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Effect of feeding groundnut haulm based complete rations on production performance of cross bred dairy cows

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Keywords: Dairy Cows
Groundnut Haulm Based Complete Ration
Conventional Ration
Milk Yield
Butter Fat.

Abstract

Dairy animal rearing is an integral part of small holder farming system in India. The traditional feeding system for dairy cattle is based on the use of straw, natural grasses supplemented with a little or no concentrates. Poor nutrition has been identified as a major constraint to productivity in smallholder dairy farming. In the present situation pastures are degraded and the area under green forage crops is shrinking due to increase in human population and urbanization. As a result the crop residues are the bulk of feeds available. They have low nutritional value, bulky and fibrous. Feeding crop residue based total mixed rations is the efficient method of utilization of crop residues and to minimize the wastage. A field trail was conducted to study the groundnut haulm based complete ration on production performance of Dairy cattle. Feeding trail was conducted for 90 days on 20 milch cows, randomly divided in to two groups; control and treatment with ten animals in each group. The control group were offered conventional ration consisting of 8 hours of grazing, green forage, paddy straw and concentrate mixture and the treatment group were fed on groundnut haulm based complete ration consisting of crushed groundnut haulm and concentrate in 60:40 ratio. The dry matter intake, average milk yield (kg/day), 4% FCM (kg/day), milk fat content were increased significantly (Pd"0.01) in the treatment group. Dry matter intake and feed cost per kg of 4% FCM did not differ significantly between two groups but were lower in the control group as compared to treatment group. Net profit over feed cost was higher in treatment group (Rs.56.02), compared to control group (Rs.41.04).

Introduction

India has a large population of 210 million cattle and 111 million buffaloes (FAO, 2012). Dairy cattle production in the country is characterized by low productivity levels mainly due to genetic and nutritional constraints. Quantitative and qualitative shortage of feeds and fodder affects the performance of milking animal. Unless feeding management is improved these animals may be limited to fully express their potential genetic superiority. It is fundamental approach to provide good quality diets to dairy cattle in sufficient amount to maximize production. Since feed cost is becoming the most important factor in livestock production, increasing self sufficiency in feed production will be an important factor in future development programs. It is estimated that approximately 500-550 Mt of crop residues are produced per year in the country (MoA, 2012). The quantity and quality of fodder available from natural pasture shows seasonal fluctuation. There is an acute shortage of feed supply during the dry season and the available feed during this period is of very poor quality. Cultivated fodder is limited to less than 4.5 per cent of the area under cultivation in country. Present area under fodder crops in India is around 8.6 million hectare. Effective utilization of the available feed resources (agricultural and agro-industrial byproducts, natural pastures and browse) and appropriate supplementation of poor quality natural pasture and crop residue based diets appear

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to be the necessary steps to alleviate the nutritional problems of dairy animals. Worldwide total crop residues is estimated to be 3.8 billion metric tons with cereals contributing 74%, sugar crops 10%, legumes 8%, tubers 5% and oil crops 3% (Lal, 2005). Crop residues will provide more than 70% of the feed resources for Indian livestock by the year 2020 (Ramachandra *et al*.,2007). These crop residues have special importance in livestock feeding as they constitute a major portion of roughages. High percentage of structural carbohydrate and low nitrogen content of these roughages result in low palatability and poor nutrient utilization in ruminants. However, incorporation of these crop residues in complete diets improves both palatability and nutrient utilization (Dhuria *et al*., 2011). The complete feed system not only ensures better utilization of nutrients from agricultural crop residues but also supplies balanced nutrients, controls the ratio of roughage to concentrate, provide uniform blend of feed, reduces feed wastage and enables use of locally available feed ingredients (Raut *et al*., 2002; Krishnamurthy and Ramaprasad, 2005).

Materials and Methods

A field trail was conducted on 20 cross bred milch cows belonging to the farmers of Palamaner village in Chittoor district of Andhra Pradesh, India for 90 days. Cows in first two lactations in early lactation were selected and were randomly divided in to two groups with 10 cows in each group. The animals in control group were offered a conventional ration consists of 8 hours of grazing, 10 kg green fodder, 4 kg paddy straw and 3 kg concentrate mixture and the animals in the treatment group were fed with complete diet made up of crushed ground nut haulms and concentrate in 60:40 proportions according to their nutrient requirements (ICAR, 1998).

The animals were housed in well ventilated conventional stalls and fed respective diets throughout the experimental period with individual feeding and watering arrangements. Daily milk yield was calculated by adding the total milk produced in the two milkings per day. 4% fat corrected milk yield was calculated (NRC, 2001)

Daily milk yield and feed intake were recorded for each animal. Milk samples were estimated every forte night for butter fat and solids not fat. Data were analyzed by standard statistical procedures (Snedecor and Cochran, 1989).

Results and Discussion

Dry matter intake

Dry matter intake per day differed significantly ($P \geq 0.01$). Dry matter intake per day was 7.97 kg, 8.16 kg in control group and treatment group respectively. DMI was within the range of ICAR 1998 standards, indicating the good palatability of the complete ration which is in agreement of the findings of Rajakishore *et al*, 2013. Feed efficiency per kg 4% FCM was better in the treatment group (1.42± 0.028), but it was not significant.

Milk yield

The average milk yield and 41% fat corrected milk per day were shown in the table. The milk yield/day and 4% FCM were higher ($P \geq 0.01$) in the treatment group (5.84±0.12, 5.87± 0.13) as compared to control group (5.39± 0.06, 5.30±0.07). The raise in the milk yield might be due to the better utilization of the feed nutrients. Higher digestibility in complete rations were also observed by Reddy *et al*. (2001) in buffalo bulls, Mahender *et al*. (2006) in Nellore lambs and Kumar *et al*. (2010) in lactating Murrah buffaloes. Babiker *et al* (2009) reported that the sugarcane bagasse based complete diet pellets improved the FCR and attributed to the absence of selective feeding by lambs. Talpada *et al*., 2002 hypothesized that complete feeds provide uniform supply of nutrients at regular interval which helps to maintain steady rumen environment resulting in better digestibility of nutrients.

Milk composition

% Milk fat increased ($P \geq 0.01$) in the group fed with ground nut haulm based complete ration when compared to those on conventional ration. Solids not fat (SNF) did not differ significantly between two groups. Higher milk fat in the group fed with complete diets were also observed by Nagalakshmi *et al*., (2006) and Raja Kishore *et al*., (2013). This could be due to the better availability of the precursors for synthesis of milk fat.

Cost economics

Feed cost per day per animal, cost of feed per kg 4% FCM were more in the control group (Table-2). Income from milk (10%) and net profit over feed cost (36%) were higher in the treatment group. It was similar to the findings of Raja Kishore *et al*., (2013) who reported that crop residue based complete rations to cattle and buffaloes reduced the cost of feeding animals for milk production.

Table 1: Milk yield and composition of milk of crossbred cattle fed on complete rations

Parameter	Control	Treatment
Milk Yield (kg/d)**	5.3928±.06736	5.8399+ .12455
Butter Fat %**	3.8920±.03055	4.0265+ .04871
4 % FCM (kg/d)**	5.3092± .07685	5.8700+ .13789
SNF (%)NS	8.1863+.04290	8.2915+.03679

Table 2: Feed intake, feed efficiency and cost economics

Parameter	Control	Treatment
DMI (Kg) per day**	7.9750±.05993	8.1667±.08946
4 % FCM (kg/d)**	5.3092± .07685	5.8700±.13789
DMI(Kg) per Kg 4% FCM^{NS}	1.5160±.02144	1.4253±.02855
Cost of feed (Rs.) per day**	91.7125±.68915	90.7317±.99394
Cost of feed (Rs.) per Kg 4% FCM^{NS}	17.4458±.24914	15.8343±.31707
Income from milk (Rs.)**± (@ 25/Kg 4% FCM)	132.75±1.92009	146.75±3.44622
Net benefit over feed cost (Rs.)	41.04±1.80939	56.02±2.76802

** ($P \geq 0.01$)
NS : non-significant

Conclusion

It can be concluded that groundnut haulm based complete rations improve milk yield, butter fat and reduce the cost of milk production with 36% net profit over conventional ration.

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Effect of supplementation of *lactobacillus* and *Saccharomyces boulardii* on the performance of broilers

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Keywords: Broilers
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Performance
Lactobacillus
Saccharomyces Boulardii.

Abstract

A trial was conducted to evaluate the effect of *Lactobacillus* and *Saccharomyces Boulardii* on performance of broilers. A total of 300 day old broilers were randomized in 5 groups, each with 6 replicates with (5x6) 10 birds per replicate. Basal diet (D1) (control) was prepared as per BIS specification. In that of *Lactobacillus* was added @ 100 and 200g/t in D2 and D3 ; *Saccharomyces Boulardii(Sb)* was added @ 500 and 1000g/t of feed respectively up to 42 days of age. The diets were iso nitrogenous and iso caloric. The results revealed, a significant ($P<0.05$) improvement in body weight gain, better FCR with increased concentrations of *Lactobacillus* (D3) and *Saccharomyces Boulardii* (D5) in feed. The results suggest that supplementation of *Lactobacillus* and *Saccharomyces Boulardii* @ 200g/t and 1000g/t of feed respectively improved the performance of the broilers by increasing the absorption capacity of the gut.

Introduction

Pathogenic bacteria are always present in the gut but the balance between pathogenic and beneficial bacteria determines whether disease will occur or not (Ivanov 2003). Mainintaing a healthy balance between all microflora with in the gut is known as Eubiosis (Jensen, 1980) and can be influenced by bacteria endemic to the micro flora. To reduce the pathogenic bacteria and to enhance growth, to minimize the disease prevalence using of antibiotic growth promoters in broiler ration is an age old practice. With increasing public concerns there is a worldwide attempt to reduce antibiotic use in Poultry production (Dibner and Richards, 2005). A convincing alternative of antibiotic has been the use of probiotics as a sub therapeutic and growth promoting agent (Yang et al., 2009).

This study was designed to study the effects of Lactic acid producing bacteria and *Saccharomyces Boulardii* i yeast on broiler performance and intestinal morphology.

Material and Methods

Commercial day old vencobb broiler chicks (n=300) were wing banded, weighed individually and randomly assigned to 5 treatments on the basis of initial body weight in a randomized complete block design. Each treatment had 60 broilers arranged in 6 replicates of 10 chicks each. The broilers were reared for a period of six weeks in battery brooders with provision of continuous lighting throughout the experimental period. The temperature was maintained at 34±1ÚC up to 7 days of the age and gradually reduced to 26±1ÚC by 21 days of age after which, chicks were maintained at room temperature. On first day chicks were offered only crushed maize and then given commercial diet from 2nd day onwards along with ad-libitum drinking water. All the birds were kept under uniform management conditions throughout the experimental period. The birds were vaccinated against Mareks disease, New castle disease and Infectious bursal disease as per the routine vaccination schedule and Dose.

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Experimental design: basal diets (D1) were prepared for both starter (0-4 weeks) and finisher (5-6 weeks) satisfying the nutrient requirement (BIS, 1992). Lactic acid producing bacteria at 100g and 200g/ton in D2 and D3 , *Saccharomyces Boulardii* strain @ 6x 10^9 /gram at 500 and 1000g/ton in D4 and D5 were supplemented to the basal diet and test diets were prepared. Representative feed samples were ground well by passing 1 mm sieve and proximate principles, calcium and phosphorus were analyzed as per AOAC (2000).

Birds were weighed at weekly interval. Body weight and feed consumption were recorded at weekly interval up to 6th week. Feed conversion ratio (FCR) was calculated as the feed consumed per kg body weight gain.

Statistical analysis

The data was analyzed using general linear model procedure of statistical Package for social sciences (SPSS)15th version and comparison of means was done using Duncan's multiple range test (Duncan, 1955) and significance was considered at P≤0.05.

Results and Discussion

The impact of *Lactobacillus* and *Saccharomyces Boulardii* supplementation on body weight, feed intake, feed conversion ratio and gut morphology on broiler chicks are shown in Table 1, 2, 3 and 4 respectively.

Significantly higher body weights were noted in groups supplemented with higher levels of *lactobacillus* (D3) and *S.boulardii* (D5). In pre starter and starter phases no significant (P≥0.05) variation in body weights, where as significantly (P≤0.05) higher but were observed in finisher phase. These were on par with Kumar *et al.,* 2013 who reported higher body weight in birds fed with diet supplemented with *Lactiflora* alone (@ 0.05% or in combination with *Sacchromyces cervacae* (@ 0.05% supplemented groups than control. Similarly improvement in the growth performance and nutrient retention due to probiotics (*Lactobacillus*) supplementation reported by Panda *et al.*, 2006 and Talebi *et al.,*2008. Whereas improvement in growth performances and nutrient retention due to supplementation of *Saccharomyces* was confirmed by Kumpretchtova *et al.*, 2000 and Zhang *et al.,* 2005. However recently Khaksefidi and Rahimi 2005, Singh *et al.,* 2009, Chae *et al.*, 2012 reported that improvement in the performance in broiler chickens by supplementation of probiotics (*Lactobacillus, Saccaaromyces and Streptococcus*) in diets.

Feed intake was significantly (P≤0.05) less in *Lactobacillus* and *Saccaaromyces* supplemented groups up to 3 weeks of age. Later (4 to 6 weeks of age) there was increase in feed intake with increase in *Lactobacillus* and *Saccaaromyces* in diet. However numerically lower (P≥0.05) feed intake values was observed than control on cumulative basis.

Alkhalf *et al.*, 2010; Rajput *et al.*, 2013 reported significant increase in live body weight of broilers in Probiotics (*lactobacillus and S.boulardii*) supplemented groups than control without any significant variation in feed intake.

