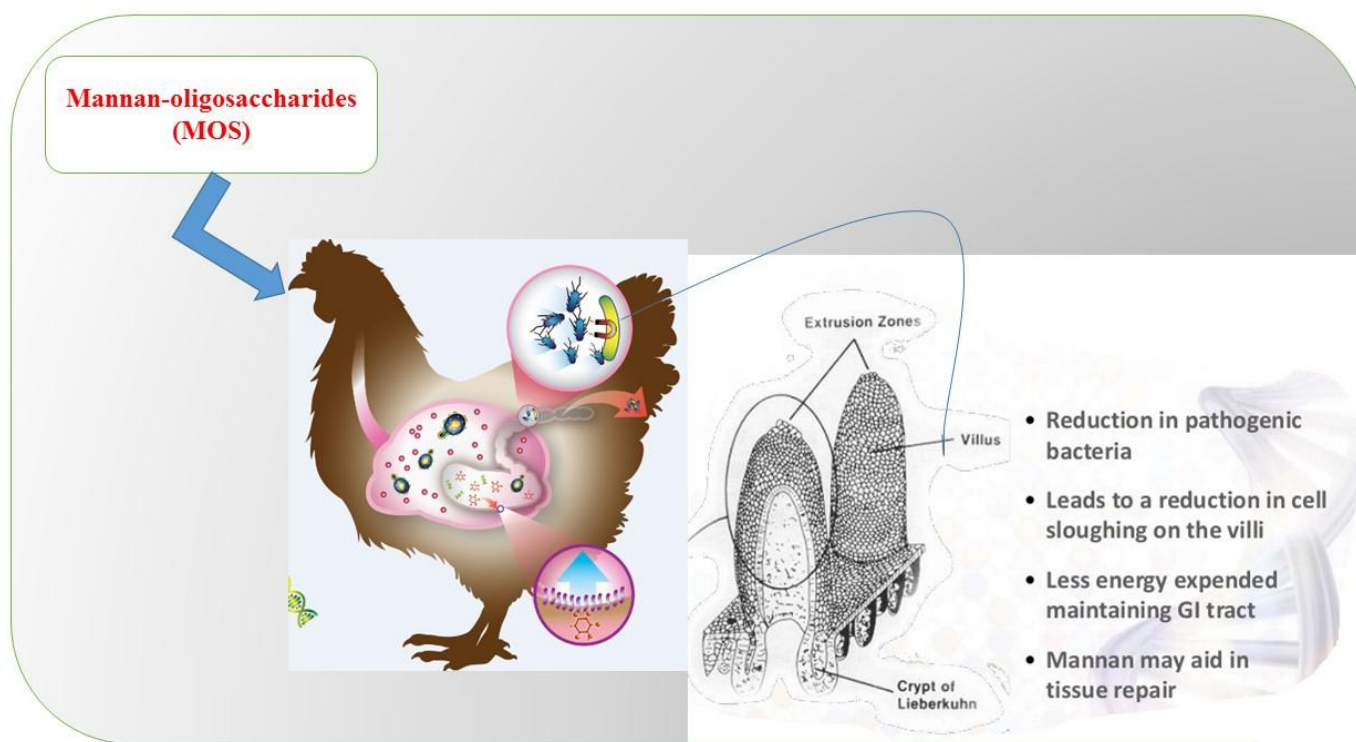


Figure 1. How do Mannan-Oligosaccharides (MOS) affect intestinal structure (MOS could prevent the colonization and attachment of pathogenic bacteria and thus reduce the adverse effects of microflora and metabolites)

Beneficial effects of mannan-oligosaccharides in poultry

Broiler Farming

Effect on growth performance and blood biochemistry. Mannan-oligosaccharide that is one of the best alternatives to antibiotic growth advancers in the poultry industry diets and which are originated from yeast outer cell wall that known as *Saccharomyces cerevisiae* (Eseceli et al., 2012). The use of MOS in broiler diets had

shown to positively impacts on performance criteria (Rosen, 2007a; Fritts and Waldroup, 2003). The range of dietary inclusion of the MOS averaged from 0.5 to 5 g /kg diet. The dose-response of MOS in different research work had showed the best dosage of MOS for optimal growth is around 2 g /kg diet as reported by Tucker et al. (2003). Iji et al. (2001b) studied the influences of different doses of MOS (0, 1, 3 and 5 g /kg diet) on the structure and function of the intestine of poultry birds within the starter

period (21-day). Results proved that poultry birds gave a high response by increasing the addition of MOS from 1 to 21 d compared to the 21-42 d period (Tucker et al., 2003). Nikpiran et al. (2014) reported that adding the MOS to the diets of poultry improved the growth performance values by enhancing the feed intake and stimulating the growth hormone and insulin release.

In a study had reported a significant decrease in the total cholesterol concentration in broiler chickens which had been supplemented with MOS @ 0.05% when compared to a control diet (Juskiewicz et al., 2003). Also, another experiment had shown that MOS could promote caecal *Lactobacillus* spp. and *Bifidobacterium* spp. growth and also elevated the height of villus and the number of goblet cells in poultry jejunum and ileum (Mohsen et al., 2014).

Effect on immune response. It is found that MOS had proved to be much more effective on antibody production against Avian Influenza Virus (AIV) in broiler chickens than Humate (HU). The immune function could be augmented with dietary Humate and MOS supplementation (Tohid et al., 2010). The innate immune system recognizes key molecular formations of the invading bacteria involving peptidoglycans, lipopolysaccharides, and possibly the structures of mannose in the cell walls of yeasts. Oligosaccharides which have mannose have been reported to impact on immune system through activating mannose-binding protein secretion from the liver. The aforementioned protein, as a result can enchain to bacteria and trigger the complement cascade of the immune system of the host as described by Newman (1994). MOS was indicated of having a beneficial effect on both immunoglobulin status and humoral immunity in general. Savage (1996) described an increase in IgG of the plasma and bile IgA in poultry grown up on diets supplemented with 0.11% MOS. The diet fed with MOS may constitute a novel and most effective plausible alternative that could reduce the spread of disease by decreasing the virus shedding and the contamination of the environment from AIV (H_9N_2) infection in poultry birds (Akhtar et al., 2016). Both *Saccharomyces cerevisiae* and its derived product is known as MOS that supplementation in poultry feed has a clear effect on the attenuation of *Escherichia coli* (*E. coli*) which induces intestinal cells disruption by reducing the intestinal inflammation and barrier dysfunction in broilers chicken. In addition to that, yeast (*Saccharomyces cerevisiae*) addition could also improved the intestinal microbiota and feed efficiency of in avian species (Wang

et al., 2016) and MOS could improve the absorption of trace minerals (Sohail et al., 2011).

Layer farming

The feed supplementation with MOS has shown to entail positive effects by improving ($P < 0.01$) the liver antioxidant status and mitigating the significant increase in the cecal pathogenic bacterial load after molt in layer birds which shows the benefits of which can be improved with MOS supplementation (Bozkurt et al., 2016). The prebiotic (mannan-oligosaccharide) supplementation can positively alter the intestinal microenvironment (Hutsko et al., 2016). In another study by Jahanian and Ashnagar (2015) found that MOS supplementation to laying hens feed under bacterial infection could improve their productive performance probably through modification in the gut's bacterial populations and improving nutrient digestibility. As described by Bozkurt et al. (2012) who had shown that egg production had efficiently improved by MOS also showed that a stimulating humeral immune response in laying hens in different climate conditions.

Turkey farming

After the broiler production industry, the turkey industry considered as the second source of poultry meat across the globe. In turkeys, 76 numbers of comparisons showed the same responses to MOS as in broilers (Hooge, 2004b; Rosen, 2007b). Hooge (2004b) claimed that MOS addition to turkey rations revealed an average increase in body weight by 2% and reduction mortality by about 25%.

So, organic enteric conditioners, such as dietary MOS, are of great importance for the turkey farming industry. Recently, antibiotic resistance had been raised in the *Escherichia coli* exist in the field which had been isolated from commercial turkey farms in North Carolina. In addition to that, a resistance to the Enrofloxacin had been showed (Bernick et al., 1999). There is no specific proof that that growth promoting doses of antibiotics control disease (Gustafson and Bowen, 1997), the debate over the Gram-negative bacteria that had been showing some resistance, as shown by *Salmonella* and *E. coli*, which caused the strongest objection to the use of antibiotic as growth promoters (Scioli et al., 1983). MOS improves the performance of turkey poults, especially during the *E. coli* challenge like antibiotics which were traditionally used (Ferket et al., 2002). An improvement in growth performance was also observed in turkeys fed diets enriched with MOS (Savage and Zakrzewska, 1996) also authors found a statistical increase in body weight gain in large white male poults which fed a diet supplemented with 0.11% MOS. Cetin et al. (2005) reported that MOS

enhanced immunoglobulin levels and caused more positive effects on growth performance, production and the turkeys' ability to resist diseases. As a result of the previous finding, it can be concluded that MOS is an interesting alternative to antibiotic growth promoters to improve performance in turkey (Parks et al., 2001) and also it has a clear effect on improving body weight gain and lowering mortality in poult (Hooge 2004a).

An alternative to antibiotic

In contrast, regarding the action mode of the chemical growth promoters (antibiotics) fermentable carbohydrates sources, oligosaccharides especially MOS, act as one of the best alternatives to the Gram-negative pathogens attachment sites, so they prevent the attachment to the enterocytes and subsequently prevents the enteric infection. The adherence step of the pathogenic microbe to the intestinal cell wall is known to be the prerequisite step to the infection (Gibbons and Houte, 1975). This can be more clarified as in *Vibrio cholera* which is incapable of starting their disease signs without the attachment step to the enterocyte, even with large numbers of bacteria present (Freter, 1969). The adhesion step causes the bacterial entrapment and colonizing. The entrapment of nutrients for growth, the concentration of the digestive enzymes and the toxins onto intestinal cell wall, and the possible prevention of antibody attachment to the pathogenic cell (Costerton et al., 1978). The cell wall of the yeast organism is mostly carbohydrates and proteins in the form of mannose, glucose, and N-acetylglucosamine that are branched and chained together (Ballou, 1970). Mannan-oligosaccharides that are derived from mannans on yeast cell surfaces are acting effective binder to the bacterial binding sites (Ofek et al., 1977). Pathogens that are mannose-specific Type-1 fimbriae are confused and adsorbed to the MOS, leaving the enterocytes without colonization. In the study of Newman (1994), that had shown that the presence of dietary mannan-oligosaccharides in the intestine had successfully discarded some pathogenic bacteria that had the possibility of attachment to the lumen of the intestine. Mannose was shown by (Oyofe et al., 1989a) to inhibit the *in vitro* attachment of *Salmonella* Typhimurium to intestinal cells of the day old broilers chicken. A study by (Oyofe et al., 1989b) had shown that dietary mannose had a successful effect on inhibiting *Salmonella* Typhimurium in intestinal colonization in broilers. (Spring et al., 2000) had shown an effort in screening different bacterial strains to examine their ability to agglutinate mannan-oligosaccharides in

yeast cell preparations (*Saccharomyces cerevisiae*, NCYC 1026). Which showed that the inclusion of MOS in the diet can improve the poultry birds' performance, especially during challenging with *E. coli*, as well as being used as growth promoter antibiotics in poultry industry? A comparison of some attributes with dietary mannan-oligosaccharides and antibiotics is shown in table 1, (Ferket et al., 2002).

Table 1. Comparison of some attributes with dietary mannan-oligosaccharides and antibiotics.

Antibiotics	Mannan-oligosaccharides
It reduces the non-specific immunological protection in the mucosa as a result of reducing both beneficial and non-beneficial bacteria (i.e. lactobacilli)	It can increase non-specific mucosal immunological protection by increasing relatively the goblet cell numbers and consequently the mucus secretion and it increases the colonization of beneficial bacteria in the gut.
It improves AME and reduces the energy needed for maintenance which consequently improves the net energy availability	It improves net energy available for production by improving dietary AME
It improves growth performance parameters under various environmental conditions	Improves growth performance parameters mainly when challenged with enteric pathogens
By suppressing enteric microflora it suppresses the competition for the nutrients.	It improves the brush border health so it enhances the absorption process.
prolonged or Improper usage can produce antibiotic resistant pathogens	It will not produce bacterial resistance
Reduces immunological stress via lowering enteric microbial load	It's important role to stimulate gut-associated system immunity by acting as a non-pathogenic microbial antigen
Decreases adverse effects of microflora metabolites by decreasing the microflora	Decreases the adverse effects of microflora metabolites by changing microflora profile
It inhibits both the viability and proliferation of some pathogens and beneficial enteric microflora	It acts as a barrier against the attachment and consequent colonization of some enteric bacteria, but it is not bactericidal.

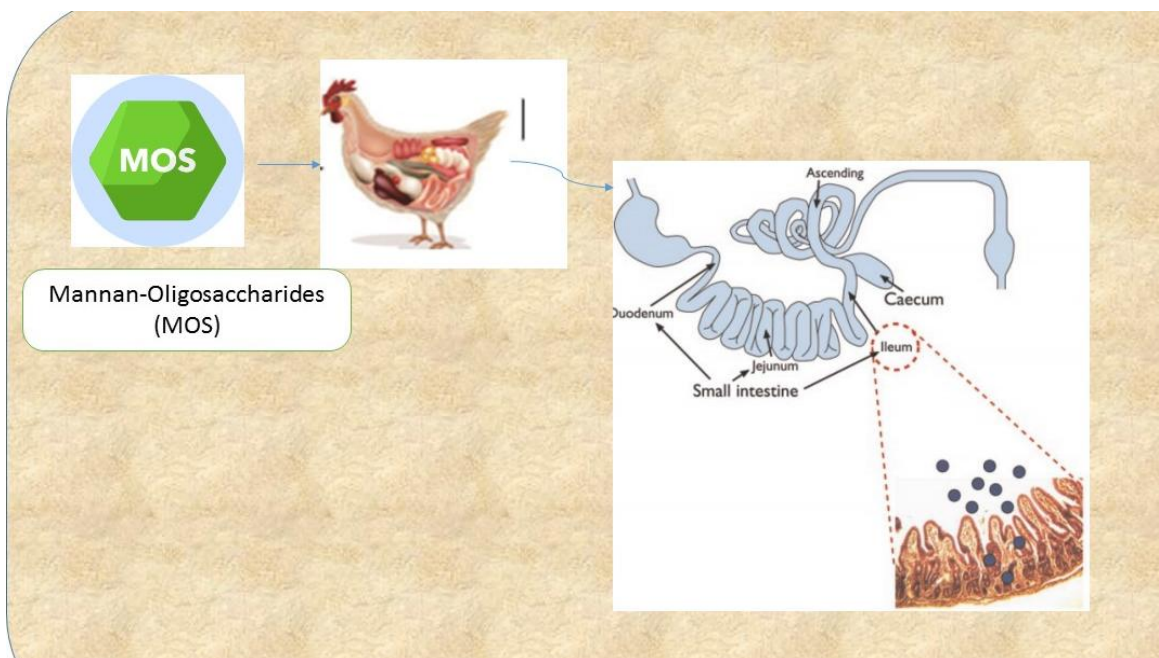


Figure 2. A flow diagram illustrating a large surface area is vital for optimal digestive function and nutrient absorption in poultry birds.

CONCLUSION

After reviewing the compiled literature it can be fully clarified that MOSs can be considered as a potential alternative to antibiotic growth promoters, and even at trace amounts @ 0.1%-0.4% practically usage as commercial feed additive in poultry nutrition would be quite effective in improving the health status and production performance of poultry. Among consumer concerns about danger increasing of antibiotic-resistant pathogens has urged the poultry nutritionist to consider “biologically safer” alternatives. After studying the published literature it is clear now that MOS is considered one of the best alternatives to antibiotic growth promoters. These mannan-oligosaccharides are non-digestible carbohydrates that may have greater benefits than antibiotics if it is used in a synergic way with other non-pharmaceutical enteric conditioners, such as fructo-oligosaccharides, probiotics, bioactive peptides, and some herbs and it would, in this manner, be a helpful additive to reduce feed cost in the poultry industry.

Acknowledgements

All the authors of the manuscript thank and acknowledge their respective Universities and Institutes.

Competing interests

Authors declared that they have no conflict of interest.

Author`s contributions

All the authors significantly contributed to compile and revise this manuscript. MS, MAA, reviewed the literature and initiated the review compilation. MEAEH, ZAB and ME, critically revise the manuscript. FA and MEAEH check the English language accuracy. Finally all authors read and approve the manuscript for publication.

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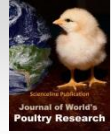
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Effect of Aging on Mitochondrial Gene Expression in Chicken Breast Muscle

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Received: 01 Aug 2017

Accepted: 04 Sept 2017

ABSTRACT

Efficient conversion of food into body mass has been associated with altered gene expression of some proteins of the electron transport chain. We evaluated the effect of age on the mRNA expression of Cytochrome oxidase III (COX III), avian adenine nucleotide translocator (avANT), avian PPAR- γ coactivator-1 α (avPGC-1 α), Peroxisome proliferator-activated receptor- γ (PPAR γ) and avian uncoupling protein (avUCP) in chicken. A total of 90 male birds each from Nandanam B2, Rhode Island Red, Aseel and White Leghorn, were divided into three replicates containing 30 birds each and used for the study. Production parameters consist of body weight, body weight gain, cumulative feed consumption and cumulative feed efficiency at fourth and eighth weeks of age were recorded. Total RNA was extracted from the breast muscle tissue of male birds and reverse transcribed into cDNA. Real-time PCR analysis was performed using specific primers for the genes. The greatest reduction was observed when comparing fourth and eighth week old birds in COX III, avANT mRNA expression levels were then followed by avPGC-1 α and increased mRNA expression levels were observed in PPAR γ followed by avUCP at eighth week of age. The study revealed phenotypic differences in production traits as well as the difference in expression of mitochondrial gene like COX III, avANT, avPGC-1 α , PPAR γ and avUCP expression level change with age in chickens.

Key words: Ageing, Mitochondria, PPAR γ , COX III, avANT, avPGC-1 α , avUCP

INTRODUCTION

Genetic improvement has greatly enhanced the production performance of broiler in recent years, by drastically reducing the slaughter age. In the poultry industry, feed efficiency is a major criterion for defining the optimum performance to broiler chicken. It is considered as one of the most important traits in poultry farming activities since feed represents about 50 to 70% of the total cost of production. Moreover, because feed cost has increased dramatically in recent years, decreasing the amount of feed per unit of weight gain will improve efficiency of production and increase profits. Efficient conversion of food into body mass was reported to be associated with altered gene expression of some proteins

of the electron transport chain (Gasparino et al., 2012). All cells need energy to perform their activities. Mitochondria are responsible for producing 90% of the energy needed for cells. Series of studies are conducted to understand relationships of mitochondrial function and biochemistry with the phenotypic expression of feed efficiency in broilers (Ojano-Dirain et al., 2004, 2005a, 2005b; Iqbal et al., 2004, 2005; Lassiter, 2006). These organelles are responsible for transforming chemical energy from metabolites into easily accessed energy to be used by the cell (Schauss et al., 2010).

Increased production of mitochondrial ROS, which occurs with advancing age, is related to greater oxidative damage in the macromolecules, as well as depletion in the energy production machinery. Birds with lower ATP

production due to lower mitochondrial efficiency in producing ATP from substrates show less efficiency or feed conversion. Therefore, mechanisms that favor a reduced ROS production may be useful in the prevention of age-related issues (Gasparino et al., 2012).

The aim of this study was to evaluate the effect of ageing on mitochondrial genes related to energy production, ATP synthesis and mitochondrial biogenesis of the genes Cytochrome oxidase III (COX III), avian adenine nucleotide translocator (avANT), avian PPAR- γ coactivator-1 α (avPGC-1 α), Peroxisome proliferator-activated receptor- γ (PPAR γ) and avian uncoupling protein (avUCP) were analyzed at the fourth and eighth weeks of age in breast muscle of different chicken breeds that are known to have differential phenotypic expression.

MATERIALS AND METHODS

Ethical approval

The experimental protocol was approved by the Institutional Animal Ethics Committee, Tamil Nadu, India.

Experimental Birds

A total of 90 male birds each selected from Nandanam B2, Rhode Island Red, Aseel and White Leghorn divided into three replicates containing 30 birds each were used for the study. Nandanam B2 is a commercial hybrid dual purpose strain developed by Tamil Nadu Veterinary and Animal Sciences University, India. The concerned breed/strain was divided into four treatment groups with three replicates in each group, containing 30 birds each. The breeds were selected based on observed high and low feed efficiency over generations in Poultry Research Station, Madhavaram Milk Colony, Chennai-51, with the aim to evaluate mRNA expression of genes that are involved in mitochondrial energy metabolism and mitochondrial biogenesis that are known to have differential phenotypic expression.

All the experimental birds were wing banded and reared up to eight weeks of age following standard management practices in cages. All the chicks were immunized against Ranikhet disease by using F₁ and Lasota strain at 7th day and 28th day respectively. Known quantity of feed was provided *ad libitum* with feed containing 3100Kcal ME/kg and 22 percent dietary crude protein. Clean potable water was provided *ad libitum*. The study was carried out during October- December months 2015 where the average daily high temperature in the study area (13.1623° N, 80.2433° E) was below 31°C. Data on phenotypic performance and gene expression studies were recorded.

Phenotypic assessment

The day old experimental chicks were weighed with 0.1 g accuracy. Body weight was again recorded at fourth and eighth weeks of age. Based on day old body weight, body weight gain was calculated. All the birds were provided with *ad libitum* quantity of experimental feed during the experimental period. At the end of every two weeks period, left over feed was weighed back and net feed consumption was estimated for each group. Feed efficiency was calculated at 4th and 8th weeks of age.