Feed conversion ratio was better (P≥0.05) at higher levels of *lactobacillus* and *S.boulardii* supplemented groups than control. This improvement in Body weight and FCR with supplementation of *lactobacillus* and *S.boulardii* might be due to maintenance of beneficial microbial population, improved feed utilization and digestion than altering bacterial metabolism.

Mechanism by which the probiotics improve growth performance include reinforcement of intestinal mucosal integrity by stimulating enzymatic activities, improving epithelial cell integrity, increasing immune response and better utilization of the diet. *lactobacillus* and *S.boulardii* has shown an improvement at the bird performance and decreased the mortality. This improvement may be related with the balanced microbial population in the gastrointestinal tract which has an important role in the health and performance of broilers.

The results of this research are similar to Paryad and Mahmoudi (2008) by incorporation of yeast @ 1.5%. Likely Zhang *et al.,* 2005 reported that yeast culture contains yeast cells as well as metabolites such as peptides, organic acids, oligosaccharides, amino acids, flavor and aroma substances, and possibly some unidentified growth factors, which have been proposed to produce beneficial performance response.

Gut morphology

observations are presented in Table 4. The current findings revealed significant (p<0.05) increase in intestinal villus height, width of villus, goblet cell number with linear increasing the level of Sb in the jejunum and ileum. These were on par with Rajput *et al.,* 2013. Maiorka (2000) and Loddi (2003) reported higher villi in the intestinal mucosa of birds fed diets with monoligosaccharides (MOS) at 7 and 21 days of age respectively.

In contrast to this Santos et al., 2004 reported

that no difference in villus height between the control group and in birds receiving diets containing probiotics based on *Lactobacillus acidophilus* and *Casei, Streptococcus lactis* and *faecium, Bifidobacterium bifidum* and *Aspergillus oryzae* or prebiotics based on MOS.

Pelicano et al. 2005 reported greater cryptal depth (CD) ($P<0.01$) in birds which received Probiotics based on *Bacillus subtilis*, smaller in those diets without additives or with probiotics based in bacterial pool.

This could be attributed to Probiotics enhancing nutrient absorption by increasing the villus height in the small intestine (Zhang *et al.*, 2005; Panda *et al.*, 2006) and thus improve broiler performance. Probiotics compete with the harmful bacteria, change the pH in the gut, prevent infection and modifies mucin biosynthesis and /or degradation , which in turn influences gut function resulting in improved nutrient uptake (Smirnov et al ., 2005).

Increase in the villus height and cryptal depth than control suggests an increased surface area capable of greater absorption of available nutrients (Caspary, 1992). Likewise, greater villus height increases the activity of enzymes secreted from the tip of the villi resulting in improved digestibility (Hampson, 1986). Cell wall components of yeast may provide protective function to mucosa by preventing pathogens from binding to villi and allowing fewer antigens to be in contact with the villi. Taller villi indicate more mature epithelia and enhance absorptive function due to increased absorptive area of the villus.

Table 1: Effect of supplementation of *lactobacillus* and *saccharomyces boulardii* in diet on body weight (g/bird/week) in broilers

Levels	Day old	1st Week	2nd week	3rd week	4th week	5th week	6th week
D1(Control)	47.10	117.8^{a}	229.4	604.8	1039.1	1258.5^{d}	2081^{d}
D2(100g/T)*	47.75	108.5^{b}	232.9	654.3	1067.0	1449.0^{c}	2229^{c}
D3(200g/T)*	47.95	113.5^{ab}	214.5	681.6	1084.5	1516.5^{b}	2390^{ab}
D4(500g/T)#	46.90	110.3^{b}	209.6	626.2	1109.8	1534.6^{b}	2262^{bc}
D5(1000g/T)#	47.05	107.9^{b}	214.2	658.4	1096.4	1601.8^{a}	2400^{a}
Sem	0.37373	0.99394	5.55163	16.03841	27.11783	32.11637	23.54034
N	10	10	10	10	10	10	10
P value	0.874	0.007	0.114	0.187	0.244	0.026	0.067

**Lactobacillus*

Saccharomyces Boulardii

Table 2: Effect of supplementation of *lactobacillus* and *saccharomyces boulardii* through diet on feed intake (g/d) in broilers

Levels	1ST Week	2nd week	3rd week	4th week	5th week	6th week	Cumulative
D1(Control)	90.1^{a}	162.7^{a}	336.2^{a}	477.4^{a}	627.3^{b}	897.0^{c}	2590.7
D2(100g/T)*	85.4^{ab}	157.6^{b}	291.2^{b}	450.2^{ab}	636.0^{b}	976.3^{bc}	2596.6
D3(200g/T)*	88.0^{a}	144.6^{c}	267.4^{c}	470.5^{a}	533.1^{c}	1021.4^{b}	2525.0
D4(500g/T)#	82.1^{b}	147.5^{bc}	314.1^{a}	464.2^{ab}	680.5^{a}	1082.8^{a}	2771.2
D5(1000g/T)#	76.1^{c}	154.3^{b}	263.9^{c}	414.3^{b}	600.6^{bc}	1114.2^{a}	2623.4
SEM	0.867	1.9485	3.348	2.714	2.932	3.483	3.416
N	5	5	5	5	5	5	5
P value	0.006	0.018	0.004	0.041	0.036	0.006	0.067

*Lactobacillus # Saccharomyces Boulardii

Table 3: Effect of supplementation of *lactobacillus* and *saccharomyces boulardii* on feed conversion ratio in broilers

Levels	1ST Week	2nd week	3rd week	4th week	5th week	6th week	Cummulative
D1(Control)	1.307	1.410	2.037	2.386	2.195	2.320	1.942
D2(100g/T)*	1.271	1.478	2.247	2.370	2.203	2.283	1.975
D3(200g/T)*	1.290	1.483	2.362	2.304	2.461	2.340	2.041
D4(500g/T)#	1.343	1.421	2.312	2.389	1.978	2.089	1.922
D5(1000g/T)#	1.418	1.388	2.325	2.298	2.406	2.154	1.998
SEM	0.024	0.028	0.036	0.034	0.051	0.034	0.067
N	5	5	5	5	5	5	5
P VALUE	0.088	0.105	0.837	0.006	0.129	0.009	0.116

*Lactobacillus # Saccharomyces Boulardii

Table 4: Gut morphology of broilers supplemented with different levels of *lactobacillus* and *saccharomyces boulardii* through diet

Parameters (μm)	D1 (Control)	D2 (100g/T)*	D3 (200g/T)*	D4 (500g/T)#	D5 (1000g/T)#	SEM
			Jejunum			
Villus height	441.25	412.69	448.98	468.47	477.63	12.90
Cryptal depth	322.98	329.46	362.54	369.41	388.22	3.77
			Ileum			
Villus height	462.12	485.54	467 .36	502.41	517 .28	13.82
Cryptal depth	337 .65	341.54	358 .80	341.24	352 .71	9.15

**Lactobacillus*

Saccharomyces Boulardii

Conclusion

Beneficial effects were seen in production parameters as well as in histological indexes of the intestinal mucosa with the use of lactobacillus and *S.boulardii* in diets of birds in finisher phases.

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Immunopotentiating action of zinc sulphate in layer chicks

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Micro Heamagglutination Inhibition Test
Phagocytic Index.

Abstract

The present study was undertaken to study the immunomodulatory effect of Zinc Sulphate in layer chicks by monitoring the specific immunological responses, to New Castle Disease and Infectious Bursal Disease Vaccines, Three groups of experimental chickens comprising of 20 chicks were taken in which, first two groups of chicks were inoculated twice with Live Newcastle Disease and Infectious Bursal Disease antigens intra occularly. The chicks of group I served as Vaccinated and II group vaccinated with Zinc Sulphate as supplement while group III experimental birds served as Unvaccinated control. At the termination of experiment, the order of seroconversion to inoculated antigens i.e. New Castle Disease and Infectious Bursal Disease were II>I>III and II>I>III while in Total Protein and Globulin levels, Net body gains and Phagocytic Index were II>I>III respectively. Challenge studies were conducted on experimental chicks employing Virulent New castle Disease Virus and Virulent E.Coli (078 serotype) to study the specific and non- specific immune responses respectively. The results indicated better survival/protection rates for Zinc Sulphate treated chicks followed by that of vaccinated group and Unvaccinated chicken groups. It is concluded that Zinc sulphate is more efficient immunomodulator in evoking specific as well as non specific immune responses in layer chicks. So, Zinc Sulphate can be recommended in field use for enhancing the immunological responses besides net body weights in layer chicks along with the Scheduled vaccination programmes.

Introduction

Poultry industry is facing a great setbacks with repeated emergence of various bacterial and viral diseases. Apart from causing mortality in young birds they produce a severe immunosuppression in surviving birds making them more prone to wide range of diseases. The reasons attributed for repeated emergence of disease may lack of quality on the part of vaccine, improper storage and handling of vaccines, lack of proper immunobiological response by vaccinated birds due to stress, inadequate nutritional diets, environmental factors and exposure to various immunosuppressive agents. The variety of stressors either singly or in combination can cause lymphoid involution, increase in Heterophill:lymphocyte ratio [1]and suppress the macrophage function[2]. The antibodies or cell mediated immune response to the infectious agent starts primarily with macrophage activity. Certain viruses and bacteria can replicate in macrophages producing different Cytopathic effects or brings about alteration in cell morphologies associated with macrophage activation. So, the immunomodulating agents in chicken have varying effects on the immune function and have potential to decrease immunosuppression in general[3]. The Zinc Sulphate as dietary supplement in the development and maintenance of immune system is now widely accepted, it consists of antistress activities[4] and enhances mononuclear phagocytic activities[5]. Zinc plays a major role in stimulation of immune system of chickens by increasing the bioavailability of zinc [6]. Zn supplementation in breeder diets has been shown to enhance immunity of their progeny[7,8]. The present study was undertaken to evaluate

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immunobiological response of layer chicks against New Castle Disease and Infectious Bursal Disease vaccines by incorporating Zinc Sulphate as supplement.

Materials and Methods

The present study was carried out for a period of 10 weeks in day old 60 healthy white Leghorn Layer chicks to monitor the serological response against New Castle Disease and Infectious Bursal DiseaseVaccines. Three experimental groups i.e. Group I Vaccinated, Group II Vaccinated and Zinc Sulphatesupplementation and Group IIIUnv accinated Control, each comprising of 20 experimental birds. In groups I and II New Castle Disease vaccine (Lasota strain) were incorporated on 7th and booster on 38th day given Intra occularly at the rate of 0.03ml/bird and later on 60th day of experiment R_2B strain of Newcastle Disease injected at the rate of 0.5ml/ bird intramuscularly. The Infectious Bursal Disease vaccine (Intermediate Plus) given on 14th and booster on 28th day of experiment incorporated Intraoccularly at the rate of 0.03ml/bird. These birds specific immunobiological response was analysed by conducting Micro HI against New Castle Disease[9] and Indirect ELISA against Infectious Bursal Disease[10] at weekly intervals and the nonspecific immune response was depicted at bi weekly intervals by phagocytic index[11] and total protein and globulin levels[12]. The overall growth of birds was analysed by taking the bodyweights at weekly intervals. At last challenge studies, was conducted by taking 10 experimental birds from each group. Each group birds were challenged virulent NDV while remaining 10 birds from each group received virulent *E.coli* culture. Symptoms, pathogenic lesions and mortality patterns were recorded and observed 14 days of post challenge. Assay of Humoral immune response was conducted using Micro Haemagglutination Inhibition test. Reciprocal of highest dilution of the serum where there was complete inhibition of HA was taken as end point. In Indirect ELISA, the plates were kept on ELISA reader to observe the optical densities at 490nm which were converted into ELISA titres by Computer programmes. Total serum proteins, albumins and globulin levels were determined.The absorbance (A) of standard (S) and test(T) were measured against Blank (B) were measured immediately on photocolorimeter with Yellow Green filter and red filter on spectrophotometer at 555nm and 630nm.

Calculations

$$\text{Total Protein gm\%} = \frac{\text{A of (T)}}{\text{A of (S)}} \text{X6}$$

$$\text{Albumin in gm\%} = \frac{\text{A of (T)}}{\text{A of (S)}} \text{X4}$$

Globulins

Globulin concentration was calculated using the formulae Globulin gm% = Total protein in gm% - Albumin in gm%.

The non specific immune response of was assessed by Phagocytic index. Each samples four smears were prepared and stained with Leishman's stain, all four slides were prepared and usually 100 phagocytic cell were counted.

$$\text{Phagocytic index} = \frac{\text{Average no. of organisms ingested per phagocytic cell in test samples}}{\text{Average no. of organism ingested per phagocytic cell in control (unvaccinated group)}}$$

Body Weights were recorded at weekly intervals for 10 weeks and average body weights for each group were calculated. Challenge studies with virulent ND virus was an isolate No.105. The isolate characterized as Velogenic New castle Disease Virus and grouped under C1(Asiatic Type) characterized by Central Veterinary Laboratory (CVL) United Kingdom [13]. The virulent NDV isolate no. 105 was used as the Challenge virus in the present study. The virulent culture of E.coli(MTCC No- 078) Procured from IMTECH Chandigarh, was grown on LB Agar Medium and processed to be given at the rate of 1ml containing 10^9 CFU after challenge, the birds were observed daily for 7 days for development of symptoms and mortality. The pertaining to various parameters were analysed statistically as per the standards methods[14].