Genotypic assessment

Mitochondrial mRNA expression of COX III, avANT, avPGC-1 α , PPAR γ and avUCP genes were studied in breast muscle tissue at fourth and eighth weeks by following the protocol below. Two male birds from each replicate were randomly selected, birds were killed by cervical dislocation, and tissue from the breast muscle (pectoralis superficialis) was collected and submerged in RNA later and kept at -80° C.

The reagents were used for RNA extraction were TRIzol® LS Reagent (Invitrogen, USA) Catalog number: 15596026, Chloroform (Sigma, USA), Isopropanol (Sigma, USA), 70 per cent ethanol prepared from 99.9 per cent absolute ethanol (Jiangsu Huasi International, China) and Nuclease free water (QIAGEN, USA)

The muscle tissues were initially triturated with, 1ml of Trizol™ reagent in a mortar and pestle. The mixture was then incubated for 5 min at room temperature and 200 μ l of chloroform was added and vortexed for 1 min. The vortexed mixture was then centrifuged at 12000 rpm for 15 min at 4°C to separate the aqueous phase. The aqueous phase was then transferred to a fresh tube and equal volume of isopropanol was added and mixed by slightly inverting the tube. The tube was then incubated at room temperature for 10 min. The mixture was then centrifuged at 12000 rpm for 10 min at 4°C and the supernatant was discarded. To the RNA pellet obtained, 1ml of 70% Ethanol was added and then stored at -80°C until further use. For immediate purposes, the tubes were centrifuged; the RNA pellet air dried and re-suspended in nuclease free water, quantified and equal volume of RNA was used across the different samples.

The quantity of RNA was measured by using eppendorf BioPhotometer Plus. The spectrophotometer was blanked with 1 μ l of nuclease free water and 1 μ L of extracted RNA was used for quantification. The final concentration of the RNA (in stock) was determined by multiplying with the dilution factor. The quantity of RNA

was measured by taking ODs at 260 and 280 and then by the ratio of 260/280.

The cDNA was synthesized from the extracted total RNA using High capacity cDNA Reverse Transcriptase kit, United States (Thermo Scientific Revert Aid H Minus First Strand cDNA Synthesis Kit #K1632) according to the manufacturer's instructions. The following biological reagents were added as presented in table 1.

Table 1. Composition of cDNA synthesis reaction

RNA	8 μ L
Primer (oligo dT)	1 μ L
5X Reaction buffer	4 μ L
dNTPs	2 μ L
Reverse Transcriptase enzyme	1 μ L
Ribolock RNase inhibitor	1 μ L
RNase free water	3 μ L
Total volume	20 μ L

The RNA pellet was air dried and re-suspended in 10 μ l DEPC water and denatured at 65^oC for 5 min and snap cooled in ice for 1 – 2 min. The cDNA master mix (10 μ l) was added to the denatured RNA on ice. The total reaction mixture was incubated at 25^oC for 5 min followed by 42^oC for 1 hour for the reverse transcription and finally at 70^oC for 5 min to inactivate the enzyme. The cDNA synthesized was then used for the amplification of the house keeping gene β actin or stored at -20^oC until further use. Quantitative PCR (qPCR) was carried out using SYBR® Green following the manufacturer's instruction. The real time plates were obtained from Roche, India. The mRNA expression levels of the mitochondrial genes COX III, avANT, avPGC-1 α , PPAR γ and avUCP. The primer sequences were adopted from Ojano-Dirain et al. (2007). The Real-time PCR mix was prepared as presented in table 2.

Table 2. Composition of Real-time PCR mix

2X SYBR Green Mix	10 μ L
Forward Primer	0.5 μ L (10pmol/ μ L)
Reverse Primer	0.5 μ L (10pmol/ μ L)
Template cDNA	2 μ L
Water	up to 20 μ L

The components were mixed gently by vortexing and were briefly centrifuged to collect all the components at the bottom of the tube. PCR reaction was performed in duplicates for each sample. The cycling protocol was 40 cycles of de-naturation at 94^oC for 2 min followed by

94^oC for 10 seconds, annealing temperature at 58^oC for 10 s with a melting program and finally held at 37^oC.

The relative mRNA expression levels of the target genes such as COX III, avANT, avPGC-1 α , PPAR γ and avUCP gene were shown as Ct values in the muscle tissue. The β -actin Ct value for each sample was subtracted from the Ct value of the target gene to normalize for the host basal levels. Following normalization the mRNA expression levels of the target genes COX III, avANT, avPGC-1 α , PPAR γ and avUCP of each breed are expressed as fold change ($2^{-\Delta\Delta Ct}$) over the respective levels in Nandanam B2 and logarithmic transformation was applied to all the genes evaluated.

Statistical analysis

The results were expressed as mean \pm Standard Error (SE). The differences between groups were assessed by using the Statistical Package for Social Sciences (SPSS version 17.0) software package for windows as per Snedecor and Cochran (1994). The difference within the means were estimated using Duncan's multiple range test (Duncan, 1955) by considering the differences at significant level ($P < 0.05$).

RESULTS AND DISCUSSION

Body weight and body weight gain

In the present study, highly significant difference was observed in biweekly body weight at the fourth and the eighth week which was due to different types of chicken. Broiler type Nandanam B2 had attained the highest body weight at eighth week of age. Nandanam B2 was followed by Rhode Island Red, Aseel and lowest in White leghorn as shown in table 3.

This finding was in agreement with the earlier results obtained by Sangilimadan et al. (2014) who had studied the performance of Nandanam B2. Few other researchers compared the performance of different breeds like Aseel and Kadaknath in their locality like Huanshi et al. (2011) and got similar results. Khawaja et al. (2012) who compared the growth performance of Rhode Island Red had revealed comparable results.

Feed consumption and feed efficiency

Effect of different types of chicken in cumulative feed consumption and feed efficiency at different periods were significantly different and better feed efficiency was seen in Nandanam B2 followed by RIR, White leghorn and Aseel. This may be due to their difference in the genetic makeup of different types of chicken as shown in table 4.

The results of this study coincided with work carried out by Jha and Prasad (2013) that had studied the performance of Aseel under deep litter and reported FCR of 5.46 upto 20 week of age. Whereas the findings in the present study were contrary to the work carried out early by Sangilimadan et al. (2014) who studied the performance of Nandanam B2 and reported that feed efficiency at 8th week of age was 2.49. Halima et al. (2006) also compared the feed consumption of native chicken and Rhode Island Red but found no significant difference in feed intake.

The primers for the COX III, avANT, avPGC-1 α , PPAR γ and avUCP genes proved in this study to be adequate for real-time PCR analysis and expression of fold change in 4th and 8th weeks of age (Figure 1,2,3,4 and 5). The analysis of the dissociation curves did not reveal the presence of any unspecific products or the formation of primer dimers, demonstrating the reliability of the data in determining mRNA expression of the genes evaluated. Mean and standard deviation of the CT values obtained in the samples of muscle tissue for analysis of the expressions of genes are shown in tables 5 and 6.

Table 3. Body weight and body weight gain of different chicken breeds during rearing periods

Genetic groups	Body weight(g)		Body weight gain(g)	
	4 th week	8 th week	0-4 weeks	0-8weeks
Nandanam B2	315.84 ^c ±5.40 ^{***} (84)	971.23 ^c ±20.87 (72)	265.75 ^c ±5.32 (84)	925.16 ^c ±24.43 (72)
RIR	219.31 ^b ±4.52 (79)	515.13 ^b ±9.19 (72)	183.99 ^b ±4.49 (79)	479.77 ^b ±9.13 (72)
Aseel	208.89 ^b ±4.16 (67)	502.81 ^b ±9.55 (55)	177.32 ^b ±4.12 (67)	471.54 ^b ±9.50 (55)
WLH	181.68 ^a ±2.71 (80)	442.95 ^a ±6.33 (69)	150.88 ^a ±2.71 (80)	412.19 ^a ±6.33 (69)
F value	139.61 ^{**}	355.13 ^{**}	139.16 ^{**}	263.74 ^{**}

a,b,c – means within coloumn bearing different superscripts differ significantly (P<0.05); **– Highly Significant (P<0.01), *** Mean Weight ± Standard Error

Table 4. Feed consumption and feed efficiency of different chicken breeds during rearing periods

Genetic groups	Feed consumption (g)		Feed efficiency	
	0-4 weeks	0-8weeks	0-4 weeks	0-8weeks
Nandanam B2	633.06 ^b ±2.15 ^{***}	1920.57 ±96.02	1.69 ^a ±0.01	1.95 ^a ±0.24
RIR	559.00 ^a ±25.85	1863.72 ±43.95	1.73 ^a ±0.19	2.78 ^c ±0.12
Aseel	521.60 ^a ±20.54	1682.20 ±47.63	1.89 ^a ±0.08	3.34 ^b ±0.04
WLH	534.85 ^a ±37.18	1789.61 ±42.64	2.94 ^b ±0.34	4.50 ^b ±0.08
F value	13.875 [*]	4.376 ^{NS}	8.533 ^{**}	193.10 ^{**}

a,b,c – means within column bearing different superscripts differ significantly (P<0.05); *–Significant (P<0.05), **– Highly Significant(P<0.01), NS-Non-Significant (P>0.05); *** Cumulative mean ± Standard Error

COX III

Expression levels of mRNA as reflected by fold change positively link with metabolic regulating factor for energy production of AMP and ATP at mitochondria, subsequently for the body weight gain and feed efficiency traits. Percent fold change was lowest in white leghorn and highest in Aseel at 8th week compared to 4th week of age. White leghorn being a layer type had a negative trend in expression level. This may be due to greater ROS

production and greater protein oxidation that are consistently found in birds with lower feed efficiency thereby decreasing the cellular efficiency.

This study coincides with the work done by Iqbal et al. (2004) who discussed that COX III mRNA levels in breast muscle were lower in poor feed efficiency compared with the birds that had better feed efficiency. Similarly, Ojano-Dirain et al. (2007) suggested that a greater ROS production and greater protein oxidation are

consistently found in birds with lower feed efficiency, indicating that this factor may alter the expression of mitochondrial genes.

The findings concurred with Barazzoni et al. (2000) who verified a reduction in COX III mRNA expression related to altered oxidative capacity of mitochondria in older animals. This would indicate that maintaining transcription levels may be essential to mitochondrial oxidative capacity and the maintenance of efficient use of nutrients. Also similar to Bottje et al., (2002) who stated that increased oxidative stress and protein oxidation in the low-FE phenotype is likely due to increased mitochondrial reactive oxygen species.

The finding was in agreement with Kemp et al., (2003) who opined that COX III plays an important role in mitochondrial energy efficiency. Also similar to Scheffler (1999) who had reported that COX III may play a key role in energy production.

Zhang et al. (2010) reported that ROS production and expression of proteins of the respiratory chain complexes involved in metabolism, with the feed efficiency of animals. Bottje and Carstens (2009) reported that the low-FE phenotype generated more mitochondrial ROS than the high-FE phenotype. The low-FE broiler phenotype exhibited site-specific defects in electron transport, resulting in increased mitochondrial ROS production and increased protein oxidation in several tissues.

av (ANT)

Fold change was increased in Rhode Island Red and decreased in Aseel and WLH birds at eighth week of age. This protein is responsible for moving ADP from the cytosol to the mitochondria and for moving ATP through the inner mitochondrial membrane (Ojano-Dirain et al., 2007). Therefore, ANT has the function of increasing the quantity of ADP to be transformed into ATP by means of ATP synthase. The mitochondrial function may be impaired by the incapacity of ADP/ATP exchange between the cytosol and the membrane, thus there may be some connection between the ANT expression with the phenotypic expression of feed efficiency (Bottje et al., 2006). In the present study, we found that older birds displayed a lower ANT mRNA expression in muscle tissue, and poorer feed conversion, just as Ojano-Dirain et al. (2007) also reported that birds with a lower ANT expression had a poor feed efficiency due to the lower ATP production efficiency. Nicoletti et al. (2005) also found a reduced ANT expression correlated to aging. According to these authors, alterations in the expression of respiratory chain subunits may represent an adaptive cellular response to the accumulated damage to proteins

and/or mitochondrial DNA that occurs due to the increased quantity of ROS in older birds.

av (PGC 1 α)

Fold change was up-regulated in Aseel and down-regulated in White leghorn and Rhode Island Red at eighth week in comparison to fourth week of age. The finding was in agreement with Nisoli et al. (2003) who stated that PGC-1 α stimulates nuclear respiratory factor-1 and mitochondrial transcription factor A expression, that in turn up-regulate expression of nuclear and mitochondrial genes that encode mitochondrial proteins. It agreed with the work done by Wu et al. (1999) who reported that PGC-1 α is the most dominant regulatory protein in mitochondrial biogenesis. In general it is coinciding with the work done by Richards (2003) who discussed about the genes associated with controlling feed intake and energy balance. Also similar to Lassiter et al. (2006) who provided evidence of increased oxidation associated with low FE and further evidence of differential protein expression associated with the phenotypic expression.

PPAR γ

Percent fold change was increased in Rhode Island Red, Aseel and WLH birds at eighth week in comparison to fourth week of age. This may be due to the advancement of age there is more fatty acid uptake and metabolism.

The result coincides with the Sato (2004) who studied chicken PPAR γ mRNA expression in abdominal adipose tissue tended to increase with age, as shown by higher expression levels at 6 week than at 1 and 2 week of age. It also coincides with the work of Rosen et al. (1999) who stated that PPAR- γ is activated by fatty acids that control adipocyte differentiation as well as fatty acid uptake and metabolism. The result contradicts with Ojano-Dirain et al. (2007) who reported that there were no differences in breast muscle PPAR mRNA expression.

av(UCP)

Fold change was down-regulated in Aseel and up-regulated in Rhode Island Red and White leghorn at eighth week in comparison to fourth week of age. This result coincides with the work done by Raimbault et al. (2001) who reported that chickens divergently selected for low feed efficiency has higher avUCP mRNA expression than in birds from a high feed efficient line. Also coincides with Bottje et al. (2006) who also stated that avUCP mRNA expression in breast muscle from low feed efficient birds may be a mechanism to reduce the higher hydrogen peroxide production. The findings were in

agreement with Abe et al. (2006) who reported that increase in avUCP content could be associated with altered ROS production by mitochondria. Also similar to Ojano-Diran et al. (2007) who reported that greater UCP mRNA expression can impair feed conversion, as it can reduce ATP production.

This study contradicts with Gasparino (2012) who observed a gradual reduction in UCP mRNA as the quails aged. Also Ferrandiz et al. (1994) suggested that with increase in age, more failures in ATP production occur due to the impaired activity of the respiratory chain complexes.

Table 5. CT values obtained in the samples of muscle tissue of different chicken breeds for analysis of COX III, avANT, PGC1 α , PPAR γ and avUCP expressions at 4 weeks of age

Genetic groups	Endogenous control β -actin.				
	COX III	avANT	PGC1 α	PPAR γ	avUCP
Nandanam B2	16.37 \pm 1.15*	29.65 \pm 2.60	30.03 \pm 2.58	21.83 \pm 2.02	29.81 \pm 2.26
RIR	24.91 \pm 0.75	29.34 \pm 0.79	30.45 \pm 0.80	32.70 \pm 0.75	29.42 \pm 0.74
Aseel	27.73 \pm 2.80	27.74 \pm 2.82	32.46 \pm 0.82	34.70 \pm 0.71	29.92 \pm 1.66
WLH	20.91 \pm 1.95	25.59 \pm 1.94	25.96 \pm 1.98	30.46 \pm 2.01	26.65 \pm 1.94

* Mean \pm Standard Error

Table 6. CT values obtained in the samples of muscle tissue of different chicken breeds for analysis of COX III, avANT, PGC1 α , PPAR γ and avUCP expressions at 8 weeks of age

Genetic groups	Endogenous control β -actin.				
	COX III	avANT	PGC1 α	PPAR γ	avUCP
Nandanam B2	29.93 \pm 2.61*	27.51 \pm 0.96	27.81 \pm 1.30	33.47 \pm 0.61	29.81 \pm 1.67
RIR	38.72 \pm 1.02	28.29 \pm 0.88	29.18 \pm 0.91	33.38 \pm 1.00	29.42 \pm 1.44
Aseel	32.52 \pm 2.62	26.17 \pm 2.45	25.73 \pm 2.50	29.99 \pm 1.18	29.92 \pm 1.09
WLH	38.69 \pm 0.38	27.69 \pm 0.48	27.40 \pm 0.36	34.56 \pm 1.17	26.65 \pm 0.36

* Mean \pm Standard Error

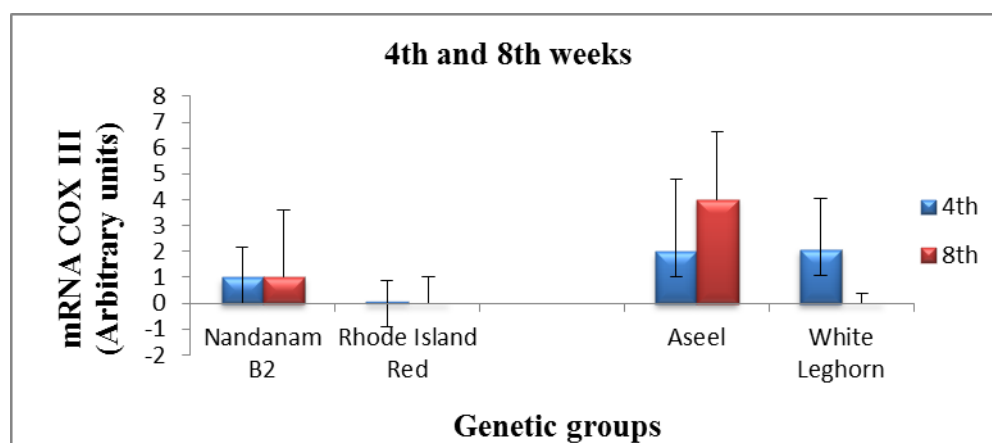


Figure 1. mRNA expression of Cytochrome oxidase III (COX III) in the breast muscle of different chicken breeds at 4 and 8 weeks of age

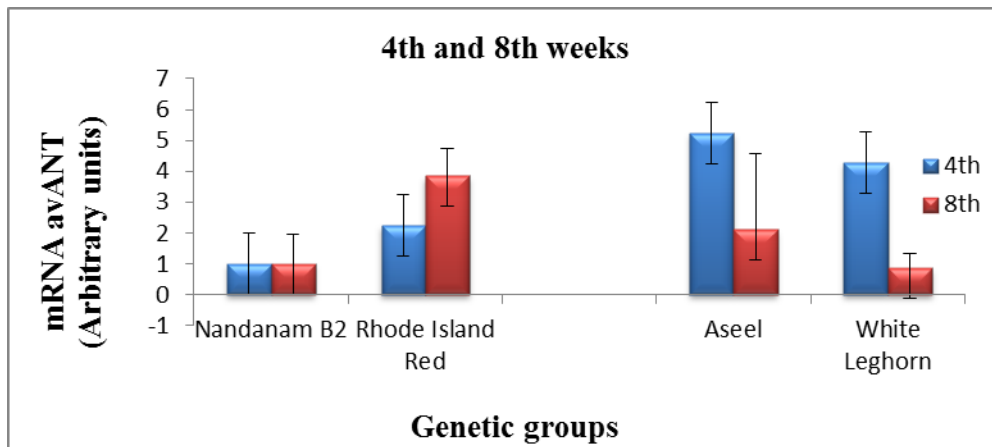


Figure 2. mRNA expression of avian adenine nucleotide translocator (avANT) in the breast muscle of different chicken breeds at 4 and 8 weeks of age

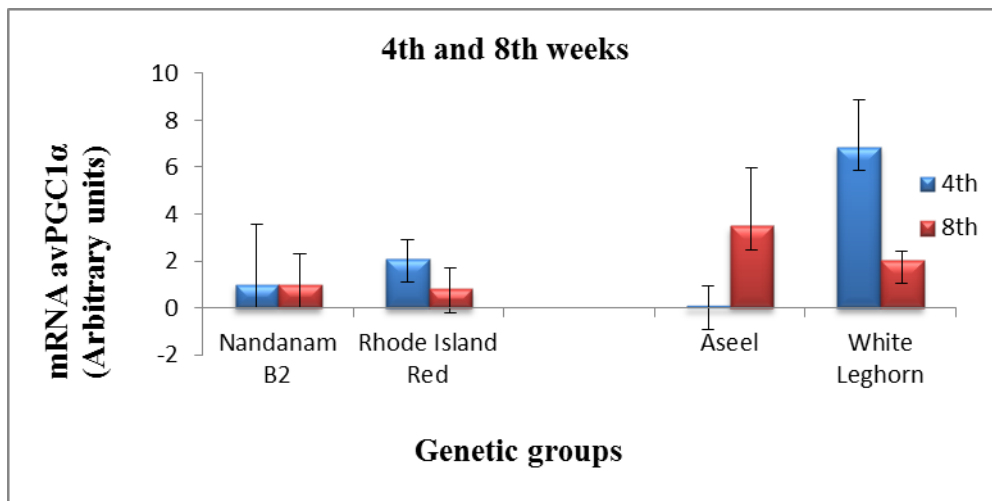


Figure 3. mRNA expression of avian PPAR- γ coactivator-1 α (avPGC-1 α) in the breast muscle of different chicken breeds at 4 and 8 weeks of age