Results

The serobiological response against New Castle Disease Vaccine and Infectious Bursal Disease vaccine was monitored by conducting Haemmagglutination Inhibition(HI) and Indirect ELISA at weekly intervals in different groups. The HI titres in Ist and 2nd week shown no significant ($P<0.05$) difference was observed between Groups I & Group II while in group III on 2rd, 3rd 4th and 5th showed decreasing HI titres and it differs significantly ($P<0.01$)from Groups I and II. On 6th week, the booster vaccination has been given at the end of the 5th week and the HI titres increased slightly in both vaccinated groups. On 7th week,

groups I and II were differed significantly at (P<0.01) level. On the 10th week of experiment, the lower HI titres of group I differ significantly at (P<0.01) from Groups II. The IBD ELISA titres also exhibited the similar results with no significant difference upto the 5th week of experiment between Group I and II while Group III which differ significantly (P<0.01) from rest of the groups. Upto the end of the experiment, the statistical analysis, indicated significant difference at (P <0.01) in titre values of group I, II and in III, i.e. Unvaccinated chicks revealed continued presence of maternal bodies to IBD virus. The average body weights of 60 experimental birds in each group revealed no significant (P<0.05) differ within groups upto 3rd week, But in 4th week, the healthy unvaccinated control group showed significantly lower body weights (P<0.01) compared to the vaccinated groups. At the termination of experiment, i.e. on 10th week, ZnSo4 treated group, showed better body weight gains over vaccinated and unvaccinated groups, and differ significantly at (P<0.01). In Challenge studies, 100% survivability was seen in both vaccinated and zinc sulphate treated groups, while 100% mortality was seen in the healthy Unvaccinated control groups. In another, non specific challenge studies, virulent cultures of *E.coli* revealed 70% and 50% of survialibility in zinc sulphate treated and vaccinated group while 100% of mortality was seen in Group III i.e. the unvaccinated control groups.

Table 1: NDV-hi titres* at weekly intervals across groups

Groups	Weeks									
	1	2	3	4	5	6	7	8	9	10
Vaccinated	3.00^{Ae}	4.00^{Ad}	4.00^{Ad}	4.00^{Ad}	4.00^{Ad}	4.667^{Ac}	4.5^{Bc}	5.833^{Ab}	5.833^{Bb}	6.167^{Ba}
Vaccinated + Znso4	3.00^{Ag}	4.00^{Af}	4.167^{Af}	4.33^{Ae}	4.033^{Af}	4.50^{Ad}	5.00^{Ac}	6.00^{Ab}	6.167^{Ab}	6.833^{Aa}
Unvaccinated control	3.00^{Aa}	3.00^{Ba}	2.00^{Bb}	1.717^{c}	0.4^{Bd}	0.00	0.00	0.00	0.00	0.00

*Mean value of Pooled sera samples of 6 birds of each group expressed in log2/0.05ml
Means in the column differ significantly (P<0.01) , Means in the row differ significantly (P<0.01)

Table 2: Efficacy of zinc sulphate on weekly weight gains

Groups	1	2	3	4	5	6	7	8	9	10
Vaccinated	14.005^{Ai}	54.5^{Ah}	142.5^{Ag}	263.75^{Af}	284.5^{Af}	327.0^{Ae}	476.5^{Ad}	530.0^{Ac}	605.75^{Ab}	675.75^{Aa}
Vaccinated+ ZnSo4(supplement)	13.99^{Ai}	48.25^{Ai}	160.0^{Ah}	245.75^{Ag}	278.5^{Af}	317.5^{Ae}	456.25^{Bd}	533.0^{Ac}	620.75^{Bb}	700.75^{Ba}
Healthy Unvaccinated	13.965^{Ai}	46.5^{Ah}	120.75^{Bg}	196.5^{Bf}	216.0^{Be}	229.725^{Be}	407.75^{Cd}	508.5^{Bc}	563.5^{Cb}	630.75^{Ca}

Means in the column differ significantly (P<0.01) ,Means in the row differ significantly (P<0.01)

Table 3: Efficacy of zinc sulphate on ibd-elisa titres at weekly intervals across groups

Groups	1	2	3	4	5	6	7	8	9	10
Vaccinated		5.623^{Ac}	5.679^{Cc}	5.897^{Bc}	7.25^{Aab}	7.13^{Bab}	8.103^{Aab}	9.508^{Aa}	6.365^{Ac}	6.324^{Ac}
Vaccinated+ Znso4		5.695^{Ab}	7.164^{Aa}	7.386^{Aa}	7.805^{Aa}	8.318^{Aa}	8.164^{Aa}	8.745^{Ba}	6.96^{Aa}	6.725^{Ab}
Unvaccinated Control		5.748^{Aa}	7.034^{Ba}	5.078^{Ba}	5.011^{Ca}	5.066^{Ca}	5.07^{Ba}	5.00^{Ca}	5.09^{Ba}	5.101^{Ba}

*Means in the column differ significantly (P<0.01), Means in the row differ significantly (P<0.01)

Table 4: Efficacy of zinc sulphate on total protein levels at biweekly intervals across groups

	Total Protein Levels				
Groups	2	4	6	8	10
Vaccinated	3.563^{Bc}	3.553^{Bc}	5.012^{Ab}	5.098^{Bb}	7.107^{Ba}
Vaccinated+ZnSo4	3.824^{Ad}	3.76^{Ad}	4.963^{Ac}	5.985^{Ab}	7.00^{Ba}
Unvaccinated control	3.313^{Ca}	3.099^{Ca}	3.372^{Ba}	3.358^{Ca}	3.229^{Ca}

Table 5: Efficacy of zinc sulphate on total globulin level across groups

Total Globulin Level					
Groups	2	4	6	8	10
VC	2.068^{Ad}	2.05^{Ad}	2.707^{Ac}	2.919^{Bb}	4.777^{Ba}
VC + ZnSo4	2.102^{Bd}	2.048^{Ad}	2.458^{Bc}	3.432^{Ab}	5.00^{Aa}
HUV	1.839^{Cb}	1.429^{Bc}	2.076^{Ca}	1.82^{Cb}	2.326^{Ca}

Means in the column differ significantly (P<0.01)
Means in the row differ significantly (P<0.01)

Table 6: Efficacy of zinc sulphate on phagocytic index at biweekly intervals across groups

Groups	2	4	6	8	10
VC	1.800	2.100	2.000	4.660	5.000
VC + ZnSo4	2.000	1.900	2.450	5.360	9.000
HUV	1.000	1.000	1.000	1.000	1.000

Means in the column differ significantly (P<0.01)
Means in the row differ significantly (P<0.01)

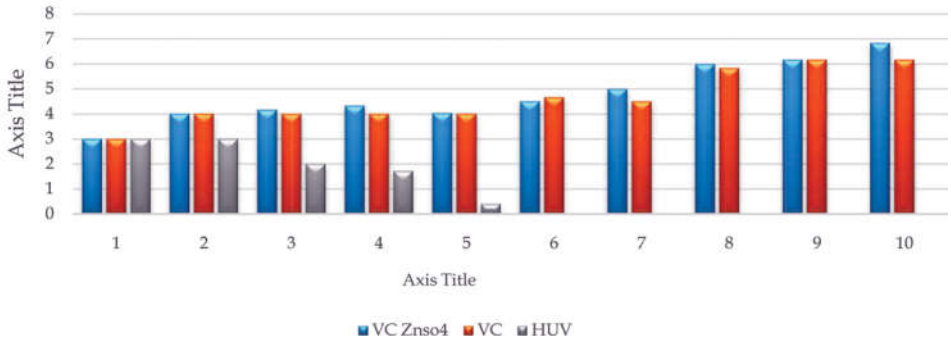

Fig. 1: Ndv-hi titres* at weekly intervals across groups

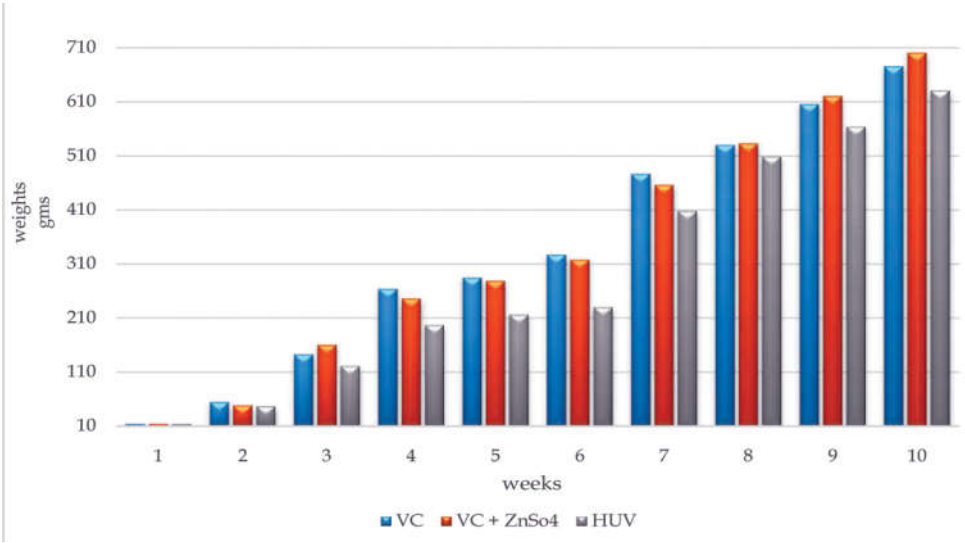

Fig. 2: Comparative efficacy of zinc sulphate on weekly weight gains across groups

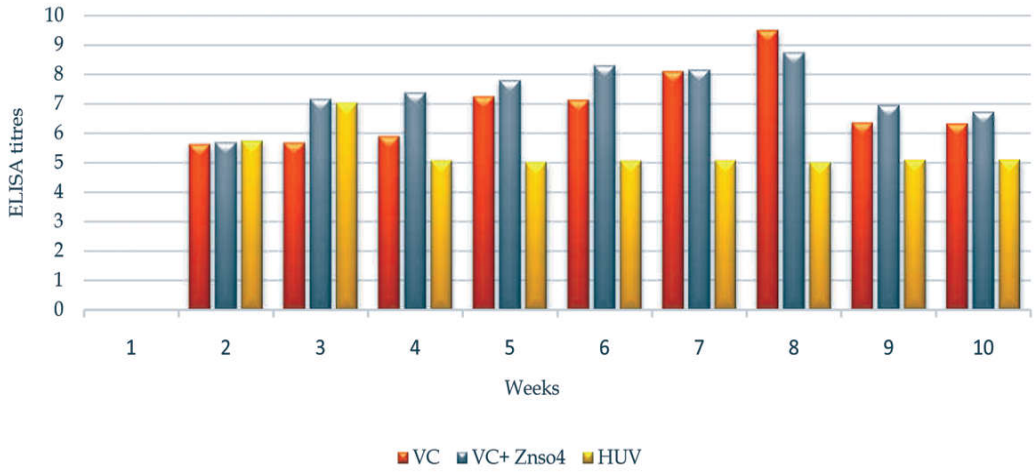

Fig. 3: Efficacy of zinc sulphate ibd-elisa titres across groups

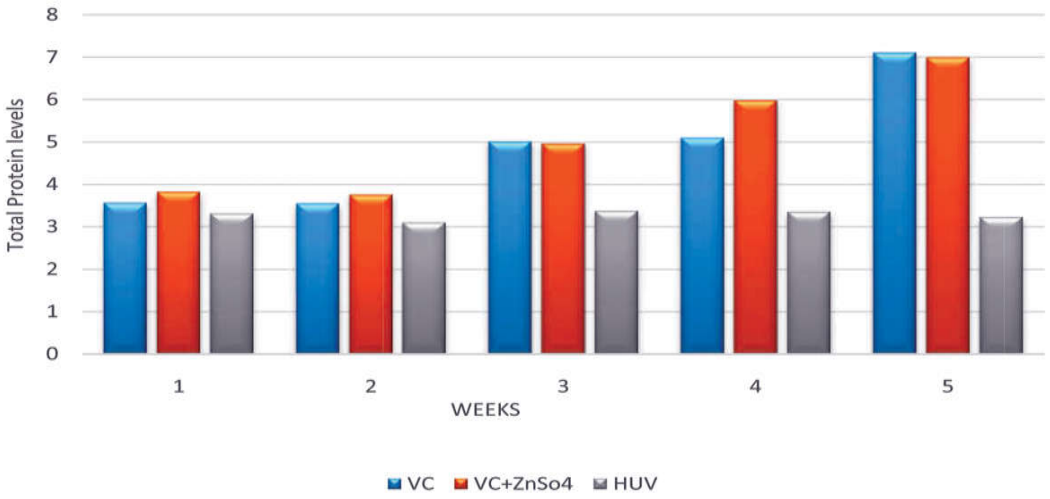

Fig. 4: Comparative efficacy of zinc sulphate on total protein levels across groups

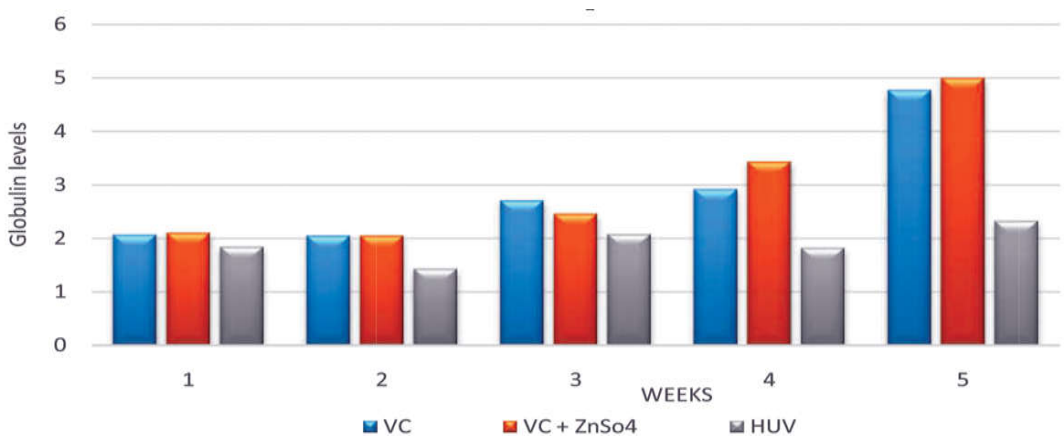

Fig. 5: Comparative efficacy of zinc sulphate on total globulin levels across groups