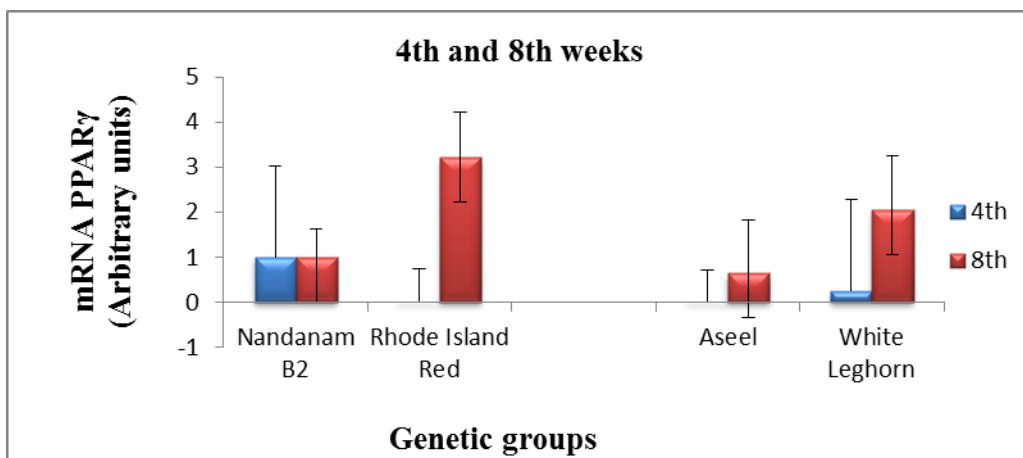


Figure 4. mRNA expression of Peroxisome proliferator-activated receptor- γ (PPAR γ) in the breast muscle of different chicken breeds at 4 and 8 weeks of age

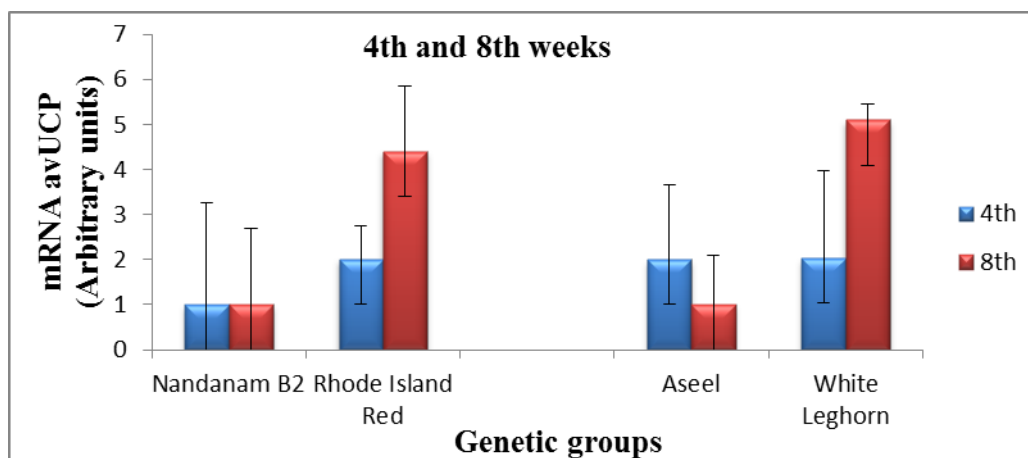


Figure 5. mRNA expression of avian uncoupling protein (avUCP) in the breast muscle of different chicken breeds at 4 and 8 weeks of age

CONCLUSION

In this study, aging influenced the expression of all genes analyzed; showing that the age of birds does influence the expression of electron transport chain genes, responsible for body energy production.

Consent to publish

All persons gave their informed consent prior to their inclusion in the study.

Competing interests

The authors declare that they have no competing interests.

Author's contributions

This study is the part of M.V.Sc. Thesis of the first author Sarada Tarai, who carried out the research under the guidance of D.Thyagarajan who has helped in technical writing of the article and its final revision. G. Srinivasan has helped during the trial, processing of samples and analysis of data. All authors have read and approved the final version of the manuscript.

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Growth Performance and Haemato-biochemical Parameters of Different Breeds of Rural Chickens

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Received: 26 Jun. 2017

Accepted: 30 Jul. 2017

ABSTRACT

A total of 2000 un-sexed day-old-chicks of each Desi, Fayoumi and Rhode Island Red (RIR) breeds were reared and maintained on deep litter system for a period of 20 weeks. In floor pens, each breed was reared separately in a single pen until 7 weeks of age when the 2000 birds had been randomly distributed between pens, with 21 to 24 birds of the same breed per pen (2.00 to 2.50 ft²/bird). The results had revealed that the average day old weight was the highest in RIR, intermediate in Desi and lowest in Fayoumi. The RIR breed had consumed more feed and therefore, gained the highest (P<0.05) weight gain than as compared to those of Desi and Fayoumi breeds at all ages of growing phase. The feed conversion was best (P<0.05) in RIR and lowest in Desi breed. Desi and Fayoumi chicks had a lower (P<0.05) mortality than the RIR breed chicks. The meat composition was found to be insignificant (P>0.05) amongst the three breeds. There was no significant (P>0.05) difference in blood glucose, triglyceride, cholesterol, calcium, protein, uric acid and alkaline phosphatase values amongst the three breeds. There was also no significant (P>0.05) difference in hematological values among all breeds. The total erythrocyte count, hemoglobin and packed cell volume increased with the advancement of age. However, erythrocyte sedimentation rate, mean corpuscular volume and mean corpuscular hemoglobin values decreased gradually with the advancement of age. It may be concluded that overall, RIR chickens had performed better than Fayoumi and Desi chickens. However, a lower mortality rate had been observed in Desi chickens.

Key words: Rural chicken, Body weight, Feed intake, Meat composition, Biochemical parameter

INTRODUCTION

Broiler chicken is an important protein source for human consumption, it also plays a major role in poverty alleviation, ensuring food security and generating family income at households within substandard management facilities (Islam et al., 2012). Over the past 50 years, poultry meat and egg production from individual birds in commercial flocks of broilers and layers has increased enormously, largely owing to genetic selection in the nucleus breeding flocks of poultry breeding companies and the rapid transfer of these gains to the commercial crossbred progeny. It should be also maintained that, the current breeding strategies for commercial poultry concentrate on specialized production lines, derived by

intense selection from a few breeds and very large populations with a great genetic uniformity of the traits under selection (Padhi, 2016; Khawaja et al., 2016). This has resulted in genetic erosion for the unselected local breeds, which are normally less productive than synthetic hybrids (Besbes et al., 2008). The rural poultry population in most countries accounts for more than 60% of the total national poultry population (Özdemir et al., 2013). However, inadequate attention has been paid either to the evaluation of these resources or to the setting up of realistic and optimized breeding goals for their improvement. As a result, many such breeds with low productivity are at a high risk of extinction under rural production systems (FAO, 2011; Hoffmann, 2011).

In Pakistan, prior to the establishment of the commercial poultry sector, rural poultry was the only source of eggs and meat supply. Although, commercial poultry sector has expanded with a rapid speed during the last three decades and highly productive birds have been imported for boosting production, yet rural poultry has not lost its value. Its importance can be judged from the fact that according to Livestock Wing of Ministry of Food, Agriculture and Livestock almost every family in rural areas and every one out of five families in urban areas have been associated with poultry production activities in various ways in the country (Government of Pakistan, 2012-2013). Meat contribution of rural poultry during 2012-13 was 0.109 million tons as compared to 9.912 million tons from commercial poultry production (Economic Survey 2012 and 2013). Keeping in view the very low cost of producing rural poultry, the net return from rural poultry could be several times more than that of birds produced on commercial scale. The contribution of rural poultry to household economy could be further enhanced through the genetic improvement of rural birds, in addition to their feeding, management and health status.

The indigenous birds maintained by the rural peoples are locally known as “Desi” and have been reported to gain 374.72 g of body weight at eight weeks of age (Khawaja et al. 2012a). The Fayoumi breed has been introduced in Pakistan since 1980 and is well adapted to local environmental conditions. This breed is known to gain about 364.10 g of body weight at eight weeks of age (Khawaja et al. 2012a). Due to its calm character and strong immunity against common diseases, farmers keep this breed at their homes and at farms (Rajput et al., 2005). Among the breeds imported in Pakistan, Rhode Island Red (RIR) has gained more popularity than the others due to its heavy growth rate i.e. 483.30g at eight weeks of age (Khawaja et al., 2012a). Moreover, its long stay in Pakistan has made it well adapted to the local environmental condition. Basically it is a dual purpose breed of American class and is getting more popularity in rural areas as “Golden birds” (Ashraf et al., 2003).

Desi, Fayoumi and RIR poultry breeds are being reared by the rural people of Pakistan indiscriminately and very little information is available with respect to growth of these breeds. Likewise, the literature on hematological and serum biochemical values of Desi, Fayoumi and RIR indigenous birds during the growing phase is also limited. Therefore, this study was planned to compare the growth performance and blood parameters of Desi, Fayoumi and RIR breeds to examine the best potential breed under local environmental conditions of Pakistan.

MATERIALS AND METHODS

Ethical approval

Bird ethics committee, poultry research institute, Rawalpindi, Pakistan, approved the protocol and conducting of the study.

Birds, management and experimental feed

A total of 2000 un-sexed day-old chicks of each Desi, Fayoumi and RIR breed were obtained from hatchery of Poultry Research Institute, Rawalpindi, Pakistan. The birds were maintained in floor pens on deep litter system for a period of 20 weeks. In floor pens, each breed was reared separately in a single pen until seven weeks of age when 2000 birds were randomly distributed between pens, with 21 to 24 birds of the same breed per pen (2 to 2.50 ft²/bird). Birds were fed manually and fresh water was made available around the clock. Nutrient content of the feed (Table 1) followed by recommendations of the NRC (1994). All birds were provided with nine hours of light per day, which was increased to 14 hours at 18 weeks with an intensity of five lux throughout. Temperature and relative humidity were between 21 to 23°C and 70%, respectively. All birds were vaccinated following a program typical of the region. Care and management of the birds followed accepted guidelines (FASS, 2010).

Table 1. Ingredients and nutrients (%) composition of diets fed to experimental birds

Dietary ingredients	Week 1 to 8	Week 9-20
Corn	35.60	42.00
Rice	23.00	12.00
Rice polish	10.00	9.48
Soyabean meal	10.00	16.00
Canola meal	8.00	6.40
Corn gluten meal (60%)	5.00	5.00
Fish meal	5.00	5.00
Lime stone	1.50	2.00
DCP	1.25	1.50
NaCl	0.33	0.27
Premix*	0.25	0.30
DL- Methionine	0.07	0.05
Total	100.00	100.00
Calculated Nutrients (%)		
ME Kcal/kg	2800	2800
CP	18.5	17
CF	3.80	4.30
EE	3.31	3.30
Ca	1.0	2.5
Available P	0.56	0.51
Lysine	1.00	0.69
Methionine	0.43	0.31

*Supplied per Kg of diet: vitamin A, 12000 IU; vitamin D3, 2200 IU; vitamin E, 10mg; vitamin K3 2mg; Vitamin B1, 1mg; vitamin B2, 5mg; vitamin B6, 1.5mg; vitamin B12, 0.01mg; Nicotinic acid, 30mg; Folic acid, 1mg; Pantothenic acid, 10mg; Biotin, 0.05mg; Choline chloride, 500mg; Copper, 10mg; Iron, 30mg; Manganese, 60mg; Zinc, 50mg; Iodine, 1mg; Selenium, 0.1mg and Cobalt, 0.1mg.

Parameter measured

The growth performance data (initial body weight, final body weight, feed intake, and feed conversion) were recorded at seven days (d) intervals. Mortality was also recorded in the rearing period. At the age of 12 and 20 weeks, meat samples of each breed from different birds were taken, dried, grounded and then subjected to proximate analysis such as percentage dry matter, crude protein, fat and total ash. Samples were analyzed using standard methods (AOAC, 2011).

Blood samples were collected from 20 birds of each breed at the age of 4, 12 and 20 weeks old and analyzed for the estimation of biochemical parameters such as glucose, triglyceride, cholesterol, calcium, protein, uric acid and Alkaline Phosphatase (ALP) and haematological parameters. For this purpose, 5 mls of blood was drawn from the brachial vein into dry clean centrifuge tubes and immediately centrifuged at 3000 rpm for 15 min. for separating serum. These samples were taken in the morning before feeding (between 8:00 to 10:00 hrs). Serum samples were stored at -20°C till time of chemical analysis. Samples were then analyzed at feed testing laboratory, Poultry Research Institute, Rawalpindi. The biochemical characteristics of blood were determined calorimetrically on UV visible spectrophotometer using commercial kits and diagnostic examinations. Total protein was quantitatively measured based on colorimetric determination as described by Cannon (1974). Glucose concentration was quantitatively measured based on enzymatic colorimetric method (Trinder, 1969). Total cholesterol concentration was quantitatively determined based on enzymatic colorimetric method of Allain et al. (1974). The uric acid was determined by the method of Bergman and Shabtay (1954) through the absorbency of the supernatant at 290 mu. The activity of ALP was determined by the method described by Bergmeyer and Wanlefeld (1980). Samples (10 μl) were incubated in alkaline buffer-substrate solution (50 mM glycine and 5.5 mM p-nitrophenylphosphate (pNPP), pH10.5) for 30 minutes at 37°C . The reaction was terminated by adding 0.02 M NaOH and ALP activity was determined as directly proportional to the amount of yellow pNPP anion liberated per unit time at 405 nm.

The anti-coagulated blood was also used to determine Red Blood Cell (RBC) count, Packed Cell Volume (PCV), Hemoglobin (Hb) concentration, and White Blood Cell (WBC) count. Differential WBC counts were made on monolayer blood films, fixed and stained with Giemsa-Wright's stain. Total RBC and total WBC count were determined manually by method using hemacytometer (Campbell, 1995). Packed

cell volume was measured by a standard manual technique using microhematocrit capillary tubes centrifuged at 2500 rpm for 5 min. Hemoglobin concentration was measured by cyanmethemoglobin method. Erythrocyte indices i.e. Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentrations (MCHC) were calculated from total RBC, PCV and Hb (Ritchie et al., 1994), respectively.

Statistical analysis

All data were determined by using the SPSS version 16, statistical analysis program. P-value of <0.05 was considered for significant differences among groups and the comparison of means was made by using Duncan's Multiple Range Test (Steel and Torrie, 1984).

RESULTS

Performance of rural chickens

The growth performance, mortality and meat composition of Desi, Fayoumi and RIR breeds during growing phase is shown in Table 2. The average day old weight was the highest in RIR (32.79g), intermediate in Desi (30.57g) and lowest in Fayoumi (27.09g). RIR breed consumed more feed and had gained the maximum ($P<0.05$) weight as compared with those of Desi and Fayoumi breeds at all ages of growing phase, which could be explained for the variation in genotype. Similarly, there was also significant variation ($P<0.05$) in feed intake among the Desi, Fayoumi and RIR chickens during growing phase. Desi breed consumed more feed, followed by RIR and Fayoumi chickens. The feed conversion was significantly poor ($P<0.05$) in Desi while it was better ($P<0.05$) in RIR breed. During the period of the 11-20 weeks feed conversion of birds seems to be better than the period of the 0-10 weeks. The results have shown that Desi and Fayoumi chicks had lowest ($P<0.05$) mortality. The mortality during the rearing period (0-10 weeks) was higher than the growing period (11-20 weeks) in all three breeds. The meat composition had shown no-significance ($P>0.05$) difference among three breeds at 12 and 20 weeks of age.

Haemato-biochemical Parameters of rural chickens

The biochemical values in all of the three breeds are shown in Table 3. There was no significant ($P>0.05$) difference in the biochemical values among three breeds. The hematological values in three rural breeds have been depicted in Table 4. There was non-significant ($P>0.05$) difference in hematological values amongst three breeds.

It is revealed from the present findings that Total Erythrocyte Counter (TEC), Hb and PCV increased with the advancement of age, being lowest in 4 weeks and highest in 20 weeks of age. However, Erythrocyte

Sedimentation Rate (ESR), MCV and MCH values decreased gradually with the advancement of age. Values of ESR in Desi, Fayoumi and RIR are inversely related with age.

Table 2. Comparative growth performance and meat composition of Desi, Fayoumi and Rhode Island Red chickens during brooding and growing periods (up to 20 weeks)

Parameters	Age (Weeks)	Breeds			P-value
		Desi	Fayoumi	Rhode Island Red	
Day old weight (g/bird)	--	30.57±0.22 ^a	27.09±0.30 ^b	32.79±0.38 ^a	0.050
Body weight (g)	0-10	577.54±9.05 ^b	547.25±7.73 ^c	673.75±11.65 ^a	0.001
	11-20	710.79±10.25 ^b	638.39±8.86 ^b	1012.61±17.21 ^a	0.000
	0-20	1288.33±15.15 ^b	1185.64±9.16 ^c	1686.36±21.36 ^a	0.000
Body weight gain (g/bird)	0-10	546.97±3.25 ^b	520.16±2.56 ^c	640.96±4.75 ^a	0.014
	11-20	680.22±5.11 ^b	611.31±3.98 ^c	979.82±7.47 ^a	0.002
	0-20	1257.76±4.52 ^b	1158.55±3.69 ^c	1653.57±5.15 ^a	0.003
Average feed intake (g/bird)	0-10	4308.44±11.35 ^a	3507.87±9.54 ^b	3490.02±8.47 ^c	0.003
	11-20	3376.25±8.96 ^b	2687.62±10.58 ^c	3787.16±9.67 ^a	0.025
	0-20	7858.81±148.32 ^a	6177.18±110.25 ^c	7521.16±201.74 ^b	0.000
Feed conversion	0-10	7.48±0.17 ^a	6.41±0.22 ^a	5.18±0.15 ^b	0.000
	11-20	4.75±0.21 ^a	4.21±0.18 ^b	3.74±0.29 ^c	0.001
	0-20	6.21±0.66 ^a	5.21±0.15 ^b	4.46±0.31 ^c	0.001
Mortality (%)	0-10	2.01±0.04 ^c	3.34±0.07 ^b	6.53±0.11 ^a	0.030
	11-20	1.87±0.03 ^b	1.88±0.02 ^b	3.35±0.08 ^a	0.000
	0-20	3.87±0.05 ^c	5.09±0.05 ^b	9.31±0.19 ^a	0.001
Meat Composition (%)					
Dry matter	12	25.13±0.12	25.45±0.15	24.82±0.17	0.140
Crude Protein	12	79.87±2.56	78.37±1.99	81.37±4.57	0.146
Crude fat	12	11.64±0.60	9.95±0.34	13.33±1.28	0.170
Total ash	12	4.52±0.05	4.55±0.02	4.50±0.12	0.150
Dry matter	20	26.18±0.11	25.52±0.19	26.85±0.24	0.164
Crude Protein	20	81.64±1.98	81.81±3.54	81.46±2.87	0.180
Crude fat	20	10.21±0.29	9.63±0.18	10.79±0.47	0.165
Total ash	20	3.93±0.10	3.79±0.09	4.08±0.15	0.189

^{a-c}Means with different letters differ significantly (P < 0.05)

Table 3. Mean values of serum glucose, triglyceride, cholesterol, calcium, protein, uric acid and alkaline phosphatase in Desi, Fayoumi and Rhode Island Red chickens at 20 weeks old

Parameters	Breeds			†Reference	P-Value
	Desi	Fayoumi	Rhode Island Red		
Glucose (mg/dl)	219.52±2.45	217.47±1.63	210.55±3.54	197-299	0.600
Triglycerides (mg/dl)	513.87±5.21	522.36±3.57	535.69±5.78	-	0.650
Cholesterol (mg/dl)	106.36±0.98	107.22±0.61	109.21±1.59	129-297	0.800
Calcium (mg/dl)	10.03±0.23	10.10±0.19	10.52±0.36	8.1-12	0.510
Protein (mg/dl)	5.10±0.08	4.99±0.10	5.25±0.15	3.0-4.9	0.475
Uric acid (mg/dl)	4.61±0.11	4.49±0.17	4.23±0.24	1.9-12.5	0.600
ALP (u/l)	1041.40±17.52	1054.12±12.22	1051.54±25.45	10-106	0.800

†Reference values of Clinical Diagnostic Division (1990)

Table 4. Normal haematological parameters in breeds of Desi, Fayoumi and Rhode Island Red breed during 4, 12 and 20 weeks of age

Parameter	Breed	Age (Weeks)		
		4	12	20
TEC (106/mm ³)	Desi	1.75±0.05	1.79±0.06	2.40±0.10
	Fayoumi	2.59±0.05	3.22±0.09	3.36±0.07
	RIR	1.80±0.20	1.97±0.04	2.61±0.15
Hb concentration (gm%)	Desi	7.70±0.17	7.73±0.15	8.54±0.14
	Fayoumi	7.00±0.50	7.62±0.10	7.84±0.16
	RIR	8.03±0.17	8.10±0.09	9.24±0.18
PCV (%)	Desi	27.63±1.12	28.26±0.55	31.15±0.67
	Fayoumi	26.46±0.61	27.28±0.40	28.00±0.60
	RIR	28.02±0.44	29.00±0.46	29.10±1.10
ESR in mm in 1st hour	Desi	1.06±0.01	0.76±0.01	0.31±0.01
	Fayoumi	3.15±0.16	2.42±0.13	2.04±0.11
	RIR	3.48±0.10	3.02±0.24	2.73±0.21
MCV (cubic micron)	Desi	166.55±6.24	163.0±2.60	128.45±7.90
	Fayoumi	103.66±2.20	86.00±1.54	84.00±1.64
	RIR	164.21±11.86	150.43±9.60	113.00±5.13
MCH (micro- micro gram or pictogram)	Desi	45.61±0.85	44.68±0.31	35.33±1.24
	Fayoumi	27.75±0.34	24.22±0.42	23.76±0.09
	RIR	48.16±8.46	43.33±2.50	35.48±1.77
MCHC (%)	Desi	27.90±1.11	27.33±0.70	27.49±0.32
	Fayoumi	26.58±0.40	28.09±0.47	28.16±0.62
	RIR	29.26±0.98	28.72±0.11	31.33±1.12

TEC= Erythrocyte Nnumber; Hb= Hemoglobin; PCV= Packed Cell Volume; ESR= Erythrocyte Sedimentation Rate; MCV= Mean Corpuscular Volume; MCH= Mean Corpuscular Hemoglobin and MCHC= Mean corpuscular hemoglobin concentration

DISCUSSION

Performance of rural chickens

The average day old body weight of RIR, Desi and Fayoumi was recorded as 32.79, 30.57 and 27.09g, respectively. A similar trend had been observed by Farooq et al. (2001) and Khawaja et al. (2012a), who reported higher day-old chick weight in RIR (35.32 and 31.30g, respectively), in comparison to Desi (33.84 and 25.90g, respectively) and Fayoumi chicken (30.74 and 20.90g, respectively). Similarly, Yeasmin and Howlider (2013) indicated that live weight at hatching averaged 34.0 and 36.1 g for the Fayoumi and RIR, with insignificant sex difference for the two breeds. Recently, Kumar et al. (2014) had reported that the RIR had significantly ($p<0.05$) higher mean day old body weight (35.42 ± 1.14 g) than that of 31.82 ± 0.85 g for Bovans White. The higher weight of newborn chicks of RIR could probably be due to the larger egg size.