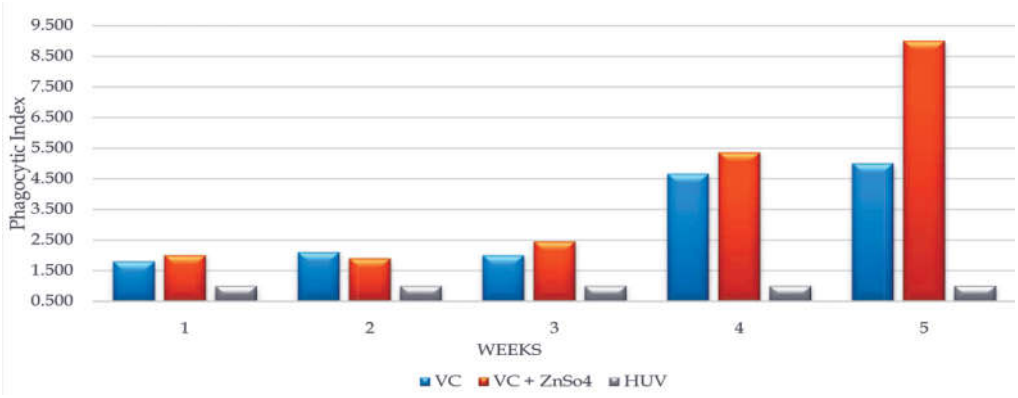

Fig. 6: Comparative efficacy of zinc sulphate on phagocytic index across groups

Discussion

In today's commercial poultry farms, major goal of many poultry producers is to attain good liveability and sustained performance. Most of the poultry feed ingredients usually contains low quantities of pesticide residues, mycotoxins, antibiotics which generally suppresses the immune status of birds, stress, hypoprotaemia, vitamins and mineral deficiency have adverse effects on immune system. Establishment of adequate levels of protective and long lasting immunity to inoculated antigens/ vaccines may require an effective immunopotentiator like adjuvants, liposomes, levamisoles, vitamins like A, E and minerals like Zn, Na, Cl and Se used for this

purpose. Specific immunomodulation implies the increase in both HI and ELISA titres in treated groups compared to untreated groups. Zinc Sulphate treated groups showed increased HI titres against New Castle Disease Vaccine compared to the vaccinated control groups [15],[16]. Their study revealed that Zinc Sulphate enhanced the HI titres significantly in healthy vaccinated and experimentally immunosuppressed birds. It also indicated that Zinc Sulphate supplemented diets improved Cell Mediated Immune as well as antibody response to Pasteurella antigens in healthy mice [17]. Marginal enhancement of ELISA titres due to Infectious Bursal Disease Vaccine were seen in Zinc sulphate treated group however, these were found to be statistically non significant with vaccinated control groups.In young birds, high Maternally Derived Antibodies titres interfere with early vaccination against Infectious Bursal Disease (IBD) using classical modified-live vaccines like Intermediate and intermediate plus and there was very marginal increase of titres [18]. In the present studies, the Zinc Sulphate treated group were not much effective against the live Infectious Bursal Disease Viral vaccinations as Maternally Derived Antibodies interfered with the vaccine and moreover, the studies conducted to know the role of Zinc on immune system in chickens revealed that bioavailability of Zinc in the form of Zinc – methionine resulted in 206% more biologically available when compared to Zinc Sulphate [6]. Supplementation of zinc in the form of chelates of methionine in breeder diet aids in development ofimmune organs and increase in antibody titers to Sheep Red Blood Cells and non-specific immunity in the progeny[10],[17]. The Scientists reported that Zinc deficiency causes hypoplasia of thymus, spleen and other lymphoid organs (bursa), and also decreases T-cell function[19]. More research is needed to throw light in enhancement of IBDV ELISA titres.The total protein and globulin levels in sera were indicated higher protein levels in sera samples which were statistically nonsignificant difference immune response in Zinc Sulphate treated groups to vaccinated groups. The reasons of poor immune response of Zinc Sulphate may be due to less bioavailability of Zinc Sulphate than Zinc methionine which experimentally proved in chicks [6]. Further, it also revealed that Zinc Sulphate was not much effective to reverse immunosuppressive condition in restoring the protein and globulin levels in sera samples of experimental birds[20]. The non immunospecific immune response was assessed by phagocytic index. The phagocytic index in Zinc Sulphate showed higher than vaccinated control groups. It was reported that adequate dietary Zinc supplementation was important for proper functioning of heterophills, mononuclear, phagocytes and T- Lymphocytes which are important for disease resistance. Zinc- deficient mice have impaired killing of intracellular parasites, which is rapidly corrected in vitro by addition of Zinc [21]. Reduced macrophage activity in phagocytosis of Candida sps. was observed in Zinc-deficient animals [22]. Net body gains were also analysed at weekly intervals and results shown that as immnopotentiator has direct action on the growth harmone or due feed conversion efficiency leads increase in the general immune status. The results of challenge test usually gives real indication of success of rate of vaccination or percentage of protection, health and immune status of birds. The resistance to challenge infection in experimental birds indicate combined effect of humoral, Cell mediated Immune response and Nonspecific immunological responses evoked by specific vaccine/antigens and immunopotentiators. In New Disease Virus challenged birds 100% mortality was seen in the Unvaccinated group[23,15]. In *E.coli* challenge studies 70% survivability seen in Zinc Sulphate treated group more than the vaccinated groups. Dietary zinc-methionine enhanced mononuclear phagocytic function against *Salmonella enteritidis* and influences clearance of *E.coli* from blood in young turkeys [24]. The immuno-modulatory role of zinc is mediated through a hormone thymulin which is necessary for lymphocyte development, metalloenzymes (DNA and RNA polymerase) and zinc-dependant deoxythymidine kinase [25, 26]. As, the Zinc supplementation in diets is important against various bacteremia, parasitic infection [27]. Deficiency of zinc leads to decrease the cellular immunity [28] Thymus [29] and spleen [30]. Abnormal T lymphocyte development is thought be the primary consequence of zinc deficiency [25]. Moreover, the Zinc Sulphate treated birds were challenged with the virulent strain of NDV virus 100% survivability was seen while *E.coli* cultures 70% survivability was seen. It was reported that zinc supplementation in breeder diets will enhance the immunity of their progeny [7,8].

Conclusion

The outcome of results on various parameters revealed better performance of Zinc Sulphate as immunomodulator in order to enhance specific and non specific immune response in chickens. Further, more studies are needed to know the appropriate

dosage of supplementation of Zinc salts in the form of chelates to enhance both specific and nonspecific immune response.

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Aflatoxicosis in livestock and poultry

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Abstract

Aflatoxin is the most studied mycotoxin, due to both its toxicity to animals and people and its high carcinogenic potential. Out of aflatoxins group, AFB1 is the most toxic. The main biological effects on farm animals, including malabsorption of various nutrients, coagulopathy, decreased tissue integrity, poor growth, poor efficiency of feed conversion, enhanced susceptibility to infection, vaccine failures, drug failures, reproductive problems in males and females and Increased sensitivity to temperature extremes. Toxic residues of aflatoxin in animal products present a hazard to public health. A variety of physical, chemical and biological approaches to counteract the aflatoxin problem have been reported in the literature on mycotoxins; but large-scale, practical and cost-effective methods for detoxifying aflatoxin-containing feedstuffs are currently not available.

Introduction

Aflatoxicosis is a disease condition caused by the consumption of aflatoxins. The name ''aflatoxin" derives from the first letter of the word Aspergillus and the first three letters of flavus. Aflatoxins are the most dangerous secondary mould metabolites produced by *fungi Aspergillus flavus* and other related species of *Aspergillus* fungi. Aflatoxins show fluoresce strongly in ultra violet light. The major members of aflotoxins are designated as B1, B2, G1 and G2. B1 and B2 fluoresces blue, while G1 and G2 fluoresces green. B1 is most hepato toxic. All four have been detected as contaminants of crops before harvest, between harvesting and drying, during storage, and after processing and manufacturing (Council for Agricultural Science and Technology, 1989).

Types of aflatoxins

Around 17 aflatoxins have been isolated (WHO, 1979), only 4 of them are well known and studied extensively from toxicological point of view. Being intensely fluorescent in ultraviolet light, the four are designated by letters B1, B2, G1 and G2 representing their blue and green fluorescence in UV light. Two other familiar aflatoxins are M1 and M2, because of their presence in milk of animals, which were exposed to B1 and B2. Of all the above-named aflatoxins, aflatoxin B I (AFB1) is most acutely toxic to various species. Toxigenic *A. flavus* isolates generally produce only aflatoxins B1 and B2, whereas *A. parasiticus* isolates generally produce aflatoxins B1, B2, G1 and G2. Aflatoxin M1 is a metabolite of aflatoxin B1 in humans and animals, which is equally potent as that of AFB1. Aflatoxin M2 is a metabolite of aflatoxin B1 in milk of cattle fed on contaminated foods. Although aflatoxins B1, B2 and G1 are common in the same food sample, AFB1 predominates (60-80% of the total aflatoxin content).

Etiology

Aflatoxins are secondary metabolites produced by the common moulds of Aspergillusflavus, A. parasiticus and A.nominus. The development of aflatoxins depends on the infestation and growth of

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the *Aspergillus* mould in grain. High carbohydrate containing grains and feedstuffs, such as peanut meal, corn, sorghum, and cottonseed are favoured by *Aspergillus* spp. Groundnuts and groundnut meal are the two agricultural commodities that seem to have the highest risk of aflatoxin contamination. Aflatoxin production is also stimulated by high zinc concentration in feed (Pattison et al., 2008).

Crops grown under warm and moist weather in tropical or subtropical countries are especially more prone to aflatoxin contamination than those in temperate zones. Water stress, high-temperature stress and insect damage of the host plant are major determining factors in mould infestation and toxin production. Similarly, specific crop growth stages, poor fertility, high crop densities and weed competition have been associated with increased mould growth and toxin production. The moisture content of the substrate and temperature are the main factors regulating the fungal growth and toxin formation. A moisture content of 18% for starchy cereal grains and 9-10% for oil-rich nuts and seeds has been established for maximum production of the toxin (WHO, 1979). Below-normal soil moisture (drought stress) has also been found to increase the number of *Aspergillus* spores in the air. Therefore, when drought stress occurs during pollination, the increased inoculum load (spores in the air) greatly increases the chances of infection.

Host susceptibility

Poultry is more susceptible to aflatoxins (Austwick, 1983). Susceptibility of poultry to aflatoxins varies among species, breeds and genetic lines. Comparative toxicological studies in avian species have shown that ducklings and turkey poultry are the most sensitive species to aflatoxins. The susceptibility ranges from ducklings > turkey poults > goslings > pheasant chicks > chickens (Muller *et al.*, 1970). Young poultry are more sensitive to aflatoxin than adults. Ducks being 10 times more sensitive than chickens. Dairy and beef cattle are more susceptible to aflatoxicosis than sheep. Young animals of all species are more susceptible to the effects of aflatoxins than mature animals. Pregnant and growing animals are less susceptible than young animals but more susceptible than mature animals. The lethal levels are different in different species (Table 1).

Table 1: Comparative LD50 or lethal values for aflatoxin B1 (WHO, 1979)

Species	Oral LD50/Lethal dose (mg/Kg)
Chick embryo	0.025
Duckling	0.3
Turkey poultry	0.5
Chicken, New Hampshire	2.0
Chicken, Rhode Island	6.3
Sheep	5.0
Pig	0.6
Cattle	0.5-1.5

Pathogenesis

The principal target organ for aflatoxins is the liver. After the absorption, highest concentration of the toxin is found in the liver (Mintzlaff *et al.*, 1974). In liver, aflatoxin B1 is metabolized by microsomal enzymes to different metabolites through hydroxylation, hydration, demethylation and epoxidation. In liver enzymatic degrada-tion of toxins takes place via the mixed function oxidase system (MFO), where toxins are converted into a more polar structure. In aflatoxicosis, however, the MFO system in the liver seems to oxidize the afla-toxin to another metabolite that reacts with the chromatin of the nucleolus protoblast, thus impairing the template activity of the chromatin to produce M-RNA. Aflatoxin binds to both RNA and DNA and blocks transcrip-tion. Aflatoxin B1 inhibit tRNA binding activity of some amino acids in protein synthesis especially the essential amino acids such as lysine, leucine, arginine and glycine. The tRNA binding, have different inhibitory effect, which interfere with the translation level of protein biosynthesis and affect cell metabolism.