This study showed that the Desi breed consumed more feed, followed by RIR and Fayoumi chickens. Kumar et al. (2014) had reported that the RIR had significantly ($p<0.05$) higher mean final body weight

(1350 ± 33.76 g) and body weight gain (1314 ± 31.77 g) than those of 1220 ± 36.55 g and 1188 ± 35.45 g for Bovans White, respectively at 22 weeks of age. The values of body weight of RIR in above study have been lower than the current study. The results showing low body weight gain in Desi birds than RIR are in line with the findings of Sahota and Bhatti (2001), who had observed lower body weight gain in Desi in comparison to RIR and White Leghorn chicks at 8 weeks of age. Halima et al. (2006) reported that day old weight, final body weight, body weight gain and mortality rate in RIR were 35.2g, 1394g, 1359g and 18.3%, respectively. In the current experiment, day old weight and mortality rate of RIR was lower (32.79 g and 9.31%, respectively) than the above study, however, final body weight (1686g), and body weight gain (1653.57g) were noted to have been higher than the above study. The poor growth rate in Desi chickens, as observed in the present study, could be attributed to genetic built up of the birds. Recently, Dutta et al. (2012) reported that initial and final weight of Fayoumi chickens at day old to five weeks of age were 32.1g and 359.5g, respectively. Day old weight is higher than the present study. All three breeds had poor feed utilization, the

Fayoumi consumed 255 g more feed per kg of body weight than the White Leghorn and RIR. The difference in growth rate of chicken is due to interplay of multiple genes and this trait could be improved through genetic selection (Khawaja et al., 2012b). These differences in body weight could also be attributed to environmental conditions such as seasons, temperature, humidity and management.

Feed conversion of birds during the period of the 11-20 weeks was found to have been better than the period of the 0-10 weeks. A probable explanation is that with the increase of the age of the birds, their activity and making voice loudly also increases, which requires more maintenance energy. Due to that, birds may utilize the feed more efficiently (Khawaja et al., 2012a). Haque et al. (1999) found that feed conversion ratio was at 5.7 and 4.9 for Fayoumi and RIR, respectively in a group of three male and 20 females during the 6 to 17 weeks which is almost similar in the present study.

The lowest mortality was recorded for Desi and Fayoumi chicks. These results are in accordance with Parveen et al. (2013), who had reported that higher mortality had been observed in RIR (16.50%) than Fayoumi (10.76%) and Desi chicken (6.78%) under field conditions. The lower mortality in these chickens could be attributed to the better adaptability of these chickens to the local environment and lower growth rate. In this study, the mortality during the rearing period was higher than growing period in all breeds; thus further improvement in the managerial practices is necessary in order to reduce the mortality among the chicks, regarding the fact that no particular infectious disease was reported during the experimental period. In Bangladesh, the mortality of different exotic breeds (Lohmann Brown, RIR and Fayoumi) under semi-scavenging conditions was at 27.6, 32.6 and 25.2%, respectively (FAO, 2005). Recently, Tadesse (2014) had reported that the average annual mortality of chicks was about 3.98% for local, 3.7% for cross breed and 3.2% for exotic breed chicks under field condition. Average mortality of growers (birds with 2 to 6 months of age) was set at 1.97% per year for local birds, 2.3% for cross breeds and 2.2% for exotic breeds. He explained that poor protection from adverse climatic conditions (very hot and cold weather) increased the severity of disease outbreaks resulting in losses of up to 70% of the flock at 12 weeks of age in field condition.

Reports regarding meat composition in rural chicken are rare in literature for the comparison to the present composition. These results are in line with the findings of Haunshi et al. (2013) who had reported that no significant difference had been observed in meat composition of

Aseel and Kadaknath rural breeds at 10 and 20 weeks of age. Similarly, Khawaja et al. (2012b) reported that meat composition had no significant ($P>0.05$) difference among pure (RIR and Fayoumi) and crossbred chickens at 20 weeks of age. Poultry meat quality may be affected by several factors such as genotype, rearing condition and feeding that have an impact on muscle metabolism as well as on chemical composition. Fanatico et al. (2005) studied slow-, medium- and fast-growing genotypes raised outdoor and slaughtered at similar live weights and found no significant differences among genotypes as for dry matter, fat and ash even if slow-growing birds were numerically lower in fat. In thigh meat the differences in fat content were more relevant than in the breast, with slow growing birds showing half of the content as opposed to fast growing. Tougan et al. (2013) reported that chicken meat quality is strongly affected by genotype whereas feeding exerts a minor effect. The appropriate choice of genotype seems to play a very important role in the quality of organic chicken products.

Haemato-biochemical parameters of rural chickens

The mean values of serum glucose (219.52 mg/dl), triglyceride (513.87mg/dl), uric acid (4.61mg/dl) and total protein (5.10mg/dl) in Desi birds in the present study are close to findings of Khawaja et al. (2012a), who had reported that serum glucose (221.80mg/dl), triglyceride (528.0mg/dl), uric acid (4.75mg/dl) and total protein (5.23mg/dl) in Desi birds. Elagib et al. (2012) reported that overall mean of total protein, uric acid and cholesterol in three Sudani chickens (Betwil, Bare Neck and Large Beladi) were found as 4.27, 7.42 and 99.97mg/dl, respectively, which are close to the values of the current study except for uric acid value tending to be higher.

Bhatti et al. (2002) reported that serum cholesterol level in different strains [Desi, Fayoumi, Crossbred (RIRxFayoumi) and Naked Neck] during pre- and post-laying period was same which implies that laying condition did not exert any extra demand on cholesterol bio-synthesis and its release in the blood circulation. In the present study, serum cholesterol of Desi, Fayoumi and RIR chickens was found within range of reference (Clinical Diagnostic Division, 1990). However, these values were lower than the values reported in crossbred (RIR male \times Fayoumi female) cockerels (187.80 mg/dl) at 12 weeks of age by Khan et al. (2011).

In the process of egg formation, the availability of dietary calcium (Ca) is critical. Ovulating hens have significantly higher Ca levels than non-reproductive females (Ritchie et al., 1994). This agrees with Elagib et

al. (2012), who compared the levels of serum calcium of Sudanese rural chickens as 14.3, 14.72 and 14.48 mg/dl for Betwil, Bare Neck and Large Beladi, respectively. There was no difference in Ca level among three breeds at laying stage in the present experiment. The birds were found to be equally affected by the stage of egg laying during which there was mobilization of Ca for shell formation. In the current experiment, the serum Ca level of three rural chickens was lower than domestic turkey (11.7-38.7 mg/dl), domestic fowl (13.2-23.7 mg/dl) and bobwhite quail (14.1-15.4 mg/dl) (Ritchie et al., 1994).

Total protein level in three rural chickens used in this study was higher (4.99-5.25mg/dl) than the reference range i.e. 3.0-4.90mg/dl (Clinical Diagnostic Division, 1990). In female birds, a considerable increase in total protein concentration occurs just prior to egg laying, which could be recognized to an estrogen-induced increase in globulins. The proteins were the yolk precursors (vitellogenin and lipoproteins), which were synthesized in the liver and transported via the plasma to the ovary where they were incorporated in the oocytes (Ritchie et al., 1994). Moreover, total proteins of hens in three chickens were lower than the normal range of the domestic turkey (5.29-7.6 mg/dl) and pheasant (male=5.65 mg/dl; female=6.06 mg/dl), but higher than the normal range of the guinea fowl (3.5-4.4 mg/dl) and common quail (3.4-3.6 mg/dl) (Ritchie et al., 1994).

In birds, uric acid is a major product of the catabolism of nitrogen, being the end product of protein/amino acid metabolism, indicates similar rate of protein/amino acid metabolism in different bird groups though genetically different (Elagib et al., 2012). Age and diet may influence the concentration of blood uric acid in birds. The uric acid values (4.23-4.61mg/dl) of three chickens in the present study are close to the values (4.16-4.63 mg/dl) determined by Bhatti et al. (2001) in Desi and Naked Neck hens. However, these values are lower than the values (7.425mg/dl) reported by Elagib et al. (2012) in Sudanese rural chickens.

During the egg shell formation process, there is an increase in activity of ALP in the blood of laying hens (Khawaja et al., 2013) due to the calcification process. The ALP value quantitatively was lower in Desi chicken (1041.40u/l) than Fayoumi (1054.12u/l) and RIR (1051.54u/l). These results are in line with the findings of Bhatti et al. (2002), who had found lower ALP values in Desi chicken (841.51u/l) than in Fayoumi (1653.04u/l) and crossbred (RIRxFayoumi) chickens (1656.5u/l). Khawaja et al. (2013) reported that the average value of ALP (1100u/l) in crossbred chickens of RIR and Fayoumi is very close to the values of present study.

Higher ESR at early age in this study was in accordance with those of Khawaja et al. (2012a). MCV values in this study for Fayoumi are lower than Desi and RIR. Similar results were reported by Khawaja et al. (2012a). Similarly, Mean Corpuscular Hemoglobin Concentrations values are similar with those quoted by Khawaja et al. (2012a). Haematological parameters in birds have been shown to be influenced by various factors such as age, sex, season and nutrition. In general haematological parameters are affected by diurnal fluctuations or changes in daily physical and metabolic activities (Piccione et al., 2005).

CONCLUSION

It can be concluded that overall, the RIR chicken performed better than Fayoumi and Desi chickens. However, a lower mortality rate had been noticed in Desi chicken. One of the most important positive characters of rural chicken is their hardiness, which is ability to tolerate the harsh environmental condition and poor husbandry practices without much loss in production. It was suggested that the low production performance of rural breeds of chickens may be improved through improvement in husbandry practices, better healthcare and also through selection and crossbreeding. Upgradation of such rural breeds of chickens through different breeding technique helps to increase the productivity of the germplasm and also their conservation in their natural habitat as the rural people will be very happy to rear them for their adoptability to harsh environment.

Competing interests

The authors declare that they have no competing interests.

Author's contributions

Abida Parveen, Tabinda Khawaja, Naveed Iftikhar and Saira Khan designed and performed the experiment. Sohail Hassan Khan analyzed data and wrote the manuscript.

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Performance, Serum Biochemical Parameters and Immunity in Broiler Chicks Fed Dietary *Echinacea purpurea* and *Thymus vulgaris* Extracts

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Received: 04 Aug 2017

Accepted: 06 Sept 2017

ABSTRACT

The objective of this study was to evaluate the effect of administering herbal extracts of *Echinacea purpurea* and *Thymus vulgaris* into broilers drinking water on performance, immune response and serum biochemical and Phyto hemagglutinin. 270 day-old Ross chicks were assigned to nine dietary treatments in a randomized manner. Each treatment was given to two replicates of 15 birds. The variables of *T. vulgaris* extract were 1% and 2% and variables of *E. purpurea* extract were 0%, 1% and 2% in drinking water. Body Weight (BW), Feed Intake (FI) and Feed Conversion Ratio (FCR) were recorded at the end of the experiment. Antibody responses against Newcastle disease viruses were measured after blood sampling at 42 days of age. The plant extracts did not affect BW, FI and FCR ($P>0.05$). Antibody titers against NDV were significantly affected by the administration of *E. purpurea* ($P<0.05$). The highest elevation was for the birds that were administered with 2% *E. purpurea* from 1 to 42 days ($P<0.05$). Administration of thyme extracts had improved serum biochemical parameters as compared with *Echinacea* and control group. It was concluded that under these research conditions, high levels of *E. purpurea* extracts had increased the broiler chickens' immunity.

Key words: Broiler, *Echinacea purpurea* extract, Immunity, Performance, *Thymus vulgaris* extract.

INTRODUCTION

Antibiotics have been widely used in poultry feed as growth promoters for more than 50 years. In the time that antibiotics were either used for curing or as growth promoters, some parts of the profitable microorganism will become damaged and resistance to diseases such as *Salmonella* and the other pathogens will decrease. Increased interest in curbing antibiotic use to reduce antimicrobial resistance has led to a growing interest in alternative growth promoters. Herbal extracts are being used as feed additives to improve performance, feed intake, secretion of digestive tract juices and immune system of animals especially under the intensive management systems (William and Losa, 2001; Amouzmehr et al., 2012).

The *Echinacea purpurea* is commonly known as an immune stimulating substance. Its palliative use in human medicine has been well established. *E. purpurea* has been shown to have non-specific immuno-stimulatory properties *in vitro* (Bauer and Wagner, 1991), including increased phagocytosis (Stotzem et al., 1992), increased cytokine production (Burger et al., 1997), and natural killer cell activity (See et al., 1997). *Thymus vulgaris* has received more attention due to its antioxidant (Bolukbasi and Erhan, 2007), antibacterial (Dorman and Deans, 2000; Ngouana Tadjong et al., 2017) anti-coccidial (Jamroz et al., 2003) and antifungal properties (Hertrampf, 2001). As, growth performance of animals is influenced strongly by the health and immune status, the objective of this study was to evaluate the effect of the utilization of *Echinacea purpurea* and *Thymus vulgaris* extracts in the feeding of

broilers on the growth performance and immune responses.

MATERIALS AND METHODS

270 day-old broiler chickens (Ross308) of mixed sex were randomly divided into nine groups (30 birds/group) and housed in pens of identical size in a deep litter system with wood shaving for flooring. Each group has 2 replicates (15 birds/pen). Strict sanitation practices were maintained in the house before and during the course of the experiment. All birds were fed a standard commercial diet based on corn and soybean meal and had free access to feed and water (Table 1). Treatment groups were:

- 1-Control group (plain water)
- 2- One mL/L (1%) of *E. purpurea* from 1 to 42 day
- 3-Two mL/L (2%) of *E. purpurea* from 1 to 42 day
- 4-One mL/L (1%) of *E. purpurea* from 21 to 42 day
- 5-Two mL/L (2%) of *E. purpurea* from 21 to 42 day
- 6-One mL/L (1%) of *T. vulgaris* from 1 to 42 day
- 7-Two mL/L (2%) of *T. vulgaris* from 1 to 42 day
- 8-One mL/L (1%) of *T. vulgaris* from 21 to 42 day
- 9-Two mL/L (2%) of *T. vulgaris* from 21 to 42 day

The live body weight of birds of all groups at 42 days of age was taken. Feed intake and their Feed Conversion Ratio (FCR) were calculated. Sera samples for subjecting to Haemagglutination Inhibition (HI) tests were obtained from all birds of each group following collection of 3mL blood (from wing vein) at 42 days of age to determine the antibody titers of Newcastle disease vaccines. Vaccination program is presented in table 2. To determine some of the

serum factors (HDL, LDL, TG, Cholesterol, Albumin, Total Protein, and Glucose), blood samples 42 days of age were collected. At 42 days of age, four birds per replicate were randomly chosen, slaughtered and their lymphoid organs (Bursa of Fabricius, spleen and Thymus) were collected, weighed and calculated as a percentage of live body weight. At d 42 of age, eight chickens per treatment were injected in the right wing with a Phytohemagglutinin (PHA) (Sigma-Aldrich, St. Louis, MO) solution (100 µg in 100 µl PBS (phosphate-buffered saline) and immediately in the left wing with 100 µl PBS. The thickness of the right and left wing web was measured prior to the injection and at 6th, 12th, 24th, 48th, and 72nd hours post PHA injection using a digital caliper; then, the chickens were killed by cervical dislocation. The wing web swelling (WWS) response was expressed as a swelling index, calculated as follows: Swelling index = [(thickness of right wing web post PHA injection – initial thickness of right wing web) – (thickness of left wing web post PBS injection – initial thickness of left wing web)] (Konieczka et al., 2017). The data were analyzed using computerized statistical program (SPSS version 15.0) to determine the Mean ±SD of antibody titer and body weight. Significance differences were denoted by P < 0.05.

Ethical approval

This study was carried out in strict accordance with the recommendations of institutional guidelines for the care and use of laboratory animals. Chickens were humanly handled in respect of the ethical standards laid down in 1964 Declaration of Helsinki and its later amendments.

Table 1. Nutritional composition of the diet

Diet composition	Starter (0-10d)	Grower (11-28d)	Finisher (29-42d)
Metabolizable energy, kcal/kg	2930	3050	3150
Crude protein %	23	19.5	18.5
Digestible lysine %	1.32	1.18	1
Methionine %	0.55	0.5	0.45
met + cys %	0.98	0.88	0.82
Threonine %	0.88	0.78	0.7
Tryptophan %	0.23	0.19	0.18
Arginine %	1.5	1.25	1.13
Calcium %	0.97	0.94	0.9
Available phosphorus %	0.48	0.45	0.42
Sodium %	0.16	0.16	0.16
Chlorine %	0.18	0.18	0.18

Table 2. Vaccination Program

Age	Day 1	Day 8	Day 8	Day 16	Day 26
Vaccine	Infectious Bronchitis-H120	Newcastle Disease-B1	Newcastle-Influenza	Infectious Bursal Disease-D78	Infectious Bursal Disease-D78
Route	Corse spray	Drinking water	Injection	Drinking water	Drinking water

RESULTS

The effects of herbal extracts on chicken's body weight gain, total feed intake, and FCR have been presented in table 3. Results have shown that the administration of *T. vulgaris* from 21 to 42 days had affected growth performance more (body weight gain and feed consumption ratio) but these two parameters weren't statistically different ($P>0.05$). As shown in table 3, antibody titers against NDV were significantly affected by administration of *E. purpurea* ($P<0.05$). The highest elevation was for the birds that administrated 2% *E. purpurea* from 1 to 42 days ($P<0.05$).

Table 4 summarizes the data obtained on the effect of experimental treatments on serum hematological parameters. No significant influence of experimental diets on albumin was observed ($P>0.05$). The serum total protein, Triglyceride and HDL of the birds treated with (2%) of *T. vulgaris* from 1 to 42 days old were better than the others ($P<0.05$). The feeding of the broilers with (1%) of *T. vulgaris* from 1 to 42 days resulted in a marked ($P>0.05$) decrease in LDL- cholesterol concentration compared to other treatments. Hemoglobin concentration and Cholesterol in groups treated *T. vulgaris* from 1 to 42 days was respectively higher and lower than the other groups ($P<0.05$) respectively. Table 5 indicates the effect of treatments on lymphoid organs' weight at 42 day. As it is shown, the Bursa weight in birds treated with *E. purpurea* had increased and the differences in Spleen and Thymus weights were not statistically significant.

The effects of the dietary *Echinacea purpurea* and *Thymus vulgaris* extracts on PHA challenge on the WWS response are shown in Table 6. PHA challenge led to a higher increase in wing web thickness in chickens fed with *E. purpurea* diets than in those fed *T. vulgaris* diets ($P<0.05$). WWS was the highest at 24 h post PHA injection in group 3, post PHA injection ($P<0.05$).

DISCUSSION

There are no appetizing effects with both *Echinacea* and *Thymus*. The feed intake and FCR of the broilers didn't show a varying influence by administration with different dosages of *Echinacea* and *Thymus* in comparison with control group. Other authors have reported also a missing effect of various supplementations of mixed herbs on the feed intake of broilers and layers (Roth-Maier et al., 2005; Nasir and Grashorn, 2010). Toghyani et al. (2010) reported that the low dosage of Thyme has had a significant effect on broiler body weight and their feed conversion ratio. But Tekeli et al. (2006) and Demir et al. (2008) had reported opposite results; they found that thyme had no influence on broilers performance. Ngouana

Tadjong et al. (2017) reported that feed intake, live body weight, weight gain and feed conversion ratio are affected by using thyme and oregano essential oil in broiler diets.