In day-old chicks, AFB1 reduces the activity of liver UDP glucose-glycogen transglucosylase resulting in depletion of hepatic glycogen stores (Shankaran *et al.*, 1970). On the other hand, there is lipid accumulation in the liver of chickens and ducklings exposed to aflatoxin (Carnaghan *et al.*, 1966; Shank and Wogan, 1966). With regard to its toxic effects on liver microsomal enzymes, AFB1 is known to decrease microsomal glucose-6-phosphatase activity (Shankaran *et al.*, 1970) whereas stimulation of microsomal enzyme activity by inducers seems to be unaffected by AFB1 (Kato *et al.*, 1970). Another effect of aflatoxin is that it causes anticoagulation of blood. This is probably because AFB1 inhibits synthesis of factors II and VII involved in prothrombin synthesis

and clotting mechanism (Bababunmi and Bassir, 1969).

Immuno-suppression is observed in animals fed aflatoxin.Aflatoxins appear to de-crease the lymphocyte response to mitogens, inhibit macrophage migration, and decrease the effectiveness of humoral mediators such as complement(Hoerr, FJ and D'Andrea,1983).

Carcinogenicity of aflatoxin has not been thoroughly studied, although trout and rat hepatomas and occasional swine undifferentiated neoplasms have been linked to aflatoxicosis. (Heathcote, JG, and Hibben,1978; Hoerr,FJ and D'Andrea,1983).

Aflatoxin B1 is excreted in urine and feces, and also in milk of lactating animals either unchanged or as various metabolites (Nabney *et al.,* 1967; Allcroft *et al.,* 1968). Only one milk metabolite, AFM1, appears to be the major metabolite of AFB1 that has shown appreciable oral toxicity (Holzapfel *et al.,* 1966).

Symptoms

Effects of aflatoxin consumption are similar in all animals; the animal's susceptibility to aflatoxin, however, varies by species, age, and individual variation (Pier, 1987). In acute clinical aflatoxicosis general signs include edema of the lower extremities, abdominal pain, and vomiting. Blood pigments may appear in the urine and mucous membranes are icteric. Feed refusal, reduced growth rate, decreased milk production and decreased feed efficiency are the predominant signs of chronic aflatoxin poisoning.

In cattle most commonly reported signs with acute toxicosis include anorexia, depression, dramatic drop in milk production, weight loss, lethargy, ascitis, icterus, tenesmus, abdominal pain, bloody diarrhea, abortion, hepatoencephalopathy, photosensitization and bleeding (Eaton and Groopman, 1994; Reagor, 1996). Other signs associated with acute aflatoxicosis include blindness, walking in circles, ear twitching, frothy at the mouth, keratoconjunctivitis and rectal prolapse (Radostits *et al.,* 2000).

In addition, chronic aflatoxicosis may impair reproductive efficiency including abnormal estrous cycle and abortions, induce immunosuppression and increase susceptibility to disease (Cassel *et al.,* 1988). The immunotoxic effect of AFB1 was expressed via the cell-mediated immune system (Raisbeck *et al.,* 1991).

In sheep and goats anorexia, depression and icterus were observed exposed to aflatoxin. The goats also developed a nasal discharge and dark brown urine was noted in the sheep (Abdelsalam *et al.,* 1989).

The clinical syndrome in pigs include rough coat, depression, anorexia, decreased feed conversion, decreased rate of gain, weight loss, muscular weakness and shivering, tremors, bloody rectal discharge and icterus (Hoerr and D' Andrea, 1983; Radostits *et al.,* 2000). Aflatoxins also suppress the immune system and thus make pigs more susceptible to bacterial viral or parasitic diseases (Diekman *et al.,* 1992).

In poultry, aflatoxin impairs all important production parameters including weight gain, feed intake, feed conversion efficiency, pigmentation, processing yield, egg production, male and female reproductive performance. Some influences are direct effects of intoxication, while others are indirect, such as from reduced feed intake (Calnek *et al.,* 1997). The direct and indirect effects of aflatoxicosis include increased mortality from heat stress (broiler breeders, Dafalla *et al.,* 1987), decreased egg production in leghorns, (Bryden *et al.,* 1980), anemia, hemorrhages and liver condemnations (Lamont, 1979), paralysis and lameness (Okoye *et al.,* 1988), impaired performance in broilers, increased mortality rate in ducks, (Bryden *et al.,* 1980), impaired ambulation and paralysis in quail, (Wilson *et al.,* 1975), impaired immunization in turkeys, (Hegazy *et al.,* 1991), and increased susceptibility to infectious diseases (Bryden *et al.,* 1980 and Calnek *et al.,* 1997).

Clinical laboratory findings

Clinical laboratory findings vary with the animal species, level of aflatoxin in the ration, and the duration of feeding. There are no consistent diagnostic changes in hematocrit, hemoglobin, and differential cell counts in animals fed aflatoxin. Leukocytosis may occur in animals with secondary bacterial infections. Serum bilirubin levels may be elevated and typically serum protein levels are decreased.

Lesions

Lesions observed at necropsy related to either acute or chronic liver disease are dependent upon the level of aflatoxin and the duration of feeding. A majority of acute liver damage observed has been the result of experimentally high doses, while chronic liver damage is a more common field observation. The liver is usually pale tan, yellow or orange. Hepatic fibrosis and edema of the gallbladder may also be observed.

Diagnosis

The diagnosis of aflatoxicosis is often difficult because of the variation in clinical signs, gross pathological conditions and the presence of infectious diseases due to the suppression of the immune system. On the farm, more than one mould

or toxin may be present in the contaminated feed, which often makes definitive diagnosis of aflatoxicosis difficult. A quick screening test for aflatoxin level in shelled corn or ground feed is the Woods' light test. A black light is held over the sample and flourescing of a metabolite in the production of aflatoxin might be observed.

Diagnosis is based on history and clinical signs, lab tests such as thin layer chromatography (TLC), mycological examination, culture samples in lab, lesions in post mortem examination, PCR, detection of aflatoxins by high pressure liquid chromatography (HPLC) and ELISA. Chromatographic methods such as TLC and HPLC are considered the gold standard and are thus the most widely used techniques in aflatoxins analysis.

Treatment

Aflatoxicosis is typically a herd rather than an individual animal problem. If aflatoxin is suspected, analyze the ration immediately. Eliminate the source at once, if aflatoxins are present. Increase levels of protein and vitamins A, D, E, and K in the ration as the toxin binds vitamins and affects protein synthesis. Practice good management to alleviate stress, reducing the risk of secondary infections. Provide immediate attention and treatment for secondary infections. Environmental stress should also be minimized.

Hydrated sodium calcium aluminosilicate (HSCAS), a sorbent compound obtained from natural zeolite, has demonstrated an ability to adsorb mycotoxins with a high affinity. Addition of this compound to feedstuffs contaminated with aflatoxins has shown a protective effect against the development of aflatoxicosis in farm animals.

Prevention

Aflatoxin levels which are considered safe in animal feedstuffs are 20 ppb or lower. A concentration of aflatoxin in feed at 100-300 ppb caused chronic intoxication signs in swine, whereas acute lethal intoxication of swine was observed at feed levels of 1,000 ppb or greater (Hoerr, FJ, and D'Andrea, 1983). Cattle and sheep are relatively more refractory to the effects of alfatoxin, pos-sibly due to rumenal microbial degeneration, whereas poultry are more sensitive to afla-toxin than swine Aflatoxicosis can only be prevented by feeding rations free of aflatoxin. Preventing aflatoxin contamination requires an on-going and thorough sampling and testing program.

Control strategies for aflatoxicosis prevention

Moisture/temperature

Monitoring and control of moisture is critical in the prevention of fungal growth and mycotoxin production. Moisture level of grains should be kept at below 13%. Aflatoxins and other mycotoxins produced by *Aspergillus* spp. are not likely to be produced at temperatures below 5 to 8^0C (Rajendra Damu et al ; 2014).

Cleaning

Periodic cleaning of all feed handling equipments with 5 to 10% bleach solution will help control mould growth as well as actually destroy, to some extent the aflatoxins present.

Pre-harvest control measures

Preharvest control measures include prevention of insect infestation, crop residues and crop rotation, irrigation and soil condition and effective drying and storage regimens.

Harvest measures

Timing of harvesting greatly influences mycotoxin production, harvesting should take place as soon as the crop is fully grown and the crop cycle is completed.

Post-harvest measures

Post-harvest strategies involved various physical, chemical and biological methods to inactivate, destroy, or remove the mycotoxin (Galvano *et al*., 2001).

Physical Methods

Antimycotic agents

Antimycotic agents like sorbic acid and sorbate; propionic acid and propionate, benzoic acid, benzoates and parabens; and acetic acid and its derivatives are the chemicals that prevent mould growth and interfere with mycotoxin production. 1% propionic acid, incorporation of 0.2% potassium sorbate, 0.7% methyl paraben and 0.2% sodium propionate completely inhibited fungal growth (Tong and Draughon, 1985).

Irradiation

Gamma or electronic irradiation is highly effective

for destroying the fungal spores. Simple exposure of contaminated grains to sunlight (UV) substantially reduces mycotoxin levels.

Processing of food

Most of the mycotoxins are generally stable at room temperature. Processing of food has been found to decrease the prior concentration. Wet milling, malting, brewing, cooking and dry and oil roasting are methods to eliminate the mycotoxins, effectively.

Chemical Methods

Ammoniation

Treatment with aqueous and gaseous ammonia or ammonium hydroxide, with or without heat and pressure to destroy the mycotoxin in contaminated food and feed is currently the best and effective method. Ammoniation not only detoxified several mycotoxins (85-100% reduction), but also inhibited mould growth (Madson *et al*., 1983).

Sodium hydroxide

Warming of grain to 1050C in the presence of 0.5% sodium hydroxide detoxified various mycotoxins in the feed.

Mycotoxin-binding agents

Numerous agents like, activated carbons (charcoal), bentonites, clay, hydrated sodium calcium alumino silicate, and zeolite, have currently been used to counteract the mycotoxicosis These sorbents are nutritionally inert and reduce the bioavailability of various mycotoxins by absorption on their surface in intestinal tract. Charcoal at 2% level had shown beneficial effects, during *in vivo* studies., HSCAS (0.5%) was effective at reducing the toxicity of aflatoxin (Harvey *et al*., 1993; Abo-Norag *et al*., 1995)

Biological Methods

Mannan oligosaccharide (MOS) extracted from the cell wall of *Saccharomyces cerevisiae* has shown broad-spectrum efficacy against most of the mycotoxins (Raju and Reddy, 2000).

Feed additives

Vitamins

Vitamin A, E and C possesses the antioxidant properties against the mycotoxin-induced damage.

Lipids

The higher levels of dietary fat reduced mortality and in some instances, improved the body weights. Lipids exerted their effects in part by interfering with absorption of the aflatoxin.

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A successful management of bovine papillomatosis with autogenous vaccine in cattle

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Keywords: Bovine Papillomas
Cauliflower Like Warts
Autogenous Vaccine
Sterility Test
Regression of Warts.

Abstract

Two cases of bovine papillomatosis were brought to the Department of veterinary surgery and radiology, CVSc, SVVU, Tirupati. It was diagnosed as bovine papillomas on clinical observations of the wart lesions. Wart samples were collected aseptically from one of the case.

An autogenous formalin killed vaccine was prepared from the collected wart samples. The formalin inactivated autogenous vaccine was adjuvented with equal volumes of aluminium hydroxide. After sterility check up the vaccine was administered to both the animals subcutaneously, cow @ 10ml and heifer calf 5ml on 0 day and then subsequently 10 days interval. The regression of the warts was started by three weeks of post vaccination and complete regression was appeared by sixth week in less severe case of heifer calf and in cow seventh week. The study represents successful management of bovine papillomas with a bovine specific autogenous vaccine. Further the autogenous vaccine prepared from one animal treated successfully to another animal.

Introduction

Papilloma viruses belong to family of Papillomaviridae affecting skin and mucosa of humans and animals. The virus normally infects epithelial cells causing benign hyper proliferative lesions (Warts, papillomas and fibro papillomas) which can progress to cancer (Campo, 2006). Bovine papillomatosis is a contagious disease of cattle occurring as warts/papilloma on skin and mucosa, caused by BPV types 1 to 10 (Vidhya *et al*, 2009).

Papilloma virus infection in cattle can result in weight loss and retarded growth. The lesions are often associated with the mammary gland and interfere with milking. It can lead to reduction in milk yield. The quality of the hide is also deteriorated. Thus the disease can lead to a serious economic loss if not diagnosed and treated promptly. A formalinized suspension of bovine warts with inactivated virus provides a vaccine for effective treatment and prophylaxis of bovine papillomatosis (Barthold et al 1976, Hunt 1984, Lesnik et al 1999, Suveges and Schmidt 2003). The present clinical study describes the use of autogenous vaccine in cattle as a successful managemental practice.

Materials and methods

Sample collection

Two animals crossbred heifer and adult cow were brought to the Department of veterinary surgery and radiology, CVSc, SVVU, Tirupati with a history of papillomas. On clinical examination small to big wart like lesions around the eyes, ears, nose, neck, shoulders, abdomen and udder varying in size from 0.5 to 50mm in diameter was observed. The lesions are characteristic varied from flat to pedunculated. The animals were apparently healthy. The case was tentatively diagnosed and the warts were collected for autogenous vaccine preparation. On clinical observation the case was suspected as bovine

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papillomatosis. Later from one animal warts were collected and processed for vaccine preparation.

Autogenous vaccine preparation

Wart lesions were collected from one of the animal (adult cow) aseptically in PBS on ice until processing according to the method described by Hunt (1984). The processing of warts were carried out with sterile scissors, washed thoroughly with sterile PBS and homogenated with sterile sand using pestle and motor. Later 10% suspension was made with sterile PBS. Then the suspension was centrifuged at 3000rpm at 4°C for 30min to remove the coarser particles. Supernatent was taken and formaline was added at a concentration of 0.5% to inactivate the virus. Vaccine thus prepared was added with equal volumes of aluminium hydroxide and left for 24 hours at 4°C for sterility check up of the vaccine samples were inoculated on blood agar, nutrient agar and macconkey agar at 37°C for 48 hours. For fungal check up the vaccine samples were inoculated on sabouraud dextrose agar media and kept in duplicates one at 37°C and another at 25°C for 3-7 days. After the sterility check up the same animals with papilloma warts were administered with the vaccine thus prepared.