Inability to improve feed conversion ratio with *E. purpurea* is in agreement with the findings of Habibian et al. (2011) who had also reported that *E. purpurea* supplementation as a feed additive didn't improve feed conversion ratio. Absence of positive effect of some herbal extracts in some experiments may be due to using a smaller dose which was insufficient to produce its effect on poultry. Improving immunity in poultry production is very important to prevent common important diseases. There was a significant effect on the immunity of treated chicks in the present study. All chicks that were administrated *E. purpurea* had significantly higher antibody titer against *T. vulgaris* ($P<0.05$). Among them, the highest (8.9) increasing was belong to 2% *E. purpurea* extract from day old to 42 days ($P<0.05$).

Our findings indicated that the dietary *E. purpurea* could not only affect the PHA-induced swelling response, but also increased the weight of Bursa. The PHA-induced WWS is a good indicator of acquired immunity and allows the assessment of leukocyte interactions during the immune response (Konieczka et al., 2017). Increasing in antibody titer of the birds administrated *E. purpurea* are consonant with the increasing of the weight of bursa. The studies of the immune system have shown that some herbs such as coneflower (*Echinacea purpurea*) were most effective in achieving immune system improvement, because this herb had increased the stimulation of non-specific immune system. It is though that immune enhancement of *Echinacea* is provided by certain polysaccharides, flavonoids and *isobutylamides* (Rehman et al., 1999). Also, herbs like thyme (*Thymus vulgaris*) that are rich in active compounds such as flavonoids extend the activity of vitamin C, act as antioxidants and may therefore enhance the immune function (Manach et al., 1996).

Obtained data from biochemical parameters showed that supplementing broiler diet with *thyme* extracts had insignificant better effect on cholesterol, triglyceride, LDL, total protein, albumin and hemoglobin ratio against supplementing *Echinacea*. In agreement with our finding, Ali (2014) and Toghyani et al. (2010) reported supplementing diets with thyme leaves powder had no significant effect ($P\geq 0.05$) on serum parameters. Thyme supplementation in broiler diets significantly increased glucose level compared to those of the control group. The possible reason for increasing serum glucose may be due to the abdominal lipids catabolism of gluconeogenesis process as feeding inclusion thyme by birds, since crushed thyme consumption in broiler chickens has been reported to increase the serum glucose as mentioned by El-Ghousein and Al-Beitawi (2009).

Table 3. Effects of *E. purpurea* and *T. vulgaris* on growth performance and antibody titer (log2 HI titer) against Newcastle virus (M ± SE)

Treatments	Body weight (g)	Feed intake (g)	Feed conversion (g/g)	Antibody titer (log2 HI titer)
Control	2477±226.1	4260	1.72	6.5 ^a ±0.15
<i>E. p</i> (1%) 1 to 42d	2412±268.3	4200	1.74	8.2 ^b ±0.024
<i>E. p</i> (2%) 1 to 42d	2409±277.6	4170	1.73	8.9 ^c ±0.21
<i>E. p</i> (1%) 21 to 42d	2410±318.8	4170	1.73	7.4 ^d ±1
<i>E. p</i> (2%) 21 to 42d	2526±230	4320	1.71	8.1 ^b ±0.18
<i>T. v</i> (1%) 1 to 42d	2389±300.5	4325	1.81	6.5 ^a ±0.14
<i>T. v</i> (2%) 1 to 42d	2434±252.7	4260	1.75	6.6 ^a ±0.18
<i>T. v</i> (1%) 21 to 42d	2567±232.8	4340	1.69	6.4 ^a ±0.13
<i>T. v</i> (2%) 21 to 42d	2460±272.1	4260	1.73	6.3 ^a ±0.19

The different superscripts on the same line are significantly different (P < 0.05); *Mean ± standard deviation

Table 4. Effect of experimental diets on serum biochemical parameters of broilers at d 42.

Factor(mg/dl)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Blood Glucose	127±4.08 ^{ac}	117.5±8.34 ^a	123.5±7.18 ^{ac}	129.25±10.5 ^{bc}	116.5±4.79 ^a	118.5±9.67 ^a	122.75±9.97 ^{ac}	131.25±12.97 ^{bc}	124.5±10.5 ^{ac}
Cholesterol	95.5±23.33 ^{ab}	97.25±17.11 ^{ab}	96±23.93 ^{ab}	102.75±16.35 ^b	95.5±19 ^{ab}	72.75±7.5 ^a	71.25±7.67 ^a	85.25±11.58 ^{ab}	84.25±11.35 ^{ab}
Triglyceride	58±6.16 ^b	53.50±9.84 ^b	56±8.52 ^b	51.25±12.12 ^{bc}	54.50±4.65 ^b	44.50±11.95 ^{ac}	38.50±13.69 ^a	41.25±19.5 ^a	42.5±11.81 ^a
LDL	47±11.9 ^c	43.25±8.9 ^{bc}	45.5±7.32 ^c	46.75±6.39 ^c	36.75±8.65 ^{abc}	27.25±4.11 ^a	31±9.09 ^{ab}	30±9.2 ^{ab}	29.25±9.28 ^a
HDL	52.25±7.5 ^a	61.25±11.5 ^{abc}	60.25±4.92 ^{ab}	64.75±4.11 ^{bc}	59.75±8.05 ^{ab}	72.50±4.79 ^{cd}	76.25±5.90 ^d	63.75±6.70 ^{bc}	68±7.11 ^{bcd}
Total protein	3.80±0.59 ^a	3.97±0.22 ^{ab}	4.09±0.11 ^{abc}	4.22±0.11 ^{bc}	3.83±0.09 ^a	4.32±0.07 ^{bc}	4.41±0.07 ^c	4.18±0.06 ^{abc}	4.05±0.19 ^{abc}
Albumin	1.42±0.09 ^a	1.47±0.03 ^a	1.41±0.06 ^a	1.39±0.04 ^a	1.38±0.06 ^a	1.46±0.03 ^a	1.44±0.03 ^a	1.39±0.1 ^a	1.47±0.04 ^a
Hemoglobin	7.85±0/32 ^a	8.08±0.39 ^{ab}	8.31±0.06 ^{bcd}	7.97±0.24 ^{ab}	8.25±0.09 ^{bc}	9.40±0.11 ^e	9.06±0.29 ^e	8.49±0.3 ^{cd}	8.69±0.16 ^d

The different superscripts on the same line are significantly different (P < 0.05); *Mean ± standard deviation

Table 5. Effect of experimental diets on weights of lymphoid organs at day 42.

Lymphoid organ weight	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Bursa/BW (gr)	0.144±0.007 ^a	0.168±0.005 ^c	0.18±0.011 ^d	0.177±0.011 ^d	0.155±0.009 ^{abc}	0.148±0.004 ^{ab}	0.15±0.007 ^{ab}	0.16±0.007 ^{bc}	0.16±0.007 ^{bc}
Spleen/BW (gr)	0.113±0.002 ^{abc}	0.11±0.002 ^{abc}	0.12±0.002 ^d	0.114±0.002 ^{abc}	0.118±0.005 ^{cd}	0.113±0.002 ^{abc}	0.117±0.005 ^{abc}	0.111±0.001 ^a	0.113±0.002 ^{ab}
Thymus/BW(gr)	0.195±0.03 ^a	0.26±0.04 ^b	0.247±0.03 ^{ab}	0.24±0.05 ^{ab}	0.242±0.03 ^{ab}	0.212±0.03 ^{ab}	0.212±0.04 ^{ab}	0.22±0.04 ^{ab}	0.227±0.04 ^{ab}

The different superscripts on the same line are significantly different (P < 0.05); *Mean ± standard deviation

Table 6. Phytohemagglutinin (PHA) injection time on the wing web swelling response in chickens challenged with PHA

Wing web reaction (PHA-P)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Stimulation index after 24 h (mm)	1.22±0.04 ^{ab}	1.55±0.08 ^f	1.76±0.1 ^g	1.48±0.07 ^f	1.52±0.06 ^f	1.35±0.05 ^{cd}	1.3±0.02 ^{bc}	1.15±0.05 ^a	1.37±0.03 ^{cde}
Stimulation index after 48 h (mm)	1.31±0.04 ^{bc}	1.69±0.06 ^g	1.71±0.05 ^g	1.71±0.1 ^g	1.47±0.04 ^{ef}	1.36±0.04 ^{cd}	1.53±0.04 ^f	1.28±0.03 ^b	1.44±0.01 ^{d^{ef}}

The different superscripts on the same line are significantly different (P < 0.05); *Mean ± standard deviation

CONCLUSION

Both herbal extracts (*Echinacea* and *thyme*) used in drinking water did not improve the performance of broilers. However, *Echinacea* extracts had significantly improved the immune response of birds to the NDV vaccine, therefore, more studies with different growth facility need to be performed to analyze the effect of herb extracts on the performance, carcass yield, hematology and immune response of poultry.

Competing interests

The authors have no competing interests to declare.

Authors' contributions

The present study was funded by H Habibi and S Firouzi. Habibi and Firouzi were also involved in the collection of data, statistical analysis and drafting of the manuscript. Both authors read and approved the final manuscript.

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Effect of Plumage Color and Body Weight on the Semen Quality of Naked Neck Chicken

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Received: 01 Jul 2017

Accepted: 03 Aug 2017

ABSTRACT

The low fertility of local chicken breeds is a major issue in backyard poultry system. The fertility rate varies among different males due to their difference in semen quality. The objective of the present study was to evaluate the effect of plumage color and body weight on the semen quality of Naked Neck chicken. The Naked Neck males (n=18) vary in three plumage colors (black=6, brown=6, white=6) and each color contains two body weight sub groups (heavy= >1600gm n=3 and light= <1600 gm n=3) were used in this study. The semen was collected and accessed individually for volume, pH, concentration, motility, livability and morphological defect. Black plumage color contained significantly more semen volume than brown color. The heavy body weight group (heavy= >1600 gm) contained significantly more semen volume (0.21±0.02 ml) and sperm concentration (1.88±0.06×10⁹ ml) than the lightweight group (light= <1600 gm). So, black plumage color roosters of can be use to enhance fertility rate of naked neck chickens.

Key words: Body weight, Naked neck chicken, Plumage color, Semen quality

INTRODUCTION

In spite of the highly developed commercial poultry system, backyard poultry system still occupies a significant position in Pakistan. The local native breeds are favored because of their adaptability, resistance and production of organic meat and eggs. Naked neck chickens have same protein requirement like other feather chickens (Daulat et al., 2015). The Naked Neck is a major native chicken breed which has high egg production, egg weight (44.86g), egg length (7.03mm), yolk weight (14.57mm), shell weight (4.29mm) and yolk height (3.29mm) (Adedeji et al., 2015) capacity with a higher heat tolerance than

other native breeds. The Naked Neck chickens have problems of low fertility and hatchability than other native breeds (Peters et al., 2008). Naked neck roosters are criticized for low semen volume, higher abnormal sperms, and higher coiled tail defective sperm (Fathi et al., 2013).

To acquire a good fertility rate, the semen quality must be excellent to ensure the fertilization process. Assessment of semen characteristics gives an excellent indicator of their reproductive potential (Mothibedi et al., 2016). The proper selection, of physically elite roosters and hens, helps to improve these breeds containing higher genetic potential (Anjum et al., 2012). Semen quality can be accessed through the direct measurement of in vitro

semen parameters like volume, concentration, motility, livability and morphological abnormalities which determines the male fertility (Liu et al., 2008). However, this procedure of semen collection and assessment is very stressful, time consuming, costly and these facilities are not available in villages. Many researchers have recommended the use of secondary sexual characteristics such as: body weight and body color of males as good indicators of semen quality in chicken (McGary et al., 2003; Wilson et al., 1979). The selection of healthy males with developed secondary sexual characteristics can improve fertility rate due to good quality semen (McGary et al., 2002). Thus, simple, reliable and indirect method for the estimation of semen quality based upon the correlation with secondary sexual traits should be evaluated (Udeh et al., 2011). To this date, there has been no information available about the selection of breeding cock for backyard poultry system in Pakistan.

Thus, the objective of the present study is to evaluate the effect of plumage color and body weight on semen quality of Naked Neck chicken for the selection of breeding cock.

MATERIALS AND METHODS

Ethical approval

Society for Protection and Care of Animals (SPCA) University of veterinary and animal sciences Lahore, Pakistan is the responsible to avoid any kind of act that is harmful to animals and make sure all rules and regulation regarding animal rights must have followed during experiment.

Experimental roosters

A total of 18 necked neck roosters (6 birds with brown color, 6 birds with black color and 6 birds with white color) were selected from avian research centre Ravi campus university of veterinary and animal sciences Lahore on base of physical appearance. Roosters do not have any physical abnormality, having red comb and wattle, shiny feathers, wet cloacae and free from any kind of parasites were selected. Roosters were divided into three groups on the bases of their colors (6 males/plumage color). Each plumage color category contained two body weight sub groups contained heavy and light body weight (>1600gm and <1600gm respectively, 3 males/body weight/plumage color). The birds in each sub group were caged separately and fed with a standard breeder diet (100g per day). The availability of water was round the clock. The photoperiod of 16L:8D was provided. The duration of experiment was six months.

Semen collection

The roosters were trained for semen collection prior the start of original experiment. Semen was collected twice weekly by abdominal massage technique in an insulin syringe (Riaz et al., 2004). The same person and same time during whole experiment to minimize the stress collected the semen 20 times.

Semen evaluation

Following parameters of individual ejaculate were estimated 20 times. The volume of ejaculate was measured by the insulin syringe. The pH was determined by digital pH meter (WTW, Germany). The concentration was determined by hemocytometer using the dilution rate of 1:500. Briefly, 2.5 ml formal saline was taken in a test tube and 5µl semen was added in a test tube after discarding 5µl of formal saline. The cover slip was placed on counting chamber and charged with diluted semen. Sperm were counted at 400× under light microscope. The number of sperm per ml of semen was calculated by using the formula.

Number of sperms/ml = number of sperms counted × 5 × 10 × 500 × 1000

The motility was determined by placing a drop of extended semen on pre wormed glass slide at 37 °C. After putting a cover slip on the percentage of motile spermatozoa was estimated under light microscope at 100×. At least three fields (the procedure was repeated three times) were observed before taking the final value. Percentage of live spermatozoa was calculated by using eosin nigrosin staining technique. The small drops of semen and eosin nigrosin stain was mixed on pre wormed glass slide at 37 °C. A uniform thin smear was made with the help of another clean glass slide. After air-drying, the slide was observed under light microscope at 400×. The spermatozoa containing unstained head was counted as live while stained head of spermatozoa counted as dead. Before taking final value two hundred spermatozoa were counted. Eosin nigrosin stained slide was observed by using oil emersion lens at 1000× of light microscope to estimate morphological defects. The two hundred sperms were counted before taking final value.

Statistical analysis

General linear procedure was performed for all plumage colors (brown, black and white) and two body weight groups i.e. heavy and light (3x2 factorial). All data were presented as Mean±SEM. All analyses were performed using statistical software SAS Enterprise Guide® (version 4.2, SAS Inst. Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

In the present experiment, the effect of body weight and plumage color on semen quality and physiological semen traits of Naked Neck chicken was evaluated. Better semen quality improves the fertility and hatchability traits which could be helpful for improvement of Naked Neck chicken breed.

Physiological traits of Naked Neck chicken semen

The values for semen parameter were as follows; volume (0.18 ml), motility (72.77%), concentration ($1.82 \times 10^9/\text{ml}$), pH (7.12), livability (83.25%), morphological defects (11.63%) (Table 1). The physiological traits of Naked Neck chicken semen were in

accordance with the results of previous studies (Ajayi et al., 2011; Fathi et al., 2000).

Table 1. Physiological semen traits of Naked Neck chicken (n=18), Lahore, Pakistan (January to June 2016)

Parameters	Means \pm SE
Volume-ml	0.18 \pm 0.009
Motility%	72.77 \pm 0.52
pH	7.12 \pm 0.007
Concentration $\times 10^9/\text{ml}$	1.82 \pm 0.03
Livability%	83.25 \pm 0.65
Morphological Defects%	11.63 \pm 0.53
Plasma Membrane Integrity%	83.71 \pm 0.71

Table 2. Effect of plumage color on semen quality of Naked Neck chicken, Lahore, Pakistan (January to June, 2016)

Items	Brown	Black	White	Means
Volume (ml)				
Heavy	0.19 \pm 0.01 ^{aA}	0.27 \pm 0.02 ^{bA}	0.21 \pm 0.02 ^{aA}	0.22 \pm 0.01 ^A
Light	0.14 \pm 0.01 ^{aA}	0.14 \pm 0.01 ^{aB}	0.13 \pm 0.08 ^{bA}	0.14 \pm 0.007 ^B
Means	0.16 \pm 0.01 ^a	0.21 \pm 0.02 ^b	0.17 \pm 0.01 ^{ab}	--
Motility (%)				
Heavy	75.00 \pm 1.62 ^{aA}	74.16 \pm 1.60 ^{aA}	72.08 \pm 1.43 ^{aA}	73.75 \pm 0.89 ^A
Light	72.50 \pm 0.75 ^{aA}	72.08 \pm 1.14 ^{aA}	70.83 \pm 0.56 ^{aA}	71.80 \pm 0.49 ^A
Means	73.75 \pm 0.91 ^a	73.12 \pm 0.98 ^a	71.45 \pm 0.76 ^a	--
pH				
Heavy	7.14 \pm 0.01 ^{aA}	7.13 \pm 0.01 ^{aA}	7.10 \pm 0.04 ^{aA}	7.12 \pm 0.08 ^A
Light	7.13 \pm 0.02 ^{aA}	7.09 \pm 0.01 ^{aA}	7.11 \pm 0.02 ^{aA}	7.11 \pm 0.01 ^A
Means	7.14 \pm 0.01 ^a	7.11 \pm 0.01 ^a	7.10 \pm 0.01 ^a	--
Concentration $\times 10^9/\text{ml}$				
Heavy	1.92 \pm 0.05 ^{aA}	2.05 \pm 0.10 ^{aA}	1.85 \pm 0.08 ^{aA}	1.94 \pm 0.04 ^A
Light	1.72 \pm 0.03 ^{aA}	1.71 \pm 0.06 ^{aB}	1.67 \pm 0.04 ^{aA}	1.70 \pm 0.02 ^B
Means	1.82 \pm 0.03 ^a	1.88 \pm 0.06 ^a	1.76 \pm 0.05 ^a	--
Livability (%)				
Heavy	84.41 \pm 1.40 ^{aA}	84.66 \pm 1.41 ^{aA}	81.54 \pm 1.90 ^{aA}	83.54 \pm 0.87 ^A
Light	85.04 \pm 1.24 ^{aA}	81.83 \pm 2.20 ^{aA}	82.04 \pm 1.50 ^{aA}	82.97 \pm 0.98 ^A
Means	84.72 \pm 0.91 ^a	83.25 \pm 1.23 ^a	81.79 \pm 1.19 ^a	--
Morphological Defects (%)				
Heavy	10.12 \pm 1.35 ^{aA}	10.04 \pm 1.52 ^{aA}	11.83 \pm 1.16 ^{aA}	10.66 \pm 0.77 ^A
Light	11.20 \pm 1.17 ^{aA}	12.62 \pm 1.53 ^{aA}	13.95 \pm 0.85 ^{aA}	12.59 \pm 0.70 ^A
Means	10.66 \pm 0.88 ^a	11.33 \pm 1.09 ^a	12.89 \pm 0.73 ^a	--

^{a-b} denote significant difference within the rows ($P < 0.05$); ^{A-B} denote significant difference within the columns ($P < 0.05$)

Effect of plumage color and body weight on semen quality of Naked Neck chicken

The semen volume was found higher ($P < 0.05$) in black than brown plumage color while it did not differ

($P > 0.05$) with white breed i.e. (black = 0.21 ± 0.02 ml, white = 0.17 ± 0.01 ml and brown = 0.16 ± 0.01 ml). The maximum semen volume was harvested from heavy black roosters than all the other groups (0.27 ± 0.02 ml, $P < 0.05$).

Mean volume was higher ($P < 0.05$) in the heavy than the light weight group (0.22 ± 0.01 and 0.14 ± 0.07 ml respectively). Mean concentration was recorded to be higher ($P < 0.05$) in heavy than light weight group ($1.94 \pm 0.04 \times 10^9$ and $1.70 \pm 0.02 \times 10^9$ /ml respectively). Concentration was observed higher ($P < 0.05$) in heavy black group than all light weight groups. Motility, pH, livability and morphological defects did not differ ($P > 0.05$) among weight and plumage color groups (Table 2). The present study has shown that plumage color affects the volume of semen in the heavy weight group only in which black group contained higher volume. The semen volume and sperm concentration were influenced by body weight while other parameters were not affected. Similar to our results El-Hammady et al. (1995) found that heavy weight cocks contain higher ($P < 0.05$) sperm concentration. Recently Galal (2007) reported the positive correlation between semen qualities and body weight. In chickens, the males are selected based on comb and wattle size, body weight and body color. Females prefer the healthy males with well developed secondary sexual characteristics may be due to high reproductive efficiency (Zuk et al., 1995). The development of secondary sexual characters has been associated with the androgen production. It has been stated that heavy weight males contain large and heavy testes which results in the production of more semen and androgens (Riaz et al., 2006). The quality of semen may vary with age, body weight of cocks, collection technique and breed. According to the Udeh et al. (2011) it was suggested that where there are no facilities for semen evaluation the secondary sexual characteristics can be used for the estimation of semen quality of cock. More over continuous reduction in genes of indigenous chicken breed may lead to high embryo mortality in backyard poultry (Ajayi et al., 2016). It was concluded that heavy body weight rooster with black plumage color contain more semen volume and sperm concentration and can be used for breeding purposes in backyard poultry system.

Author's contribution

Adnan Jabbar Ansari and Waseem Abbas were main responsible for tabulation of experimental data and article writing under supervision of Amjad Riaz, Muhammad Akram and Yasir Allah Ditta.

Competing interests

The authors declare that they have no conflict of interest with respect to the research, authorship, and/or publications of this article.

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Potential of Commercial Spice Mixes to Enhance the Quality and to Extend the Shelf Life of Raw Chicken Breasts

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Received: 30 Jul 2017

Accepted: 29 Aug 2017

ABSTRACT

Raw chicken harbors spoilage microorganisms such as the Mesophilic Aerobic Bacteria (MAB), Lactic Acid Bacteria (LAB), Spoilage Yeasts (SY) and *Pseudomonas*, which limit product shelf life. This study compared the potential of three spice mixes (“Tandoori”, “Kalia” and “Massala”) to extend the shelf life of raw chicken. Chicken breasts were marinated with each of the spice mixes (3% w/w), and subsequently refrigerated for up to 15 days. Marinated and non-marinated samples were withdrawn at three-day intervals and analyzed for enumeration of MAB, LAB, SY and *Pseudomonas*. After three days, chicken marinated with “Tandoori” and “Kalia” spices had a significantly ($P < 0.05$) lower load of MAB (5.51-6.06 log cfu/g) compared with untreated control breasts (6.58 log cfu/g) although by Day 15, there were no significant differences ($P > 0.05$) observed in the final MAB counts between treated samples (7.51-7.88 log cfu/g) and untreated controls (7.88 log cfu/g). There were also no significant ($P > 0.05$) differences in the counts of *Pseudomonas* (2.65-3.64 log cfu/g), LAB (2.56-4.20 log cfu/g) and SY (2.60-4.15 log cfu/g) over the 15-day storage. Since the onset of microbial spoilage is marked by MAB reaching 7 log cfu/g, the microbiological shelf-life of marinated and non-marinated chicken breasts were estimated at 12 and 6 days respectively. However, based on the sensorial attributes, both marinated and non-marinated chicken received poor acceptability scores after six and three days respectively. Commercial spice mixes can thus extend the refrigerated shelf-life of raw chicken by three days to a maximum of six days.

Keywords: Breast, Chicken, Quality, Shelf-life, Spice Mixes

INTRODUCTION

The consumption of chicken has increased worldwide because it forms a major part of the human diet. Chicken has nutritional characteristics such as low lipid content and a high concentration of polyunsaturated fatty acids (Bourre, 2005). Chicken meat can however perish rapidly if it is not stored, processed, packaged or distributed correctly (EFSA, 2013). Raw meat can either be spoiled by microbial activity or by oxidative processes due to the high content of PUFA and high peroxidation index (Sebranek et al., 2005; Dal Bosco et al., 2016). Spoilage microorganisms associated with meat, especially lactic acid bacteria can cause undesirable changes in

meats. Those changes make the chicken unattractive and unfit for human consumption (Gram et al., 2002; Doulgeraki et al., 2012).

Thus food industries worldwide have resorted to the addition of synthetic preservatives to meat products to prevent the uncontrolled growth of spoilage organisms and to increase their shelf life. In recent years, there has been a significant concern over the safety of these chemicals, thereby influencing consumers' preference for natural products such as spices and plant extracts over chemical preservatives (Govaris et al., 2010).

A spice can be referred to as a seed, fruit, root, bark, berry, bud or vegetable which is used in food to enhance its flavour (aroma and taste), colour or texture as well as to

preserve the product from deterioration. The use of spices is very important in raw or fresh meat because the latter is mostly susceptible to spoilage (Thomas et al., 2012). Many studies have stated that spices have antioxidant properties by the virtue of their phenolic components (Konczak et al., 2010). Spices with known antimicrobial activity, are cloves, cinnamon and oregano as they contain eugenol, cinnamaldehyde and thymol respectively (Wang et al., 2011). Not only do spices delay the onset of microbial spoilage, but also enhance the safety of food by inhibiting the growth of foodborne pathogenic microorganisms (Devatkal and Naveena, 2010). For instance, Radha Krishnan et al. (2014) showed that spices, containing a high amount of phenolic compounds, decrease lipid oxidation and inhibit the growth of microorganisms, thus increasing the shelf life of poultry (Radha Krishnan et al., 2014). There are many studies, which have either investigated the antimicrobial activities of crude forms of spices (Smith-Palmer et al., 1998; Hara-Kudo et al., 2004; Skrinjar and Nemet, 2009; Aggarwal et al., 2015) or the essential oils of spices (Hammer et al., 1999; Dorman and Deans, 2000) against foodborne pathogens and spoilage microorganisms. As the Mauritian cuisine is diverse and is an amalgamation of European, Chinese and Indian cuisines, a wide variety of spices are used as ingredients and seasoning in Mauritian cooking. Moreover, spices are also used as decoctions in Mauritius (Mahomoodally et al., 2012). However, to our knowledge no studies have been attempted to test the potential of spice mixes, locally available in Mauritius to control the growth of spoilage bacteria in food systems. This study therefore aims at assessing the potential of commercially available spice mixes to enhance the quality and extend the shelf-life of raw chicken breast.

MATERIALS AND METHODS

Sample collection and treatment

A preliminary survey targeting a convenience sample of 50 consumers was conducted in order to identify different spice mixes typically used in households. A questionnaire was designed for that purpose and was administered face to face. The three most frequently used spice mixes identified were “Tandoori”, “Massala” and “Kalia” and chicken breast was identified as the most preferred part of poultry meat used by consumers.

Chicken breasts were bought from a chilled retailed outlet and transported to the laboratory in a cooler bag. The skin was removed and the chicken breasts were cut into cubes, weighing approximately 10g, with dimensions of 2 cm x 2 cm x 2 cm. Chicken samples were then either left untreated (U) or homogeneously mixed with

“Tandoori” (Tt), “Kalia” (Tk) or “Massala” (Tm) at a final concentration of 3% w/w as determined in the preliminary survey. Samples were subsequently placed in closed plastic containers and were kept at 4°C for up to 15 days.

Microbiological analysis

Marinated and non-marinated samples were withdrawn for microbiological analysis every three days for a period of 15 days. Each sample was aseptically placed in a sterile stomacher bag to which 90 ml of sterile buffered peptone water was added. The mother sample and its dilutions were placed on Plate Count agar medium (OXOID), *Pseudomonas* agar (OXOID), *Lactobacillus* MRS agar (OXOID) and Potato Dextrose agar (PDA) for the enumeration of Mesophilic Aerobic Bacterial (MAB) counts, *Pseudomonas* counts, Lactic acid bacteria (LAB) counts and Spoilage Yeast (SY) counts respectively. The plates for the *Pseudomonas* counts and Lactic acid bacterial counts were incubated at 37°C for 24±2 h and 37°C for 48±2 h respectively. PDA plates were however incubated at room temperature (ca. 24°C) for five days. All the colonies were then enumerated with a colony counter.

Physicochemical analysis

For determination of pH, treated or untreated chicken samples (10 g) were minced and mixed with 90 ml of distilled water for 30 minutes using a magnetic stirrer. The pH of the mixture was then measured using a digital pH meter (Mettler Toledo). For the determination of the water activity of samples, a hand-held water activity meter (Novasina, Japan) was used. For the determination of instrumental surface colour (CIE L*a*b), chicken samples were minced and placed in a clean petri dish which was then inverted. Triplicate measurements of surface colour were then taken using a chromameter (Minolta CR-410, Konica Minolta, Japan). Drip loss of chicken samples was also determined by measuring cumulatively the volume of exudate lost after two hours, one day and 15 days through refrigerated storage. To determine drip loss, 10 g of marinated and non-marinated samples were initially weighed and then placed in a sealed ziplock bag and kept in the refrigerator at 4°C. Their weight was then determined immediately (D₀), one day (D₁) and 15 days (D₁₅) through refrigerated storage. All the physicochemical analyses were carried out in three independent replicates and their measurements were taken in triplicates.

Sensory evaluation

Marinated and non-marinated chicken samples were prepared and stored at chilling temperature as described

previously. A sensory evaluation questionnaire was designed and sensory analysis was conducted with 10 untrained panellists at 3-day intervals for a period of 15 days. On each day of the analysis, samples were taken out and rated for different sensory parameters such as colour, aroma, texture and general appearance on a scale of 1-10, where 1 being the least accepted and 10 being most accepted.

Statistical design and analysis

The statistical analyses were conducted using the General Linear Model in MINITAB version 16.0 to determine the differences for the different treatments on the different days of storage. Significant differences were considered at the 95% confidence level ($P < 0.05$).

Ethical approval

The authors solemnly declare that publication ethics and good conduct were adhered to during preparation, reviewing, processing and proofreading of this article. No ethical clearance was needed to conduct the work.

RESULTS AND DISCUSSION

Consumer use of spices

The most commonly used spice mixes among Mauritians were “Tandoori” (24.0%) followed by “Kalia” (21.9%) and “Massala” (17.7%), as these spices are ingredients used in many traditional Mauritian dishes. As a matter of fact “Tandoori chicken” is one of the favourite dishes of Mauritians and it is of Indian origin (Kioon, 2015). “Chicken Kalia” is considered as one of the most authentic Mauritian recipes and is also widely appreciated by Mauritians (Kioon, 2010). These spice mixes were most preferred by survey participants thanks to the virtue of their unique compositions and flavour. The survey also revealed that the spices were added to chicken at a ratio of approximately 30g to 1 kg of chicken or ca. 3% w/w of chicken.

Microbiological analysis

The MAB counts of chicken samples had increased from an initial population of 5.4 log cfu/g to a maximum of 7.9 log/cfu over the 15-day refrigerated storage (Table 1).

For samples that were marinated with “Tandoori”, “Kalia” and “Massala” spice mixes, MAB counts were consistently lower than their untreated counterparts by a maximum of 1.0 log cfu/g. The exact mechanism of antibacterial action of spices and derivatives is not yet clear (Lanciotti et al., 2004), although it has been hypothesized that hydrophobic and hydrogen bonding of

phenolic compounds to membrane proteins, partitioning of the lipid bilayer (Juven et al., 1994), perturbation of the permeability of bacterial cell membranes (Cox et al., 1998), membrane disruption (Caccioni et al., 2000), destruction of electron transport systems (Tassou et al., 2000) and cell wall perturbation (Odhav et al., 2002) could play a role. MAB counts of samples treated with “Tandoori”, “Kalia” and “Massala” however reached levels of 7.9, 7.6 and 7.7 log cfu/g after 15 days respectively indicating that the spice mixes used in the study had not significantly suppress growth of mesophilic aerobes ($P > 0.05$). On the other hand, other authors have successfully demonstrated the effectiveness of spices and spice mixes to sustainably control spoilage and pathogen growth in food. Shelef (1983) indicated that high levels of spices inhibited growth of spoilage microorganisms in food such as chicken and fish. Grohs and Kunz (2000) tested the effectiveness of spice mixtures to inhibit the growth of various meat-spoilage microorganisms (*Bacillus subtilis*, *Enterococcus* spp., *Staphylococcus* spp., *E. coli* K12 and *Pseudomonas fluorescens*) and to stabilize the colour and smell of fresh-portioned pork meat.

Table 1. Mesophilic aerobic bacterial (MAB) counts (log₁₀ cfu/g) of spice-marinated and non-marinated chicken samples over a 15-day storage period

Days	Treatments			
	U	Tt	Tk	Tm
0	5.4±0.2	5.4±0.6	6.0±0.0	5.9±0.1
3	6.6±1.2	5.5±0.5	6.1±0.1	6.7±0.5
6	7.2±0.8	6.3±0.9	7.0±0.2	7.0±0.2
9	7.5±0.8	6.4±0.8	7.1±0.1	7.1±0.2
12	7.7±1.0	7.3±1.5	7.2±0.1	7.2±0.5
15	7.9±1.2	7.9±1.2	7.6±0.4	7.7±0.6

U: Untreated, Tt: “Tandoori”, Tk: “Kalia”, Tm: “Massala”; Data represent mean values of three replicates ± standard deviation; Counts within the same row representing the same day of storage were not significantly different ($P > 0.05$).

Contrary to our findings, the authors showed that these spice mixtures were effective shelf-life extenders. The disparity in our results could be partly attributed to differences in the variety of spices used, the composition of spice mixes tested, the cultivar of spice vegetables and the marination procedure. Indeed, several scientific reports attributed the differences in the inhibitory effect of spices to variation in the resistance of different microorganisms to a given spice and of the same microorganism to different spices (Akgul and Kivanç, 1988). It is also worth mentioning that there is a considerable body of research on the antibacterial effectiveness of essential oils of a wide range of spices against different spoilage and pathogenic bacteria and their results consistently showed that

individual or combinational extracts exhibited strong antibacterial activity (Arora and Kaur, 1999; Elgayyar et al., 2001; Zhang et al., 2015). The high potency of the extracts noted in these studies could be attributed to the fact that they tested aqueous or alcohol-based extracts of spices and herbs. In our study on the other hand, we used commercial spice blends, which were crude mixtures of different dried spice vegetables.

Shelf-life can be defined as the period in which a product remains safe and suitable for consumption. This means that it has not deteriorated in quality or spoiled in any way that the consumer would find it unacceptable (EFIC, 2013). The limit of acceptability or the onset of spoilage of poultry products is usually marked by mesophilic aerobes attaining populations of 7.0 log cfu/g in fresh poultry (Cox et al., 1998). The microbiological shelf-life of non-marinated chicken was thus estimated to be < 6 days while the shelf-life of chicken marinated with “Tandoori” was estimated to be < 12 days. “Kalia” and “Massala” were not as effective shelf-life extenders as “Tandoori” as the chicken products had a shelf-life of < 6 and < 9 days respectively. Similarly Khanjari et al. (2013) and Kuswandi et al. (2014) also observed that the microbiological shelf-life of untreated chicken was ca. 6-7 days at refrigeration temperature. *Pseudomonas* spp., Lactic acid bacteria and Spoilage yeasts counts on non-marinated chicken increased from an initial density of 2.9, 2.6 and 2.6 log cfu/g to a maximum of 3.2 log cfu/g although the difference between the final and initial density was not significantly different ($P > 0.05$) (Table 2).

Table 2. The initial and final population density of *Pseudomonas* spp., Lactic acid bacteria (LAB) and Spoilage Yeasts (SY) (log cfu/g) of spice-marinated and non-marinated chicken

Parameter	Days	U	Tt	Tk	Tm
<i>Pseudo.</i>	0	2.9±0.0	2.6±0.8	2.7±0.1	2.8±0.2
	15	3.2±0.1	3.0±0.1	3.0±0.0	2.9±0.0
LAB	0	2.6±0.1	2.6±0.0	2.6±0.7	2.7±0.6
	15	3.2±0.1	3.1±0.4	4.2±0.6	4.2±0.6
SY	0	2.6±0.0	2.4±0.9	3.0±0.5	2.9±0.2
	15	3.2±0.5	3.8±1.1	4.0±0.7	4.2±0.6

U: Untreated, Tt: “Tandoori”, Tk: “Kalia”, Tm: “Massala”; Data represent mean values of three replicates ± standard deviation; Counts within the same row representing the same day of storage were not significantly different ($P > 0.05$).

For chicken marinated with one of the different spice mixes, *Pseudomonas* spp. had increased to a maximum of 3.0 log cfu/g although these final counts were not significantly different from the final densities of their untreated counterparts ($P > 0.05$). The *Pseudomonas* species isolated from poultry could likely be *P. fluorescens*, *P. putida* or *P. fragi* (Russell, 2009). *Pseudomonas* spp. has generally been considered to be the

predominant Specific Spoilage Organism (SSO) in poultry (Barnes and Impey, 1968; Cerveny et al., 2009). SSO is defined as the part of the total microbiota responsible for spoilage of a given product within the spoilage domain, which is the range of product characteristics and storage conditions within which a given SSO causes product rejection (Dalgaard, 1995). In fact, Davies and Board (1998) indicated that *Pseudomonas* spp. made up approximately 85% of the entire bacterial population on poultry refrigerated for about two weeks and fluorescent and non-pigmented strains of *Pseudomonas* spp were mostly found in spoiled chicken. Jay et al. (2007) and Rukchon et al. (2014) also mentioned that the primary spoilage organism in chicken kept at low temperature reportedly belongs to the genus *Pseudomonas*. However, in the current study, *Pseudomonas* spp. did not appear to be the predominant spoilage bacteria as it had increased only by 0.35 log cfu/g compared to other bacterial species. It is possible that other microbial species that were not enumerated in this study could have been responsible for product spoilage since there are over 25 bacterial genera that make up the microbiota of poultry (Lahellec et al., 1975). Lahellec et al. (1975) indicated that in a study of 5920 isolates from chicken carcasses, pseudomonads were found to constitute only 30.5% of the microbial biota while the rest consisted of *Acinetobacter*, *Flavobacterium* and *Corynebacterium* in relative abundances of 22.7%, 13.9% and 12.7% respectively and yeasts and *Enterobacteriaceae* in relatively lower in numbers.

The counts of LAB in marinated samples ranged from 3.1 - 4.2 log cfu/g, compared with 3.2 log cfu/g in the non-marinated chicken although these final counts were not significantly different from the final densities of their untreated counterparts ($P > 0.05$). The higher population of LAB noted in samples marinated with “Kalia” and “Massala” could be attributed to the lower pH of these spice mixes. Both of these spices have chili powder in varying proportions (Cuizinemaurice, 2014), and chili powder is known to have a low pH of 4.4 (Peter and Babu, 2012). Adding these spices to chicken is likely to lower the pH and favour the growth of acidophilic and acid-tolerant microorganisms such as lactic acid bacteria (Hutkins, 1993; 2009). This could partly explain the higher population density attained by LAB in marinated chicken over none-marinated chicken. Moreover, lactic acid bacteria are spoilage microorganisms that can occur in spices such as onion and garlic powder, and these ingredients are present in varying proportions in “Kalia” spice mix (Cuizinemaurice, 2014). Davies and Board (1998) reported that even moderate levels of lactic acid bacteria in poultry can in fact result in the release of off-flavours and deterioration of the colour of chicken (Franz

et al., 2010). Hence selecting spices having low counts of lactic acid bacteria, particularly the hetero-fermentative variety, is important for manufacturing of products with an extended shelf-life (Sperber, 2007).

Although spoilage of poultry meat has been largely attributed to bacteria (Corry, 2007), yeasts can also be present in the microbiota. Russell (2009) mentioned that fungi are usually of less importance in poultry spoilage except when antibiotics are employed to suppress bacterial growth. In fact, yeasts have been reported to attain population density as high as 10^6 cfu/g on fresh chicken carcasses during storage (Hinton et al., 2002). In our study, we observed that spoilage yeasts proliferated to a greater extent in spice-marinated chicken (3.8-4.2 log cfu/g) than in the none-marinated samples (3.2 log cfu/g) although these differences were not significant ($P > 0.05$). The higher counts of yeast organisms on marinated chicken noted in our study could be because of the presence of indigenous microorganisms already present in these commercial spice blends. Indeed, spices and their plants can be contaminated with microorganisms during cultivation, processing and packaging (Ito et al., 2008). In addition, we also observed that the addition of spices depressed the water activity of chicken meat by a maximum of 0.23 (Table 4) thus potentially encouraging the growth of spoilage yeasts compared to bacteria (Beuchat, 1983). The yeast isolates could likely belong to the genus *Candida*, *Rhodotorula*, *Debaromyces* or *Yarrowia*, as these are predominant yeasts in poultry (Jay et al., 2007). Viljoen et al. (1998) also indicated that *Candida* and *Debaromyces* were the two most dominant genera of yeasts on both fresh and spoiled carcasses although *Rhodotorula* was not found on any spoiled carcasses. Ismail et al. (2000) further mentioned that the two most abundant species of *Candida* and *Debaromyces* were *C. zeylanoides* and *D. hansenii* on fresh and spoiled poultry.

Physicochemical characteristics of chicken pH of chicken samples

The pH of chicken marinated with “Tandoori”, “Kalia” and “Massala” had decreased significantly ($P < 0.05$) from 10.00 to 6.51, 7.18 and 7.24 respectively and did not change significantly ($P > 0.05$) over the 15-day storage period. On the other hand, the pH of untreated samples had decreased significantly ($P < 0.05$) over the 15-day period from 10.00 to 7.86 (Table 3).

The changes in the pH of chicken during refrigerated storage is comparable to the findings of Zhang et al. (2015) who had observed that spice extract treatments comprising of clove, rosemary and clove + rosemary spice extracts reduced the pH to final values of 5.62, 5.58 and 5.48 respectively which were lower than the pH of the control (6.66). Istrati et al. (2015) also observed that the pH values of beef treated with six different marinades comprising of wine, spices such as black pepper and garlic, and herbs such as thyme and marjoram, decreased from an initial value of 5.70 to a minimum value of 4.90. On the other hand, the same author showed that the pH of untreated beef increased to a final value of 6.16. Zhang et al. (2015) mentioned that the pH increase of control untreated samples could have been caused by the utilization of amino acids by bacteria, which are released during protein degradation following depletion of stored glucose. Indeed, accumulation of ammonia and products of amino acid decomposition are thought to result in an increase in pH (Gill, 1983).

Water activity of chicken samples

The mean a_w of untreated chicken meat was 0.93 while the mean a_w of chicken marinated with the different spices ranged from 0.83 to 0.91 on the day of addition of the spice mix (Table 4). However, over the 15-day storage period, the water activity of chicken treated with “Tandoori”, “Kalia” and “Massala” decreased from 0.83 to 0.65, 0.89 to 0.64 and 0.91 to 0.65 respectively. No significant difference was observed among the water activity of samples treated with the difference spice mixes ($P > 0.05$) (Table 4).