Vaccine dose and administration

The adult animal was administered with 10ml of vaccine dose subcutaneously in the shoulder region and the heifer calf with 5ml subcutaneously similar manner. The second dose was given 10 days after the first dose. Then later third dose was given 10 days after the second dose and then subsequently fourth and fifth doses were given 10 days apart and observed for regression of warts.

Results

Initially the cases were diagnosed as papillomas based on clinical presentation and wart like lesions on different parts of the body (Fig.1 and 3). The papilloma lesions were started regression after third dose of post vaccination of animals and the complete regression was observed by sixth week of post vaccination in heifer calf of less severity of the case and by the seventh week in severely affected cow (Fig. 2 and 4).

Fig. 1: Papilloma case before autovaccination (Heifer calf)

Fig. 2: After autovaccination (heifer calf)

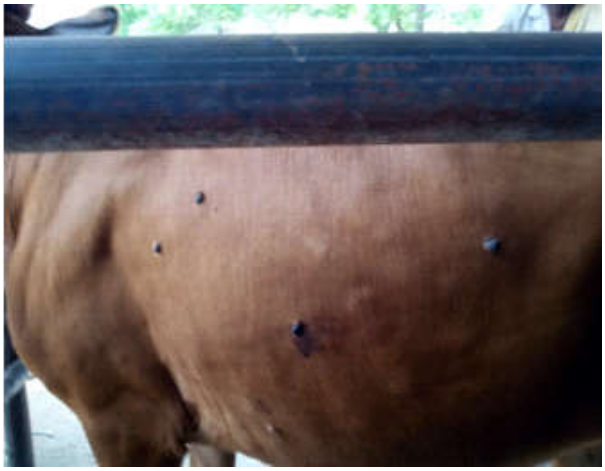

Fig. 3: Papilloma case before autovaccination (adult cow)

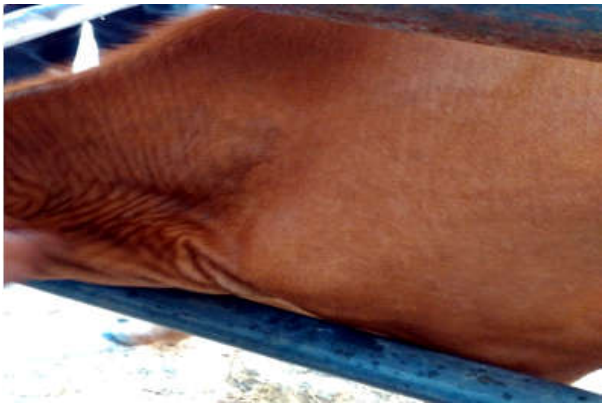

Fig. 4: After autovaccination (adult cow)

Discussion

The results of the present study showed the

successful recovery of the papillomatosis. of the two animals, the recovery in heifer calf by sixth week of post vaccination and cow by seventh week with species specific autogenous vaccine. Similar findings were reported by Hamad M.A. et al (2012), Thaiya et al (2009) and Suveges and Schmidt (2003). In the present study the autogenous vaccine prepared from cow resulted in complete recovery of not only the particular animal but also complete recovery of other heifer calf also. But Ndarathi and Mbuthia (1994) made an observation that a single wart sample from one calf was used to prepare the vaccine, thus prepared vaccine was failed to cure other infected animals. Further Campo (1999) and Campo (1997) also reported that Bovine papilloma viruses 1, 2 and 5 cause fibropapillomas and the vaccine prepared using all the three virus types results in successful treatment of Bovine papillomas. In the present cases the young animals were being affected and this inagreement with Radostits et al (1994) that older animals have been reported to be resistant to the infection and it could be due to immunity acquired from apparent and inapparent infections. Thaiya et al (2009) suggested that the wart samples should be collected from all infected ones and with those material common vaccine can be prepared for successful treatment of Bovine papillomas.

Further owners of the both the animals were advised for good managemental practices.

Acknowledgement

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Quality improvement of fish ball in curry processed at elevated Temperature

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Keywords:
Types of Fish Balls
Setting
Tgase Enzyme
Fish Ball with Wet Ingredients
Thermal Processing.

Abstract

Process of F_0 value of 6 minutes was found to be sufficient for fish ball in curry product and fish balls without curry. To improve the quality of fish ball in curry product, different methods such as effect of setting, types of starch, levels of wet ingredients, different types of ingredients and pack and levels of transglutaminase enzyme were adopted to find out a suitable method and subjected to biochemical (pH and moisture), physical (gel strength and expressible water percentage) and organoleptic evaluation. Of the different methods tried, levels of wet ingredients and different types of ingredients and pack showed an improvement in texture. Texture of fish balls showed an improvement with a reduction in the levels of wet ingredients.

Plain fish balls without the curry (dry pack) showed superior texture followed by plain balls with curry, fish balls with wet ingredients packed dry. Although wet ingredients incorporated fish balls in curry had lower textural values (A grade in folding test and organoleptic textural score of 8.4), it was liked by the panelists as they preferred soft texture to rubbery ones.

Introduction

Non-starch polysaccharides (NSP) are some important anti-nutritive components in plant based feed stuffs. Exogenous enzymes can hydrolyze these NSP into smaller units that can be utilized by pigs (Partridge and Bedford, 2000). Similarly phosphorus from plants is of low bio-availability to swine and poultry as a result of phytate, the principal form of phosphorus storage in plants, being relatively indigestible by non-ruminants (NRC, 1998). Exogenous supplementation of feeds with phytase has demonstrated the ability to increase phosphorus bio-availability and thus growth rates in pigs by cleavage of phosphorus molecule from phytase. Since four decades, concern about antibiotic resistance has increased worldwide (Cromwell, 2002). The ban on some Antibiotic Growth Promoters lead to think on phytogenic feed additives which include herbs and their residues, essential oils, botanicals, extracts etc. The mode of action of plant active substances include improvement of endogenous enzyme secretions, stimulation of the appetite, improvement of the digestibility and absorption of nutrients, promote proliferation of beneficial bacteria like Lactobacillus spp. in the gut. Hence the present experiment was planned with the objective of studying the effect of exogenous enzymes on pig performance either with or without herbal residues and their role in gut pathogen inhibition in finishers.

Materials and methods

Five experimental diets (Table 1) were formulated as per NRC (1998) requirements and were evaluated Frozen surimi was taken out and thawed before use. Fish ball in curry was prepared according to the recipe of Joshi *et al*., (2011) with a slight modification, but with

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slight modifications i.e., such as usage of surimi instead of fish mince, overnight setting, reducing the quantity of wet ingredients, usage of dry ingredients instead of wet ingredients, use of different types of starch, incorporation of transglutaminase enzyme.

Curry paste was prepared according to the recipe of Joshi *et al*., (2011). The curry paste was mixed with oil and heated for 2 minutes, and mixed with water in 1:1 ratio and boiled for 5 minutes.

Fish ball paste was prepared by mixing all the ingredients. Fish ball paste weighing 10g was moulded into round balls and steamed at 100^0 C (0 psi) for 15 minutes.

The fish ball and liquid curry so prepared were used for further studies.

Retort pouches (150mm × 200mm) of 300g capacity having a configuration of 12µ PET, 9µ Al foil, 15µ bi-axially oriented nylon and 70µ CPP duly laminated were used for packaging of fish ball in curry.

Fish balls were kept for overnight at 0^0 C, brought to room temperature, steamed at 100^0C (0 psi) for 30 minutes, packed in retort pouch along with curry (fish ball 100g and curry 200g), sealed, washed, stacked in retort and subjected to thermal processing at 115^0C for 45 minutes, steam was shut off and simultaneously air and water was pumped inside the retort to maintain the internal pressure bought 25 psi and cooling continued inside the retort till the temperature at cold spot of the product inside the pouch reached below 60^0C. Afterwards the pouches were wiped dry and stored until used fish ball in curry product was subjected to physical, chemical and organoleptic analysis. In the case of control, fish balls were prepared without setting, steamed for 30 minutes, packed in retort and subjected to thermal processing at 115^0C for 45 minutes. In the case of different types of starch, five sets of fish ball paste were prepared by mixing the different types of starches such as corn starch, modified starch, wheat starch, tapioca starch and control was without starch. The rest of the followed was as above (minus setting procedure). In the case of different levels of wet ingredients, quantity of wet ingredients was reduced in each set in a sequential manner and finally in the last set wet ingredients was not added.

All the other ingredients were kept constant. The rest of the followed was as above (except starch variation). In the fourth experiment, three sets of fish ball were prepared by mixing various types of ingredients such as wet ingredients, dry ingredients and without ingredients along with surimi, starch and salt. The rest of the followed was as above. Similarly in another experiment fish balls were packed (150g), sealed, subjected to thermal processing at 115^0C for 67 minutes for both plain fish balls and wet ingredients incorporated fish balls. The product so prepared was subjected to physical, chemical and organoleptic analysis. In the fifth experiment, five sets of fish ball paste were prepared by mixing the various ingredients with different concentrations of transglutaminase enzyme in the fish ball paste such as 0.1%, 0. 2%, 0.3%, 0.4% and control was without enzyme. The rest of the followed was as above.

Table 1: Standardized recipe of fish ball

Sr. No.	Ingredients	Quantity in gram
1	Surimi	1000
2	Salt	20
3	Starch	150
4	Curry paste*	400
5	Total	1570

Table 2: Recipe of curry paste

Sr. No.	Ingredients	Quantity in gram	
		WI	PB
1	Fried onion paste	246.06	-
2	Dried onion powder	-	-
3	Fried tomato paste	49.21	-
4	Garlic paste	33.46	-
5	Dried garlic powder	-	-
6	Chilly powder	8.24	-
7	Turmeric powder	3.69	-
8	Coriander seed powder	6.15	-
9	Garam masala	8.7	-
10	Green chilly paste	8.95	-
11	Coriander leaves paste	8.95	-
12	Dried coriander leaves powder	-	-
13	Ginger paste	4.05	-
14	Dried ginger paste	-	-
15	Salt	22.5	-
16	Total	399.96	-

Note: WI – wet ingredients; PB – plain fish ball

Table 3: Recipe of curry paste for liquid curry

Sr. No.	Ingredients	Quantity in gram
1	Onion	526.6
2	Tomato	394.9
3	Garlic paste	27.2
4	Chilly powder	6.6
5	Turmeric powder	2.6
6	Coriander seed powder	4.0
7	Garam masala	3.3
8	Green chilly	7.3
9	Coriander leaves	7.3
10	Ginger paste	3.3
11	Salt	18.3
12	Total	1001.4

Results and Discussion

Based on the heat penetration studies , an F_0 value of 6 min was found to be sufficient The come-up time of wet ingredients incorporated fish ball in curry product packed in retort pouch to achieve a processing temperature of 115^0C was found to be 50 minutes, cooling period of 23 minutes was noted and the total process value was determined to be 74 minutes at 115^0C. The come-up time of plain fish ball in curry product packed in retort pouch to achieve

a processing temperature of 115^{0}C was found to be 51 minutes, cooling period of 22 minutes was noted. The total process period at 115 ^{0}C was found to be 77 minutes. The come-up time of plain fish balls packed in retort pouch to achieve a processing temperature of 115^{0}C was found to be 74 minutes, cooling period of 16 minutes was noted and the total process period was found to be 90 minutes at 115^{0}C. The come-up time of curry paste incorporated fish balls packed in retort pouch to achieve a processing temperature of 115^{0}C was found to be 73 minutes, cooling period of 20 minutes was noted and the total process period was found to be 93 minutes at 115 ^{0}C Of the five methods tried, three methods, i.e., effect of setting, effect of different types of starch and different levels of transglutaminase enzyme did not improve the qualities of fish ball in curry after thermal processing, as compared to steamed ones. In all these methods, the values of pH, folding test grades and organoleptic qualities were found to be decreased; the values for moisture and expressible water percentage were found to be increased after thermal processing.

Similar observations have been made by Saralaya *et al.* (1978) for canned fish sausages in brine, oil pack and dry pack and Runglerdkriangkrai *et al.* (2006) for canned fish balls in brine.

In the case of fish ball prepared with different levels of wet ingredients, the steamed fish balls (control) prepared with normal procedure was found to better as compared to the thermally processed fish balls prepared with different levels of wet ingredients. However, the fish balls prepared with lower levels of wet ingredients were found to be as good as steamed fish balls (control).

The pH of fish balls prepared with different levels of wet ingredients did not show much variation in the values of pH ranging from 6.01 ~ 6.4. It can be seen that there was a slight increase in pH as the levels of wet ingredients reduced. This may be due to the increase in the amount of moisture content and reduction in the quantity of tomato paste, thereby increasing the pH value to the alkaline side. Balange (1999), Desai (2003) and Temburne (2005), reported a pH of 5.9, 5.98 and 5.98 respectively for the fish ball in curry product, which were steamed (100^{0}C, 0 psi).

The moisture content of fish ball paste prepared with different levels of wet ingredients did not show much variation with the values ranging from 59 ~ 64%. However, the moisture content of fish balls was found to be increased after steaming. Similar trends were also observed in the case of thermally processed fish balls. This increase in moisture content may be due to the absorption of moisture from the curry.

As the quantity of wet ingredients reduced, there was a slight increase in the moisture content. This may be due to the relative increase in the quantity of surimi, as the quantity of wet ingredients were reduced, thereby resulting in the relative increase in moisture content.

With the reduction in the wet ingredients, there was found to be a decreasing trend in the expressible water percentage. The decrease was very slight in the initial stages followed by a steep decrease in the last two stages.

These results correlate well with the trends observed for folding test grades. It may be possible that the higher percentage of moisture present in the samples with higher content of wet ingredients, might interfere the formation of viscous paste and subsequent high gel products. With the higher level of moisture as well as non protein component, the myosin component become relatively lesser in quantity, leading to a low gel product with a high expressible water percentage (Suzuki,1981; Shahidi and Botta, 1994).

Unlike in the earlier experiment, it was found that there was not much decrease in the expressible water percentage (3.9 and 4 ~ 4.1% for control and samples with low and zero level of wet ingredients).

The present investigation indicates that there was not much difference in the folding test grades of fishballs subjected to thermal processing when the level of wet ingredients were reduced from 87.49 to 60.75g. However, with the further reduction there was improvement in the folding test grades of fish balls showing higher grades similar to that of control (steamed at 100 ^{o}C, 0 psi for 30 minutes). This trend is reflected in the trend observed for the expressible water percentage. This may be due to the higher levels of moisture added by way of wet ingredients of curry paste contributing to the lower folding test grades. In this connection, the criteria chosen for deciding the grades of surimi can be considered as a factor affecting the quality of the product. Among these factors, moisture content of surimi is an important parameter affecting the gel strength of kamaboko and different grades of surimi are classified based on the moisture content as super class, first class, second class and off grade (Suzuki, 1981).

Similar reports on the effect of high moisture content leading to difficulty in the preparation of meat paste from *Acetes* having low viscosity and also in moulding have been noted by Patil (2000). Bhatkar (1998) also noted similar problem while standardizing the level of moisture (10 ~ 50%) in the fish chikuwa paste subjected to microwave pasteurisation and arrived at an optimum level of moisture i.e., 35% of the fish chikuwa paste mixture.

The decrease in the folding test grades and increase in the expressible water percentage of fish balls after subjecting to thermal processing may be attributed to the loss of total SH plus SS groups which occurs by oxidation to cysteic acid or splitting to hydrogen sulphide (Nakai and Li-Chan, 1988; Yamazawa *et al*., 1979). H_2S formation from the free reacting SH groups of actomyosin starts at about 80^0C and increases exponentially with rising temperature (Hamm and Hofmann, 1965).

Organoleptic evaluation indicated that with the decrease in the content of wet ingredients incorporated in ball, there was a gradual increase in textural scores up to 34.5 g and with the further reduction, textural scores were higher. The last sample without the incorporation of wet ingredients had scores almost same as those of control i.e., steamed (100 °C, 0 psi) fish balls. This trend correlates very well with the trends observed for folding test grade and expressible water percentage.

However, the scores of taste, odor and color were significantly affected as the content of wet ingredients decreased in the fish balls. The scores of taste, odor and color were highest with the fish balls with higher content of wet ingredients unlike those with the reduced levels and particularly low scores for those without incorporation of wet ingredients.

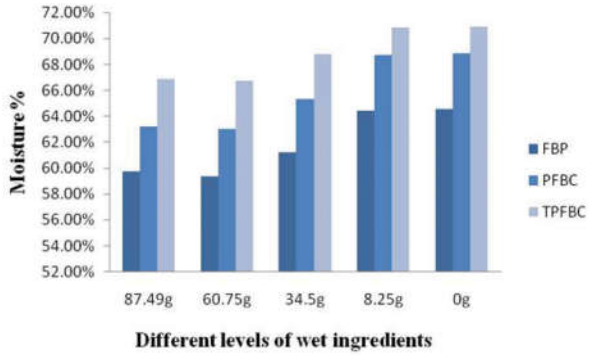

Fig. 1: Moisture percentage of fish ball product with different levels of wet ingredients

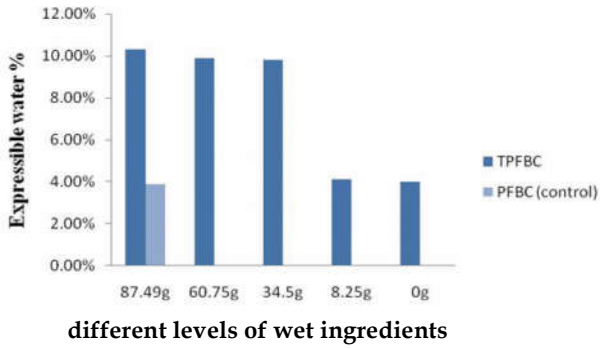

Fig. 2: Expressible water percentage of fish ball product with different levels of wet ingredients

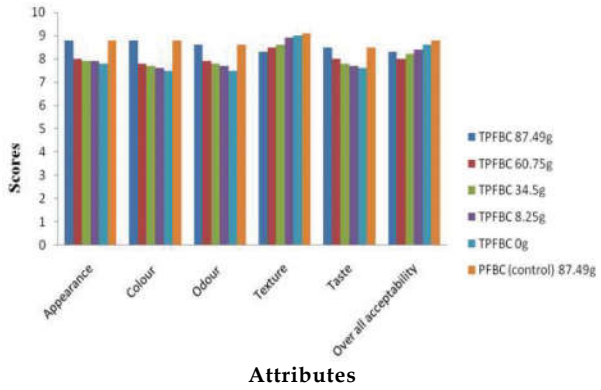

Fig. 3: Organoleptic evaluation of fish ball product with different levels of wet ingredients

In the case of different types of ingredients and types of pack, it was found that, the pH of plain fish balls were higher than the fish balls with wet ingredients. The pH of plain fish ball, thermally processed without curry was found to be higher. This may be due to the absence of tomato paste and increase in the relative concentration of surimi, thereby resulting an increase in the moisture content. The pH of plain fish balls after thermal processing with liquid curry was found to be decreased which may be due to the absorption of organic acids from the curry. Similar trend have been observed in the case of wet ingredients also. The pH of fish balls was found to be decreased on the acidic side after thermal processing without curry and it may be due to the decrease in moisture content of fish balls. The pH of fish balls prepared with dry ingredients was found to be lower after thermal processing. The reason may be the contribution of organic acids by the tomato paste and low moisture content. Balange (1999), Desai (2003) and Temburne (2005), reported a pH of 5.9, 5.98 and 5.98 respectively for the fish ball in curry product, which were steamed (100^0C, 0 psi).

The moisture content of thermally processed fish balls with wet ingredients packed along with curry showed an increase in moisture content as compared to the fish balls which were steamed at 100^0C, 0 psi for 30 minutes. However, the moisture content of fish balls with wet ingredients, subjected to thermal processing without curry showed a decrement in the moisture content. The higher levels of moisture in wet ingredients incorporated fish balls packed along with curry, and thermally processed, might be due to the entry of moisture from the curry. Similar trends have been observed in the case of plain fish balls, however, the moisture content in plain fish balls was higher as compared to that of fish balls with wet ingredients. This could be due to the relative increase of surimi as a consequence of no addition of wet ingredients and thereby increase in the moisture content in plain fish balls as compared to fish balls

with wet ingredients. The moisture content present in steamed fish balls with dry ingredients was higher as compared to the other two types. This might be due to the uptake of moisture by the dry ingredients incorporated fish balls from the curry. However, the moisture content of fish balls with dry ingredients packed with curry in retort pouch decreased after subjecting to thermal processing which may be due to the lack of high gel strength forming ability of fish balls and thereby reducing the capacity to hold the moisture.

The expressible water percentage of fish balls indicate that plain balls with and without curry and fish balls with wet ingredients but without curry had lower expressible water percentage as compared to the fish balls with wet ingredients along curry. This may be due to the absorption of moisture from the curry thereby reducing the gel strength and resulting in higher expressible water percentage.

In the case of fish balls prepared with dry ingredients, the expressible water percentage values were very high and the organoleptic scores were very low and the panelists indicated that the texture was very soft. This may be attributed to the fact that with the increase in non-muscle protein components, there is relative decrease in myosin group of proteins and consequently the gel strength was lower; also the dried components which were added in large quantity having lost their functional properties may not contribute to the proper emulsion formation, and thereby interfere with the emulsion formed by the fish myosin group of proteins and affect the gel strength consequently. This might have reduced the ability to hold the moisture resulting in higher values of expressible water percentage (Shahidi and Botta, 1994).

The folding test grades of fish balls indicate that the plain fish balls with and without filling medium and fish balls with wet ingredients but without filling medium had high folding test grades as compared to the fish balls with wet ingredients along with curry. This may be due to the absorption of themoisture from the curry resulting in lower folding test grades. The plain balls had higher pH and without the curry paste ingredients, there was no contribution of organic acid from tomato and moisture from the wet ingredients of curry paste. This would have created an optimum pH of 7.2-7.3 and an optimum moisture level (super A class 79%) in the surimi. Similar justification can be attributed for higher folding test grades and lower expressible water percentage of plain balls with curry and wet ingredients but with curry. Apart from this, plain fish balls and wet ingredients incorporated fish balls did not show uptake of moisture leading to optimum moisture level.

Saralaya *et al*. (1978) reported a decrease in gel strength of canned fish sausages in natural casing (processed at 115.6 ^{o}C for 75, 45, 60 minutes for dry pack, wet pack and oil pack respectively). The difference in this and the present study may be due to the difference in preliminary treatment, composition, type of filling medium.

The decrease in the folding test grades and increase in the expressible water percentage of fish balls after subjecting to thermal processing may be attributed to the loss of total SH plus SS groups which occurs by oxidation to cysteic acid or splitting to hydrogen sulphide (Nakai and Li-Chan, 1988; Yamazawa *et al*., 1979). H_2S formation originated from the free reacting SH groups of actomyosin starts at about 80^{o}C and increases exponentially with rising temperature (Hamm and Hofmann, 1965).

The above factor responsible for gel strength reduction due to thermal processing may not be operational in plain balls, plain balls with curry and curry paste incorporated fish balls where an environment of reduced moisture level exists due to the entrapment of the moisture within the gel matrix.

In the case of fish balls prepared with dry ingredients, the scores were very low and organoleptic scores were very low and the panelists indicated that the texture was very soft.

The organoleptic scores indicated that the textural scores of plain balls with & without curry and wet ingredients incorporated fish balls but without curry were higher compared to other samples. However, the textural scores of these fish balls were slightly lower than those of steamed (100^{o}C, 0 psi) fish balls, i.e., the corresponding control samples (9.80-9.82).

Saralaya *et al*. (1978) reported that the textural score of canned pink perch fish sausage (in natural casing) had lower values, indicates fair quality. The decrease in the gel strength of fish balls after subjecting to thermal processing may be attributed to the loss of total SH plus SS groups which occur by oxidation to cysteic acid or splitting to hydrogen sulphide (Nakai and Li-Chan, 1988; Yamazawa *et al*., 1979).

Runglerdkriangkrai *et al*. (2006) also reported a decrease in textural scores of fish balls processed at 116^{o}C for 30 minutes as compared to steamed (unsterilized) samples.

The organoleptic scores of appearance, taste, odor and color for plain fish balls with and without curry were lower as compared to fish balls with wet ingredients. This may be due to the less quantity of wet ingredients added in it, thereby reducing the taste and odor. The organoleptic scores of fish balls prepared with dry ingredients were lower as

compared to fish balls with wet ingredients and plain balls. The scores of texture, odor and taste were very low and the panelists indicated that the fish balls were softer in texture, not good in taste, with an unfavorable odor.

The overall acceptability scores were found to be higher in the case of plain balls without curry followed by plain fish balls with curry, wet ingredients incorporated fish balls without curry and wet ingredients incorporated fish balls with curry. The overall acceptability scores of fish balls with dry ingredients were found to be lower. Although wet ingredients incorporated fish balls in curry had lower textural values (A grade in folding test and organoleptic textural score of 8.4), it was liked by the panelists as they preferred soft texture to rubbery ones.