Table 3. pH of spice-marinated and non-marinated chicken

Days	U	Tt	Tk	Tm
0	10.00 ± 0.41 ^{Aa}	6.51 ± 0.21 ^{Ac}	7.18 ± 0.05 ^{Ab}	7.24 ± 0.13 ^{Ab}
15	7.86 ± 0.18 ^{Ba}	7.60 ± 0.33 ^{Aa}	7.09 ± 0.14 ^{Ab}	6.88 ± 0.17 ^{Ab}

U: Untreated, Tt: “Tandoori”, Tk: “Kalia”, Tm: “Massala”; Data represent mean values of three replicates ± standard deviation; Means within a column with different uppercase superscripts differ significantly ($P < 0.05$); Means within each row with different lowercase superscripts differ significantly ($P < 0.05$).

Table 4. Water activity of spice-marinated and non-marinated chicken

Days	U	Tt	Tk	Tm
0	0.843 ± 0.023 ^{Aa}	0.687 ± 0.043 ^{Ab}	0.800 ± 0.017 ^{Aa}	0.540 ± 0.013 ^{Ac}
15	0.773 ± 0.020 ^{Ba}	0.613 ± 0.036 ^{Ab}	0.570 ± 0.027 ^{Bb}	0.387 ± 0.047 ^{Ac}

U: Untreated, Tt: “Tandoori”, Tk: “Kalia”, Tm: “Massala”; Data represent mean values of three replicates ± standard deviation; Means within a column with different uppercase superscripts differ significantly ($P < 0.05$); Means within each row with different lowercase superscripts differ significantly ($P < 0.05$).

The decrease in the water activity of treated samples over the storage duration could be due to the steady loss of free water by osmosis due to the osmotic pressure exerted by the spice marinades (Gurtler et al., 2014). Moreover, since the spice mixes used in the study have an inherently low water activity of ca. 0.3 (Peter and Babu, 2012), their use in the marinating of chicken could have depressed the water activity of the chicken meat by simple osmosis, or dehydration. When spices are applied to high moisture food products such as raw chicken, the dry spices attempt to reach equilibrium with the food product with which it is in contact by drawing available water from within the flesh to the outside while the spices try to permeate into the food interior (Parish, 2017). The result is a reduction in the water activity (a_w) of the chicken products. Since the minimum threshold to support the growth of spoilage fungi (0.80) is lower than the threshold for bacteria (0.93), the marinated chicken products could more likely support the growth of spoilage yeasts over spoilage bacteria as already indicated above. After 15 days of refrigerated storage, the a_w of untreated chicken had decreased from 0.93 to 0.86 and this is likely due to considerable drip loss reaching as high as 20.1% (Table 6). From our observations, we could infer that drip loss is inversely related with water activity; the higher the drip loss, the lower the water activity of the chicken product. On the other hand, Oliveira et al. (2015) noted that different thawing treatments of chicken breasts resulted in different degrees of drip loss with no effect on the a_w .

Surface colour characteristics of chicken samples

The lightness (L^*) values of untreated samples had decreased from 54.3 to 51.3 over the 15-day refrigerated period (Table 5). Galobart and Moran (2004) similarly observed that L^* values for refrigerated poultry fillets decreased following 48-h of storage and attributed it to the considerable drip loss. Indeed, we also observed a maximum drip loss of 13.9 and 20.1 % in untreated samples after 1 and 15 days of refrigerated storage (Table 6). Galobart and Moran (2004) also mentioned that further decreases in L^* values during prolonged storage could relate to meat drying and shrinkage. In fact, we also observed that longer storage of up to 15-days resulted in concomitantly lower L^* values (51.3), higher drip loss (20.9%) and lower water activity (0.77) compared to the initial L^* values (54.3), drip loss (0.6%) and lower water activity (0.84) of untreated samples. Indeed, the extensive drip loss after 15 days was observed in the form of white exudation from the chicken meat.

The L^* values for marinated chicken were lower than their untreated counterparts and ranged from 40.9 to 46.9 on the day of application. Indeed, untreated chicken initially appeared pale pink and translucent while the marinated chicken exhibited the colour of the added spices

i.e. appeared reddish, brownish or yellowish with the addition of the “Tandoori”, “Kalia” and “Massala” spice mixes respectively.

Table 5. Comparison of the initial and final surface colour characteristics of spice-marinated and non-marinated chicken during a 15-day storage period

Treatment	Days of storage	Colour parameters		
		L^*	a^*	b^*
U	0	54.3±2.13	9.3±1.01	6.1±0.68
	15	51.3±3.68	9.6±1.62	7.8±0.89
Tt	0	44.1±1.80	13.6±2.19	4.3±0.98
	15	41.6±1.70	7.8±0.75	9.9±0.88
Tk	0	43.7±1.43	5.1±0.53	19.1±1.29
	15	42.9±1.74	6.6±1.09	18.1±0.88
Tm	0	46.9±1.39	6.9±0.17	24.7±1.20
	15	45.6±1.91	7.8±1.07	24.6±2.91

U: Untreated, Tt: “Tandoori”, Tk: “Kalia”, Tm: “Massala”; L^* : Lightness, a^* : Redness, b^* : Yellowness; Data represent mean values ± standard deviation

As expected, the redness (a^*) value for samples marinated with Tandoori (13.6) was higher than either untreated (9.3), Kalia (5.1) or Massala (6.9) marinated samples since tandoori spices are red in colour due to the presence of sweet paprika (Cuizinemaurice, 2014) and occasionally due to the presence of synthetic dye E124 also known as cochineal red (EFSA, 2015). Sweet paprika has a characteristic red colour due to the presence of red-pigmented carotenoids such as capsanthin, capsorubin, zeaxanthin and cryptoxanthin (Zachariah and Gobinath, 2008). However, the redness (a^*) of chicken marinated with Tandoori had decreased after 15 days reaching a final mean value of 7.8. The reason for this downshift may be due to the loss of oxy-myoglobin in the meat as well as compositional changes undergone in the Tandoori spice mix (Khan et al., 2015).

The initial b^* values were highest in chicken treated with Massala (24.7) and Kalia (19.1), compared to either untreated (6.1) or Tandoori (4.3) chicken, due to the different shades of yellowness of the two spice mixes. Indeed, Massala and Kalia spice mixes both comprise of different proportions of turmeric, which is also known as the “yellow root”. After 15 days, chicken marinated with Massala or Kalia still had a persistent yellow colour with b^* values of 24.6 and 18.1 respectively. Untreated and tandoori-marinated chicken had slightly higher b^* values of 7.80 and 9.94 respectively probably due to acquisition of a slightly brownish colour. Colour changes undergone during storage of poultry arise when by-products generated during lipid oxidation interact with the myoglobin pigment (Khan et al., 2015).

Drip loss

Water within meat exists inbound, immobilized or free forms. Bound water molecules associate with electrically charged reactive groups of muscle proteins (Montgomery, 2007), while immobilized water molecules are attracted to the bound molecules in layers that become successively weaker as the distance from the reactive group on the protein becomes greater (Mills et al., 1989). Free water refers to water molecules that are only held by weak forces (Montgomery, 2007). Drip loss can be determined by quantifying the amount of free water lost in raw chicken meat, cooked whole meat or cooked comminuted meat products (Hertog-Meischke et al., 1997; Lawrence et al., 2003; Otto et al., 2006) and usually gives an indication of the juiciness (Montgomery, 2007). In this study, drip loss of 7.8-16.7% was observed after one day of refrigerated storage for marinated and non-marinated samples (Table 6). Indeed, drip loss can be expressed as milligrams per gram (mg/g) of sample or as a percentage (Montgomery, 2007). However, after 15 days of refrigerated storage, the cumulative % drip loss attained 17.6-30.2% probably due to extensive exudation by osmosis.

Table 6. Cumulative drip loss (% w/w) of marinated and non-marinated chicken

Days of storage	U	Tt	Tk	Tm
0	0.0	0.0	0.0	0.0
1	13.9	10.1	16.7	7.8
15	20.1	19.9	30.2	17.6

U: Untreated, Tt: "Tandoori", Tk: "Kalia", Tm: "Massala"

Extensive drip loss could also probably explain the lowering of the water activity of treated samples from 0.91 to 0.65 and untreated samples from 0.93 to 0.86. Another reason for the increase in the drip water loss is that immediately after refrigeration, the surface of the chicken becomes colder than inside the cell and hence the rate of moisture loss increases, leading to a surge in the loss of water (Garcia et al., 2010). Qiao et al. (2002) studied the effect of marinating on drip loss and colour of broiler breast fillet. A marinade made of water (92.5%), salt (5%), and phosphates (2.5%) was applied for 24 h. Results showed considerable variation in drip loss, pH and meat colour. Drip loss of raw chicken fillets was positively correlated with lightness of raw fillets while water-holding capacity was negatively correlated with lightness (Qiao et al., 2002). Montgomery (2007) mentioned that the pH can greatly affect moisture binding in meat. However, in our study, we did not observe any clear-cut association between pH of samples and extent of drip loss.

Sensory evaluation

The four sensory attributes, which generally influence consumers' decision to purchase fresh chicken meat, are colour, juiciness, flavour and texture/tenderness. Both marinated and control samples had a pleasant texture and appearance on the initial day of storage with $\geq 90\%$ of the panelists indicating moderately high to very high acceptance of the products. After three days of storage, treated and untreated samples were equally well received by the panelists with more than 90% showing moderately high to very high acceptance. This observation is very much congruent with findings of Radha Krishnan et al. (2014) who also showed that the sensorial quality of chicken breasts left untreated or treated with spice extracts fared well in all three attributes up to three days of storage. However, the sensory quality of all samples began to deteriorate after six days of refrigerated storage with more than 70% of the panelists indicating low to no acceptance of the products tested. In fact, after six days of storage, none of the samples were accepted due to their odour, texture and appearance as all of them released very pungent putrid odours and had a sticky appearance. This finding is in agreement with that of Radha Krishnan et al. (2014) who also demonstrated a lower acceptability score for control samples after 6 days. However, contrary to our findings, Radha Krishnan et al. (2014) indicated that the sensory attributes of samples treated with spices only deteriorated significantly after 12 days of storage as opposed to 6 days noted in our study. After 15 days, we observed that all samples appeared sticky and slimy. In fact, the slime layer was the result of individual white colonies forming on the spoiled breast fillet that eventually coalesce to form a biofilm (Russell, 2009). Kong et al. (2007) observed that pork marinated with spices started to produce off-odours characteristic of putrefaction after seven days of storage and more pronounced discoloration was observed on the 14th day for both control and treated pork. Kong et al. (2007) attributed the off-odors to lipid oxidation and ammonia production from breakdown of proteins. Russell (2009) on the other hand mentioned that off-odors do not result from breakdown of the proteins in skin and muscle, rather are released from the direct microbial utilization of low molecular weight nitrogenous compounds such as amino acids, which are present in skin and muscle. Among off-odor producers in general, there is a selection of bacterial species that forms part of the microbiota of fresh poultry (Thomas et al., 1987; Erkmén and Bozoglu, 2016). These include psychrotrophic bacteria such as *Pseudomonas* spp., *Acinetobacter*, *Moraxella* and *Shewanella putrefaciens* (Thomas et al., 1987). *S. putrefaciens* isolates tend to produce sulphide-like odours as this organism is known to produce hydrogen sulphide,

methyl mercaptan and dimethyl sulphide (Thomas et al., 1987). As Ayres et al. (1950) indicated, the release of off-odors generally precedes the development of sliminess, with the former being first detected when the population reaches about 7.2-8.0 log cfu/g or log cfu/cm². Indeed, we also observed significant slime formation after 15 days coinciding with aerobic plate counts reaching 7.9 log cfu/g. Slime formation is an evidence of superficial spoilage that tends to occur because the inner portions of poultry tissue are generally sterile or contain relatively few organisms. The spoilage biota therefore, is restricted to the surfaces (Tellez et al., 2013) and grows in an environment of high humidity such as in the refrigerator.

CONCLUSION

The spice mixes “Tandoori”, “Kalia”, and “Massala” are frequently used in Mauritius for marination of chicken. The spice mixes variably inhibited the growth of mesophilic aerobic bacteria achieving a maximum reduction of 1.0 log cfu/g, relative to untreated controls. Since a population density of TVC exceeding 7 log cfu/g indicates the onset of spoilage, Tandoori and Massala were found to extend the microbiological shelf-life of the product by 6 and 3 days respectively. However addition of the commercial spice mixes did not improve the sensory attributes of marinated chicken over their untreated counterparts and the sensory shelf-life of both treated and untreated chicken breasts were < 6 days due to significantly reduced acceptability scores.

Acknowledgements

The authors wish to thank Mrs Zaynab Joomun-Baboorally for technical assistance in the project.

Competing interests

The authors declare that they have no competing interests.

Author`s contributions

SS made substantial contributions to conception of the study, acquisition, analysis and interpretation of data and writing-up of the first draft of the manuscript. AR contributed in the design of experiments and reviewing of the manuscript. HN was involved in critically reviewing, revising and formatting the manuscript. HN and AR have given final approval of the version to be published.

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Efficacy of Combined Vaccine against Salmonellosis and Infectious Coryza in Poultry

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Received: 06 Aug 2017

Accepted: 11 Sept 2017

ABSTRACT

In the present study, efficacy of two prepared combined vaccines against salmonellosis and infectious coryza in poultry has been studied. Two vaccines were prepared using *Salmonella* Typhimurium and Enteritidis combined with *Avibacterium paragallinarum* serovars A, B, and C. one vaccine was adjuvanated with aluminium hydroxide gel and the other adjuvanated with montanide ISA71. The two vaccines were assayed in six weeks old Specific Pathogen Free (SPF) white Lohman layer chickens by injecting two doses of each vaccine 3 weeks apart. These chickens were challenged with either *Salmonella* virulent strains or *Avibacterium paragallinarum* different serovars 3 weeks post second dose. Antibody titers in sera of chickens against different antigens were higher in groups vaccinated with montanide oil vaccine than those vaccinated with aluminium hydroxide gel vaccine as detected by different serological tests; ELISA, micro-agglutination test and haem-agglutination inhibition test. Protection rate against challenge test were 80% and 85% for *Salmonella* and (80%; 90%, and 70%) and (90%; 100%, and 90%) to *Avibacterium paragallinarum* serovars A, B, and C respectively for combined vaccine adjuvanated by aluminum hydroxide gel and montanide ISA71. The protection rate was 15% against *Salmonella* Typhimurium and Enteritidis and 0% against infectious coryza among the unvaccinated chicken group. it could be concluded that producing a vaccine from locally isolated *Salmonella* and *Avibacterium* (*Haemophilus*) *paragallinarum* strains adjuvanated with montanide ISA71 is recommended to aid in controlling avian salmonellosis and Infectious coryza at the same time.

Key words: Aluminum hydroxide gel, Chicken, Infectious coryza, Salmonellosis, Vaccine.

INTRODUCTION

Salmonella is a persistent pathogen in the environment, able to easily survive and proliferate. The most commonly isolated serovars worldwide from various animal sources continue to be *Salmonella* Enteritidis and *Salmonella* Typhimurium which, besides producing gastroenteritis, are found in asymptomatic carriers in a wide variety of animal species. Of these, *Salmonella* Enteritidis is the most prevalent one followed by *Salmonella* Typhimurium (52.3% and 23.3% of the cases, respectively) (López-Martín et al., 2016). *Salmonella* has

remained to be one of the most frequently detected causative agents in the food-borne outbreaks reported (26.6% of outbreaks). Eggs and egg products are frequently associated with *Salmonella* outbreaks. *Salmonella* Enteritidis and to a lesser extent, *Salmonella* Typhimurium are associated with egg-related outbreaks (EFSA, 2004).

Avian Infectious Coryza is a serious respiratory tract infection of domestic fowls caused by an opportunistic pathogen *Avibacterium paragallinarum* having an economic implication on the poultry industry and ornamental bird's population (Priya et al., 2012).

Infectious Coryza is a contagious bacterial disease of poultry; it is a common bacterial disease in the commercial poultry (Gayatri et al., 2010). It mainly affects the upper respiratory tract of chickens. The meat of the affected chicken is condemned if it is infected with *A. paragallinarum* (Blackall et al., 2005).

Combined vaccines have the advantage of protection against more than one disease at the same time, besides, reducing vaccination expenses, decreasing the stress of vaccination for different vaccines, number of vaccination performed and saving time. So this study evaluates the efficacy of a prepared combined vaccine against salmonellosis and infectious coryza using two different adjuvants; aluminium hydroxide gel and montanide ISA 71.

MATERIALS AND METHODS

Bacterial strains

Salmonella Typhimurium and *Salmonella* Enteritidis

These two strains are local field isolates kindly obtained from Department of Bacterial Sera and Antigens, Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo, Egypt. These strains were used for preparation of vaccines under test.

Avibacterium paragallinarum

The reference strains *Avibacterium paragallinarum* strain W (serovar A-1) and Modesto strain (serovar C-2) were obtained from MSD Animal Health/Intervet International bv., Boxmeer, The Netherlands; and reference strain 0222 (serovar B-1) was obtained from Dr. R.B. Rimler, USDA National Animal Disease Center, Ames, Iowa, USA. Local field strain (A) has been originally isolated by Anaerobic Vaccines Research Department, VSVRI from an outbreak of Infectious Coryza in a laying flock in Egypt, confirmed using species level and serotype using serological tests with standard antisera against reference serovars.

Experimental birds

SPF one day old chicks. Forty chicks were used for safety testing of the prepared vaccines.

SPF white Lohman layer chickens. A total number of 150, six weeks old SPF white Lohman layer chickens were obtained from SPF Farm at Koom Osheem Fayoum province, Egypt. They were housed in batteries with the network floor. All birds were ascertained first to be free from *Salmonella* and coryza (organism and antibodies). They were fed on free balanced rations, and used for evaluation of prepared vaccines.

Vaccine preparation

Two combined vaccines were prepared according to Blackall et al. (1992) and Charles et al. (1994). Briefly, ST and SE were cultured on specific media. Equal volumes of each culture (adjusted to contain 1×10^8 CFU/ml) were mixed together and inactivated by adding 0.5% Formalin. Also cultures of *Avibacterium paragallinarum* serovars A, B and C were prepared (adjusted to contain 1×10^6 CFU/ml) and equal volume of each serotype were mixed and inactivated by adding 0.5% Formalin and 0.01% (w/v) of thimerosal was added as a preservative agent. Then the above cultures were combined together and divided into 2 parts; one part adjuvanted with 20% (v/v) aluminium hydroxide gel and the other part with Montanide ISA-71 (30:70 v/v).

Experimental design

A total of 150, six weeks old SPF white Lohman layer chickens were divided into three groups 50 chicks per each. Group 1 contained fifty chickens were vaccinated with the prepared combined aluminium hydroxide gel vaccine in a dose of 0.5 ml S/C. Group 2 contained fifty chickens were vaccinated with the prepared combined montanide ISA71 vaccine with dose of 0.5 ml S/C. Group 3 contained fifty chickens injected 0.5 ml S/C with normal saline, left as a control group.

Birds in group (1) and group (2) were boosted with the same vaccine (by the same route and dose) 3 weeks after first immunization. Serum samples were obtained regularly before immunization, weekly for 3 weeks after the 1st vaccination and every 2 weeks post boosting for 22 weeks. Then pooled and stored at -20 °C till used for following up the induced antibodies.

Quality control testing of the prepared experimental vaccines

Sterility test. The prepared vaccines were tested to be free from any external contaminant (aerobic and anaerobic bacteria, fungus and mycoplasma) according to OIE (2016).

Safety test. Safety of the prepared vaccines was monitored through the injection of a double field dose (1 ml) of the vaccine subcutaneously in each of 20 one day old SPF chicks. The chicks were observed daily for two weeks for any signs of local reactions, clinical signs or deaths.

Determination of immune response to the prepared vaccines Serological evaluation of humeral immune response of the vaccinated chickens against *Salmonella* Typhimurium and *Salmonella* Enteritidis

Micro-agglutination test (MAT)

Antibody titer in vaccinated and unvaccinated chickens was followed up on regular intervals post vaccination applying Micro-agglutination test (MAT) using sonicated antigen, according to the method described by Thaxton et al. (1970) and Brown et al. (1981).

ELISA

The developed humoral immune response against ST and SE in the vaccinated chickens was measured by ELISA in the sera using *Salmonella* antibody test kit (BioChek Poultry Immunoassays cat # CK117 for *S. enteritidis* and CK118 for *S. typhimurium*) according to Haider et al. (2007). Calculation of the antibody titers in ELISA were performed in relation to S/P ratio according to the following formulae:

$$\text{S/P ratio} = \frac{\text{Sample mean} - \text{Negative control}}{\text{Positive control} - \text{Negative control}}$$

Calculation of Antibody Titer $\text{Log}_{10} \text{Titer} = 1.13(\text{Log}_{\text{S/P}}) + 3.156$.

Antibody titer = AntiLog

Serological evaluation of humeral immune response of the vaccinated chickens against *Avibacterium paragallinarum* serovars A, B, and C

Haemagglutination inhibition test

Antibody response in vaccinated and unvaccinated chickens was followed up on regular intervals post vaccination applying Haemagglutination Inhibition (HI) test using sonicated antigen, according to the method described by Blackall et al. (1990).