Based on this studies, it can be concluded that plain fish balls (dry pack), plain fish balls in curry (wet pack), curry paste incorporated fish balls processed (dry pack) processed in retort pouch at 115^0 C for 45 minutes were found to retain the textural scores. However, the scores were slightly lower than the steamed samples. Curry paste incorporated fish balls with curry had moderately good textural scores but were lower than the above

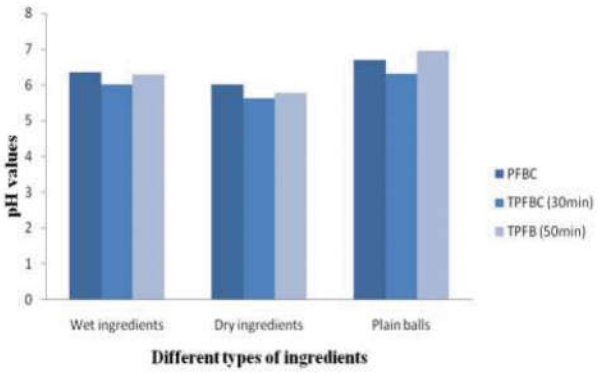

Fig. 4: pH of fish ball product with different types of ingredients

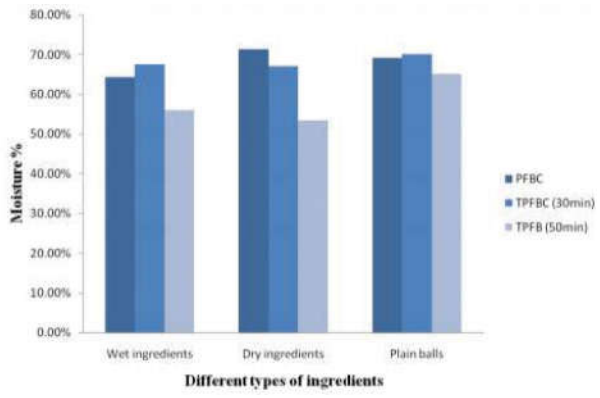

Fig. 5: Moisture content of fish ball product with different types of ingredients

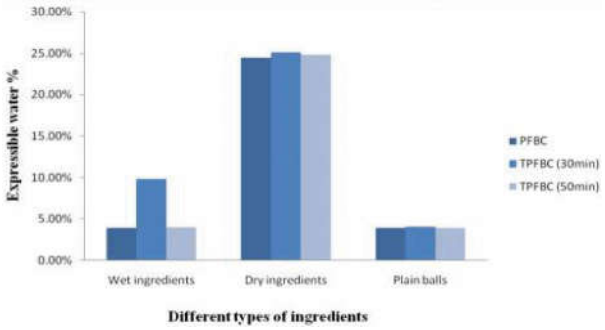

Fig. 6: Expressible water percentage of fish ball product with different types of ingredients

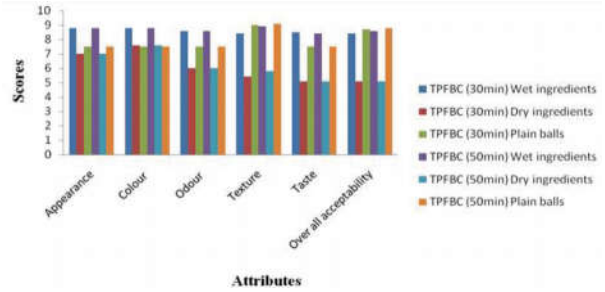

Fig. 7: Organoleptic evaluation of fish ball product with different types of ingredients

three samples. The textural scores of fish balls of the above four types were lower than the fish balls steamed. Hence the all fish balls of the above types can be used for manufacture and sale bye the industry depending upon the choice of consumer in respect to textural scores. Plain fish balls can be improved further with respect to other quality characteristic like taste and color by addition of oleoresins and natural red color capsicum in the fish ball paste instead of the curry paste ingredients. Although wet ingredients incorporated fish balls in curry had lower textural values, it was liked by the panelists as they preferred soft texture to rubbery ones.

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Physio-biochemical alterations due to stress in poultry

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Physio-Biochemical
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Abstract

Stress is inevitable for poultry even in state of the art facilities available in modern poultry industry. Birds have limited body resources for growth, reproduction as well as to cope with different environmental stressors. So, they seem to be particularly sensitive to different stressors including the environmental ones resulting alteration in their behavior and physiological responses. Present manuscript describes the different types of stressors and their effect on physio-biochemical parameters along with physiological mechanism to cope with stress.

Introduction

Stress, a response to adverse stimuli, is difficult to define and understand because of its nebulous perception. The word 'stress' is derived from the latin word 'strengene' which means to draw tight. Stress has been defined by several workers. According to Webster's collegiate dictionary (1981), stress is physical, chemical or emotional factors that cause bodily of mental disturbance and may lead to disease condition finally if not taken care immediately. As per Dobson and Smith (2000), it is the inability of an animal to cope up with its environment, a phenomenon which is often reflected in a failure to achieve genetic potential. Rosales (1994) defined stress as the cumulative detrimental effect of various factors on health and performance of animals. In fact, stress represents the reaction of body to stimuli that disturb normal physiological equilibrium or homeostasis, often with detrimental effects as shown by Khansari et al. (1990). According to Stott (1981), stress is the result of environmental forces continuously acting upon animals which disrupt homeostasis resulting in new adaptations that can be detrimental or advantageous to the animal.

Nonetheless, the most acceptable definition is that "stress is the nonspecific response of the body to any demand", whereas stressor can be defined as "an agent that produces stress at any time". Therefore, stress represents a biological response of body to stimuli that disturb its normal physiological equilibrium or homeostasis.

Generally, stress is used to describe the detrimental effects of variety of stressors on the health and productive performance of poultry. However, it is not always harmful unlike general perception and belief. Birds have limited body resources for growth, reproduction as well as to cope with different environmental stressors (Rosales, 1994). So, poultry seems to be particularly sensitive to different stressors including the environmental ones resulting alteration in their behavior and physiological responses. Further, under the stress conditions, there is redistribution of body resources including energy and protein at the cost of decreased growth, reproduction and health (Beck, 1991). Hence, understanding the physio-biochemical alteration is crucial for devising control measures to combat stress for successful poultry production and welfare.

Types of stress in poultry

Stress is inevitable for poultry even in state of the

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art facilities available in modern poultry industry. The common sources of stress, which can be grouped under, one or more of the following categories (Freeman, 1987).

i. Climatic stress (extreme heat and cold, high humidity)
ii. Environmental stress (bright light, wet litter, poor ventilation)
iii. Nutritional stress (shortages of nutrients, feed intake problems)
iv. Physiological stress (rapid growth, process of maturing sexually)
v. Physical stress (catching, immobilization, injections, transport)
vi. Social stress (overcrowding, poor body weight uniformity)
vii. Psychological stress (fear, harsh care takers)
viii. Pathological stress: Exposure to infectious agents is a common source of stress, however challenges may not result in overt disease. When sub-clinical infections due to poor bio-security and sanitation persist, excessive activation of the immune system will result in a condition known as immunological stress. This condition results in a series of changes in nutrient metabolism induced by mediators of the immune response.

In addition to the categories of stress mentioned above, all the possible types of stressors can be broadly classified under two categories (a) avoidable stressors (b) unavoidable stressors

Avoidable stressors

Overcrowding, poor ventilation, wet litter, toxins in feed, Starvation, high ammonia level, dehydration, poor management etc.

Un-avoidable stressors

Extreme weather, handling, vaccination, transportation, rapid growth, debeaking, lighting medication etc.

It is to be noted that effective stress management involves complete elimination of avoidable stressors and minimizing the load of unavoidable stressors on the birds.

High ambient temperature in the tropics, like that of ours in India accompanied by high relative humidity is one of the most important stressor. Birds are more susceptible to high environmental temperature than low environmental temperature due to absence of sweat glands in the feathered body, fatty nature and high body temperature (40.1 0 C to 41.6 0C). The degree of susceptibility to tropical heat stress is higher in broilers than layers. Among broilers males are more susceptible to heat stress than females (Marin, et.al.2002). Good layers housed in cages are more susceptible than poor layers reared on deep litter.

Common causes of stress in poultry

Some of the most common causes of stress in poultry as categorized by Rosales (1994) are summarized as follows:

a. Poor brooding conditions (low temperatures, cold water)
b. Contaminated premises (built-up litter, early exposure to various disease agents)
c. High stocking density (limited feeder and dringker space)
d. Temperature exteremes (cold and heat)
e. Handling, weighing, vaccination, grading and transport (pain, physical damage)
f. Beak trimming (handing pain)
g. Lack of body weight uniformity (magnified differences in the packing order)
h. Rapid growth (Strict nutrient demand)
i. Quantitative feed and water restrictions (frustration, hunger)
j. Postvaccinal reactions (reduced feed intake, fever)
k. Feed quality problems (variation in nutrient content)
l. Long or uneven feed distribution (split feeding)
m. Sex separate feeding (pressure to restrict body weight gains)
n. Harsh caretakers (poor husbandry)
o. Inadequate ventilation (deterioration of the air quality)
p. Clinical or subclinical diseases (reduced feed intake, fever, pain)
q. Poor litter conditions (wet and cold)
r. Sexual maturity and onset of egg production (drastic stimulation with feed and light)

Physio-biochemical alteration due to stress in poultry

Several workers have reviewed the effect of stressors in fowl (Brown 1967, Freeman, 1971). They recoded the following physio-biochemical alteration in poultry:

i. Atrophy of the thymus and atrophy of the bursa of fabrics in young birds,
ii. Enlargements of the anterior pituitary and the adrenal glands.
iii. Depletion of the adrenal cholesterol.
iv. A rise level of plasma corticosterone, insulin or glucagon.
v. Increased reliance on glucose as an energy source.
vi. Hypoglycemia (increased glucose utilization).
vii. Decreased growth and increased muscle degradation.
viii. Release of acute-phase cytokines (monokynes and lymphokynes)
ix. Impaired growth of cartilage and bone.
x. Synthesis of specific heat shock proteins.
xi. Decreased voluntary feed intake (anorexia)
xii. Increased body temperature
xiii. Changes in the level of plasma metabolites (e.g. glucose, tryglyceride, non-estrified fatty acids and lactate). Epinephrine content in yolk of donor hens also serves as a very good tool to reflect stress load in layer stock.
xiv. Changes in the numbers of circulating leucocytes profiles (heterophil: lymphocyte ratios and basophil and eosinophil numbers).
xv. Immunosuppression
xvi. Excess fat deposition in the abdomen (abdominal fat pad).
xvii. Ascites (water belly) in high producing broilers.

Physiological mechanism of stress regulation in poultry

Exposure of birds to stress is an inevitable event in poultry husbandry, when the threshold level of stress is crossed it results in distress to birds. Then the birds show stress syndromes, which are classified into three stages.

1. Stage of alarm reaction (Neurogenic system).
2. Stage of resistance or adaptation (Endocrine system).
3. Stage of exhaustion.

Neurogenic (sympatho- adrenal) system (Short-term regulation of stress)

This system consists of sympathetic (post ganglionic) nervous system and adrenal medullary tissue. It controls the rapid response of the birds i.e. fight or flight or alarm (emergency) reaction. This reaction lasts only a short time. It is characterized by increased rates secretion of the catecholamine from the adrenal medulla. These catecholamines prepare the bird for "Fight or Flight" reaction and commanding a rapid release of glucose in blood, depletion of liver glycogen, increased peripheral vasomotor activity, altered ventilation rate and increased neural sensitivity (Siegel. 1980). Catecholamines also stimulate the activity of hepatic adenyl cyclase, the enzyme required for the production of cAMP (Robinson and Sutherland, 1971). cAMP regulates the number of energy reaction (physiological processes) and directly increases the formation of antibody (Braun et al. 1971).

Endrocrine system (Long-term regulation of stress)

Involvement of endocrine system in stress regulation is called the 'stage of resistance'. This system is comprised of hypothalamus-pituitary adrenal axis (HPA). It is characterized by adrenal cortical hypertrophy and increased synthesis and release of adrenal glucocorticoids, known as corticosterone in bird (Siegel, 1980). Activation of the HPA is a longer-term adjustment by the animal to the surrounding changes. Selye (1936) called it General Adaptation Syndrome (GAS).

The endocrine mechanism of stress regulation is started with the stimulation of hypothalamus and release of ACTH from anterior pituitary, which causes the increase of adrenal cortical steroid secretions. Continuous stimulation to adrenal cortex leads to chronically high levels of corticosteroid hormone. This hormone is responsible for the formation of glucose from body's reserve of carbohydrates, lipid and proteins. Corticosteroids contribute to many of the disease associated with long-term stress, such as, cardiovascular and gastronistestinal disease, hypercholesteraemla, metabolic rearrangements and antibody suppression. (Siegel, 1985).

Other hormones

i) Glucagon: The á cells of the pancreas are the source of glucagon, are stimulated in alarm response in both mammals and birds (Freeman. 1980).
ii) Thyroid hormone: Hormone produced by thyroid glands are also involved in stress regulation (Siegel, 1980).

Stage of exhaustion

Finally, if the bird does not recover from the stressor

and the availability of body reserves and hormones from the adrenal gland are inadequate, a third or exhaustion phase leads to fatigue of the homeostatic mechanisms and death (Maxwell,1993).

Future course of stress managemental practices in poultry

Research work in the area of stress physiology should be directed in future in the following directions.

Need good indicator of physiological stress

The physiological indicator of stress such as atrophy of the thymus and bursa of fabrics in young birds, enlargements of the anterior pituitary and the adrenal glands are good indicator of stress but there are inherent problems with their detections. These organs cannot be weighed in live birds and require slaughter of the animal. Therefore, a suitable technique of physiological indicator of stress is currently needed.

Suitability of the technique

Suitability of technique is also an important factor, particularly for blood sampling of bird for hormone estimation. Practical problems can be associated with techniques themselves.

Mechanism of stress

To study the mechanism of stress (climatic and environmental stress) laboratories should be strengthen with some specific facilities like climatic chamber.

Identification of a physiological cue

initiating the vicious cycle of events in birds that are under stress.

Amelioration of stress in birds at physiological level

Development of a anti-stress kit for birds.

Suitable managemental practices

The production efficiency of poultry is severely affected by various kind of stressors. It has significant effect on economic production of poultry. Appropriate managemental practices can be investigated to reduce the different type of stress in poultry and getting the best returns from them. The research work should begin right from construction of poultry shed, along with feeding, disease control and other managemental options.

Conclusion

The goal of poultry scientist should be to strike a balance between the hypo-and hyper-stress and to find as much eustress as possible and to minimize distress. While devising strategies for stress control, it is necessary to eliminate all avoidable forms of stressors and maintaining unavoidable stressors under control. Thus, the ultimate aim of successful poultry husbandry is not eliminate stress but to maintain it at optimum level for good production efficiency. A targeted multi-disciplinary futuristic approach is advocated so that the problem of stress is well tackled.

Multifarious efforts should be made to develop suitable technology to overcome the problem of follicular atresia as this is one of the main channels responsible for the drop in egg production under stressful conditions.

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