Enzyme-Linked Immunosorbent Assay (ELISA)

It was done according to Ryuichi et al. (2012) for *Avibacterium paragallinarum* serovars (A, B, and C). Optical Density (OD) was measured at 490 nm by using a micro plate reader (DYANA Tech., USA). The S/P ratio was calculated and expressed as ELISA titer.

$$\text{S/P ratio} = \frac{\text{Sample mean} - \text{Negative control}}{\text{Positive control} - \text{Negative control}}$$

Efficacy test (Challenge)

Challenge by *Avibacterium paragallinarum* serovars A, B and C

All challenge was done by intra sinus inoculation with 0.1 ml overnight broth culture of *Avibacterium paragallinarum* serovars A, B and C challenge dose containing 1×10^6 CFU/ml. Clinical signs of Infectious Coryza were recorded from day-1 to day-7 after inoculation. The presences of any nasal discharge and facial edema in challenged chickens were recorded. A protected chicken was defined as a chicken that had shown no clinical signs.

Challenge by *Salmonella Typhimurium* and *Salmonella Enteritidis* strains

Via administrating the vaccinated chickens 3 weeks post boosting dose by a dose of 1 ml virulent ST and SE broth culture containing 1×10^8 CFU /ml (OIE, 2016).

Fecal shedding

Shedding of *Salmonella* was detected in the fecal samples collected from challenged vaccinated and non-vaccinated chicks up to 4 weeks post challenge.

Statistical analysis

The level of protection present in the vaccinated groups were analyzed and compared with parametrical correlation using Student's T test (significant difference at $P < 0.05$) (Snedecor and Cochran, 1980).

Ethical approval

All animal procedures were approved by the Animal Ethics Committee at Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo, Egypt.

RESULTS

Safety and sterility of prepared vaccines

Both of two vaccines were found to be safe and sterile.

Humeral immune response of the vaccinated chickens against *Salmonella Typhimurium* and *Salmonella Enteritidis*

Table 1 and 2 illustrated results of MAT and ELISA which are parallel to each other as the antibody titers started rising 2 weeks post first vaccination and reached peak sixth week post boosting. It was clear that MAT and ELISA titer for combined montanide ISA 71 vaccine was higher or double the titer of combined aluminium hydroxide gel vaccine for both antigens. The obtained results shown in tables 1 and 2 were analyzed statistically using Student's T test and it was found that there is a significant difference at $P \leq 0.05$ between group 2 (vaccinated with combined montanide ISA71 vaccine) and group 1 (vaccinated with combined aluminium hydroxide gel vaccine).

Humeral immune response of the vaccinated chickens against *Avibacterium paragallinarum* serovars A, B, and C

Results of Haem-agglutination Inhibition (HI) test and ELISA as shown in table 3 and 4 were in accordance to those of *Salmonella* organisms of both vaccines. As antibody titers start raising two weeks post first vaccination and reached peak six weeks post boosting. The obtained results in tables (3 and 4) were analyzed statistically using Student's T test and it was found that

there is a significant difference at $P \geq 0.05$ between group 2 (vaccinated with *combined* montanide ISA71 vaccine) and group 1(vaccinated with combined aluminium hydroxide gel vaccine).

Concerning ELISA titers for *Avibacterium paragallinarum* serovars (A and C) in both vaccines as shown in table 4, we paralleled with that of HI, also there was a statistically significant difference in ELISA titer between both vaccines.

Table 1. Measurement of antibody against *Salmonella* Typhimurium and Enteritidis in sera of vaccinated and unvaccinated layer chickens using microagglutination test.

Weeks post vaccination	Group (1)		Group (2)*		Control
	Serovar Typhimurium	Serovar Enteritidis	Serovar Typhimurium	Serovar Enteritidis	Serovar Typhimurium and Enteritidis
0	0	0	0	0	0
2WPV	40	40	40	40	0
3WPV	40	40	80	80	0
Boostering					
2 WPB	80	80	160	80	0
4WPB	160	160	320	320	0
6WPB	320	320	640	640	0
8WPC	320	320	320	320	0
10WPC	320	320	320	320	0
12WPC	160	160	320	320	0
14WPC	160	160	160	160	0
16WPC	80	80	160	160	0
18WPC	80	80	80	80	0
20WPC	40	40	40	80	0
22WPC	20	20	20	40	0

Group (1): SPF layer chickens vaccinated with combined aluminium hydroxide gel vaccine; Group (2): SPF layer chickens vaccinated with combined montanide ISA71 vaccine; Control: Unvaccinated group; WPV: Weeks post vaccination; WPB: Weeks post boosting; WPC: weeks post challenge; * Significant at $P < 0.05$; The antibody titer in MAT was expressed as Geometric Mean Titer (GMT)

Table 2. Measurement of antibody against *Salmonella* Typhimurium and Enteritidis in sera of vaccinated and unvaccinated layer chickens using ELISA

Weeks post vaccination	Group (1)		Group (2)*		Control
	Serovar Typhimurium	Serovar Enteritidis	Serovar Typhimurium	Serovar Enteritidis	Serovar Typhimurium and Enteritidis
0	93	100	93	100	100
2WPV	975	850	1530	1443	112
3WPV	1453	1413	2553	2721	111
Boostering					
2WPB	2189	2189	3517	3617	128
4WPB	2344	2544	3782	3982	123
6WPB	2763	2791	4543	4484	130
8WPV	2675	2547	3925	3855	143
10WPC	2320	2250	3845	3745	135
12WPC	2230	2130	3667	3686	156
14WPC	1970	1940	3253	3354	122
16WPC	1515	1465	2180	2370	129
18WPC	1325	1298	2020	2120	125
20WPC	1250	1110	1890	1970	123
22WPC	1140	1020	1680	1730	128

Group (1): SPF layer chickens vaccinated with combined aluminium hydroxide gel vaccine; Group (2): SPF layer chickens vaccinated with combined montanide ISA71 vaccine; Control: Unvaccinated group; WPV: Weeks post vaccination; WPB: Weeks post boosting; WPC: weeks post challenge; * Significant at $P < 0.05$

Table 3. Geometric mean of Haem-agglutinating Titer against *Avibacterium paragallinarum* serovars A and C in sera of vaccinated layer chickens.

Weeks post vaccination	Group (1)		Group (2)*		Control
	Serovar A	Serovar C	Serovar A	Serovar C	Serovar A and C
0	0	0	0	0	0
2WPV	40.32	40.31	28.50	32	0
3 WPV	40.23	40.31	35.78	43.11	0
Boostering					
2WPB	40.8	57.01	57.01	80.63	0
4WPB	50.79	57.01	71.83	90.50	0
6WPB	57.01	71.83	101.59	114.04	0
8WPB	57.01	71.83	101.59	114.04	0
10WPB	57.01	71.83	101.59	114.04	0
12WPB	50.79	57.01	90.50	101.59	0
14WPB	40.34	57.01	90.50	101.59	0
16WPB	40.87	50.79	90.5	101.59	0
18WPB	35.91	50.79	80.63	90.50	0
20WPB	28.50	40.31	80.63	90.50	0
22WPB	28.50	40.31	80.63	90.50	0

Group (1): SPF layer chickens vaccinated with combined aluminium hydroxide gel vaccine; Group (2): SPF layer chickens vaccinated with combined montanide ISA71 vaccine; Control: Unvaccinated group; WPV: Weeks Post Vaccination; WPB: Weeks Post Boostering; *Significant at P < 0.05

Table 4. ELISA results (S/P ratio) of vaccinated and unvaccinated layer chickens against *Avibacterium paragallinarum* serovars A and C.

Weeks post vaccination	Group (1)		Group (2)*		Control
	Serovar A	Serovar C	Serovar A	Serovar C	Serovar A and C
0	0.031	0.023	0.044	0.021	0.002
2WPV	1.304	1.474	1.292	1.344	0.011
3 WPV	1.388	1.476	1.549	1.598	0.233
Boostering					
2WPB	1.454	1.455	1.936	1.942	0.043
4WPB	1.474	1.519	1.975	1.936	0.022
6WPB	2.190	2.274	2.095	2.011	0.056
8WPB	2.130	2.235	2.164	2.274	0.044
10WPB	2.091	2.064	2.278	2.274	0.070
12WPB	1.782	1.940	2.087	2.164	0.033
14WPB	1.566	1.885	2.011	2.036	0.056
16WPB	1.431	1.850	1.975	2.011	0.099
18WPB	1.519	1.770	1.907	1.942	0.043
20WPB	1.472	1.549	1.869	1.936	0.065
22WPB	1.199	1.454	1.848	1.907	0.023

Group (1): SPF layer chickens vaccinated with combined aluminium hydroxide gel vaccine; Group (2): SPF layer chickens vaccinated with combined montanide ISA71 vaccine; Control: Unvaccinated group; WPV: Weeks Post Vaccination; WPB: Weeks Post Boostering; *Significant at P < 0.05

Results of Challenge test

As shown in tables 5 and 6, the protection rates in chickens vaccinated either with combined aluminium hydroxide gel vaccine or combined montanide ISA71 vaccine were 80% and 85% for *Salmonella* organisms. On the other hand it was (80%, 90% and 70%) for combined aluminium hydroxide gel vaccine and (90%, 100% and

90%) for combined montanide ISA71 vaccine against *Avibacterium paragallinarum* serovars A, B, and C. Meanwhile, the protection rate was 15% against *Salmonella* Typhimurium and *Salmonella* Enteritidis and 0% against infectious coryza among the unvaccinated chicken group.

Fecal shedding of *Salmonella* Typhimurium and *Salmonella* Enteritidis from challenged chickens

Fecal shedding of *Salmonella* Typhimurium and *Salmonella* Enteritidis as shown in table (7), from chickens vaccinated with either combined aluminium hydroxide gel vaccine or combined montanide ISA71

vaccine in the 1st, 2nd and 3rd weeks post challenge were (25%, 12.5% and 12.5%) and (22.22%, 11.11% and 0%) respectively while in the 4th week the fecal shedding disappeared. Regarding the control unvaccinated birds the fecal shedding were 66.66%, 66.66%, 33.33% and 33.33% in the 1st, 2nd, 3rd and 4th weeks post challenge respectively.

Table 5. Protective Efficacy of combined vaccine against salmonellosis in SPF chickens challenged with virulent strains

VACCINE	Serovar	No. of inoculated chickens#	Survived chickens	Protection %
Group (1)	Typhimurium	10	8	80
	Enteritidis	10	8	80
Group (2)	Typhimurium	10	8	80
	Enteritidis	10	9	90
Control	Typhimurium	10	1	10
	Enteritidis	10	2	20

*Protection % = (Survival birds/ total number of birds) x 100; Group (1): SPF layer chickens vaccinated with combined aluminium hydroxide gel vaccine; Group (2): SPF layer chickens vaccinated with combined montanide ISA71 vaccine;# Challenge with virulent *Salmonella* Typhimurium and *Salmonella* Enteritidis; Control: Unvaccinated group.

Table 6. Protective Efficacy of combined vaccine against infectious coryza in SPF chickens challenged by *Avibacterium paragallinarum* serovars A, B, and C

VACCINE	serovar	No. of inoculated chickens#	Survived chickens	Protection %
Group (1)	A	10	8	80
	B	10	9	90
	C	10	7	70
Group (2)	A	10	9	90
	B	10	10	100
	C	10	9	90
Control	A	10	0	0
	B	10	0	0
	C	10	0	0

Group (1): SPF layer chickens vaccinated with combined aluminium hydroxide gel vaccine; Group (2): SPF layer chickens vaccinated with combined montanide ISA71 vaccine; # Challenge with virulent *Avibacterium paragallinarum* serovars A, B, and C; Control: Unvaccinated group.

Table 7. Results of fecal shedding of *Salmonella* Typhimurium and *Salmonella* Enteritidis from layer chickens after challenge

Chicken groups	No. of birds positive for isolation / total No. of living birds			
	1 st week	2 nd week	3 rd week	4 th week
Group (1)	2/8 (25%)	1/8 (12.5%)	1/8 (12.5%)	0/8 (0%)
Group (2)	2/9 (22.22%)	1/9 (11.11%)	0/9 (0%)	0/9 (0%)
Control	2/3 (66.66%)	2/3 (66.66%)	1/3 (33.33%)	1/3 (33.33%)

Group (1): SPF layer chickens vaccinated with combined aluminium hydroxide gel vaccine; Group (2): SPF layer chickens vaccinated with combined montanide ISA71 vaccine; Control: Unvaccinated group.

DISCUSSION

Avian salmonellosis is a large group of acute and chronic diseases of poultry caused by any one or more member of genus *Salmonella*. However, particular *Salmonella* Enteritidis is the most prevalent one followed by *Salmonella* Typhimurium (Capita et al., 2003).

Infectious coryza is an acute respiratory disease of chickens caused by the bacterium *Avibacterium paragallinarum*. The greatest economic losses associated with infectious coryza results from poor growth performance in growing birds and marked reduction (10-40%) in egg production in layers (Blackall and Matsumoto, 2003).

Charoenvisal et al. (2017) examined efficacy of four commercial Infectious Coryza vaccines available in Thailand for protection rate against Thai field isolates serovar A, B, and C. The study revealed that the protection rate of Infectious Coryza vaccines depended on the strains isolated from each country.

So in this study combined vaccines have the advantage of protection against more than one disease at the same time, beside, reducing vaccination expenses, number of vaccination performed and saving time. The efficacy of a prepared combined vaccine against salmonellosis and infectious coryza using two different adjuvants; aluminium hydroxide gel and montanide ISA 71 was monitored in sera of vaccinated chickens using HI, MAT and ELISA. It was clear that antibody titers in sera of chickens for all tests were paralleled to each other in starting and increasing titer and also after boosting as illustrated in tables 1, 2, 3, 4 and 5 which agree with that obtained by Akeila et al. (2014). Who evaluated a combined vaccine against *A. paragallinarum* and *S. Enteritidis* and found that antibody titers reached the maximum levels at the 6th WPV in the vaccinated groups.

With serovar B vaccines, a HI test was not done as it is based on a hyaluronidase-treated antigen and formaldehyde-treated RBC and gave only very low HI titers following vaccination (as compared with serovar A or C vaccines) but the vaccinated birds were significantly protected against homologous challenge, These results correlate with other studies done by Yamaguchi et al. (1991).

The protection rates against *Salmonella* Typhimurium and Enteritidis as measured by challenge test were 80% and 85% in chickens vaccinated with combined aluminium hydroxide gel vaccine and combined montanide ISA71 vaccine are respectively, as shown in table 5.

Also the protection rates against *Avibacterium paragallinarum* serovars A, B, and C were 80%, 90% and 70% in chickens vaccinated with combined aluminium hydroxide gel vaccine and were 90%, 100% and 90% of the montanide ISA71 vaccine respectively (Table 6).

Meanwhile, the protection rate was 15% against *Salmonella* Typhimurium and Enteritidis and 0% against infectious coryza among the unvaccinated chicken group and these results agreed with by Akeila et al. (2014) who reported 73.3% and 93.3% protection rate against *S. Enteritidis* and *A. paragallinarum*, respectively in a combined vaccine containing both organisms.

The fecal shedding of *Salmonella* Typhimurium and Enteritidis in the 1st, 2nd and 3rd weeks post challenge in chickens vaccinated with combined aluminium hydroxide gel vaccine was 25%, 12.5% and 12.5% , while it was 22.22%, 11.11% and 0% for those vaccinated only with montanide ISA71 vaccine, respectively. The fecal shedding disappeared in the 4th week.

Regarding the control unvaccinated birds the fecal shedding were 66.66%, 66.66%, 33.33% and 33.33% in the 1st, 2nd, 3rd and 4th weeks post challenge and these result agreed with Nourhan et al. (2015) who found that fecal shedding of *Salmonella* organisms in vaccinated group of chickens with *S. Kentucky* reached 8.33% while the unvaccinated control group at 3 week post challenge revealed fecal shedding of 25 %. No shedding was detected at the fourth week post challenge in the vaccinated group, while there was 16.6% shedding in control unvaccinated group.

So, the SPF layer chickens vaccinated with combined vaccine against salmonellosis and infectious coryza adjuvanted with montanide ISA71 gave high immune response and protection which is capable of improving vaccine efficacy via the induction of a strong and long lasting immunity. Also it is an excellent adjuvant stimulating humoral and cellular responses. This product is recommended for producing a potent vaccine able to protect layer chickens against salmonellosis and infectious coryza.

CONCLUSION

From the above results it could be concluded that producing a vaccine from locally isolated *Salmonella* and *Avibacterium* (Haemophilus) *paragallinarum* strains adjuvanted with montanide ISA71 is recommended to aid in controlling avian salmonellosis and infectious coryza at the same time.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

All authors participated in making the design, performing the experiment, analyses of the data, and writing the paper.

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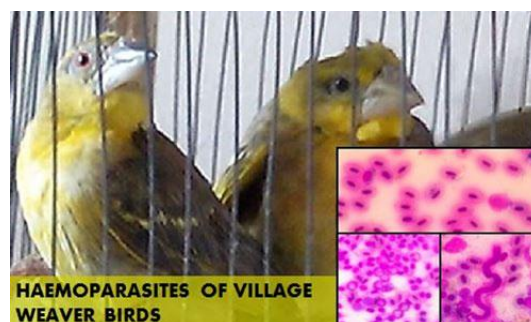
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Abayomi (2000), Agindotan et al. (2003), Vahdatpour and Babazadeh (2016), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; Chukwura, 1987a,b; Tijani, 1993, 1995), (Kumasi et al., 2001).

--Examples (at References section):

a) For journal:

Lucy MC (2000). Regulation of ovarian follicular growth by somatotropin and insulin- like growth factors in cattle. *Journal of Dairy Science*, 83: 1635-1647.

Kareem SK (2001). Response of albino rats to dietary level of mango cake. *Journal of Agricultural Research and Development*. pp 31-38. DOI:XXX.

Chikere CB, Omoni VT and Chikere BO (2008). Distribution of potential nosocomial pathogens in a hospital environment. *African Journal of Biotechnology*, 7: 3535-3539. DOI:XXX.

Tahir Khan M, Bhutto ZA, Abbas Raza SH, Saeed M, Arain MA, Arif M, Fazlani SA, Ishfaq M, Siyal FA, Jalili M et al. (2016). Supplementation of different level of deep stacked broiler litter as a source of total mixed ration on digestibility in sheep and their effects on growth performance. *Journal of World`s Poultry Research*, 6(2): 73-83. DOI: XXX

b) For symposia reports and abstracts:

Cruz EM, Almatar S, Aludul EK and Al-Yaout A (2000). Preliminary Studies on the Performance and Feeding Behaviour of Silver Pomfret (*Pampus argentens euphrasen*) Fingerlings fed with Commercial Feed and Reared in Fibreglass Tanks. *Asian Fisheries Society Manila*, Philippine 13: 191-199.

c) For edited symposia, special issues, etc., published in a journal:

Korevaar H (1992). The nitrogen balance on intensive Dutch dairy farms: a review. In: A. A. Jongebreur et al. (Editors), *Effects of Cattle and Pig Production Systems on the Environment: Livestock Production Science*, 31: 17-27.

d) For books:

AOAC (1990). *Association of Official Analytical Chemists. Official Methods of Analysis*, 15th Edition. Washington D.C. pp. 69-88.

Pelczar JR, Harley JP, Klein DA (1993). *Microbiology: Concepts and Applications*. McGraw-Hill Inc., New York, pp. 591-603.

e) Books, containing sections written by different authors:

Kunev M (1979). Pig Fattening. In: A. Alexiev (Editor), *Farm Animal Feeding*. Vol. III. Feeding of Different Animal Species, Zemizdat, Sofia, p. 233-243 (Bg). In referring to a personal communication the two words are followed by the year, e.g. (Brown, J. M., personal communication, 1982). In this case initials are given in the text.

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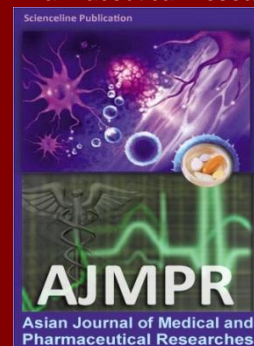
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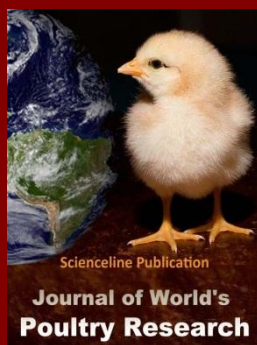
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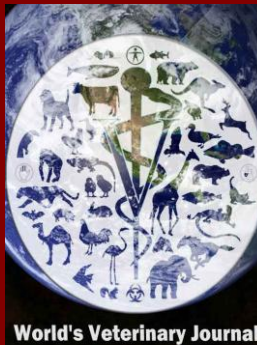
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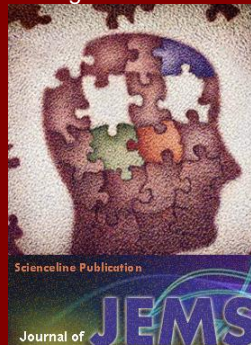
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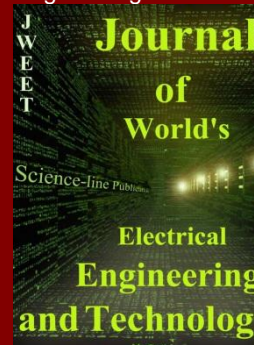
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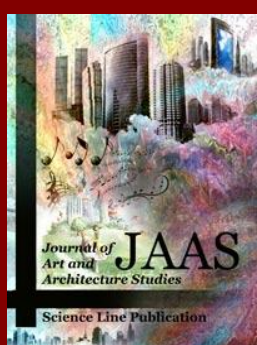
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