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Diagnosis and Therapeutic Management of Diabetes Mellitus in Canine

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ABSTRACT

Diabetes mellitus (DM) is a common endocrine disorder affecting both canines and felines, characterized by persistent hyperglycemia and glucosuria due to either decreased production or decreased action of insulin or both. In this study, during the period of two years, a total of 2500 dogs were presented to the clinics and out of these 254 dogs were having primary complaint of polyuria and polydipsia. Among these a vast majority of the dogs were diagnosed with chronic kidney disease (58.2%) followed by hepatic disease (24.4%) and pyometra (09%) and only seven animals (2.7%) were diagnosed with DM based on persistent hyperglycemia (390.71mg/dL) and glucosuria (3+). Majority of these animals were females (n=5) and rest were males (n=2). The most commonly presented breeds were Dachshund (57.1%) followed by Labrador retriever (28.5%), pug and Shih-tzu (14.2% each) respectively. Most prevalent age group during this study was 4-5 years as all the females affected were falling in this age group. Rest of the two males were of 09 and 11 yrs, respectively. Clinical signs in these dogs included polyuria, polydipsia, weight loss in all the animals. Inappetence (71.4%), polyphagia (28.5%) and vomiting (28.5%) were seen uncommonly. Complications associated with DM in this study include cystitis (28.5%) and cataract (28.5%), respectively. Treatment was done based on American Animal Hospital Association (AAHA) guidelines with exogenous administration of insulin along with strict dietary management and implementation of exercise in routine schedules. Remission time in these dogs was 15 d and average dose of insulin was 0.63 IU/kg. The present study concluded the need of more elaborative studies as the present sample size was less and the importance of dietary management and exercise among pet owner.

Keywords: Cataract, Diabetes mellitus , Polyuria, Polydipsia, Polyphagia, Weight loss.

INTRODUCTION

Diabetes mellitus is defined as a complex endocrine disorder characterized by chronic hyperglycemia and glucosuria resulting either from decreased production or decreased action of insulin or both (Niessen *et al*, 2022). Hyperglycemia is defined as an increased blood glucose concentration of more than 125 mg/dL, but clinical signs are usually not detectable until the renal tubular resorption threshold for glucose is exceeded, *i.e.,* more than 180 mg/dL. DM is a common endocrinopathic disorder among dogs and is characterized by polyuria, polydipsia, polyphagia, and weight loss. Apart from these clinical signs, associated complications include cystitis, latrogenic hypoglycemia, diabetic neuropathy, diabetic nephropathy, and cataract formation (Nelson and Couto, 2019). The estimated prevalence of DM among the dog population ranges between 0·0005 and 1·5 per cent (Wilkinson, 1960).

Etiology among the dogs tends to be multifactorial, with beta cell loss, particularly due to immune-mediated mechanisms, vacuolar degeneration, and pancreatitis as the major ones (Haritha *et al,* 2024; Davison *et al,* 2003). Apart from these genetic predispositions, infection, insulin-antagonistic drugs, and obesity have also been identified as causal factors. The result among the majority of these is loss of beta cells. Genetic predisposition is proved by the fact that certain breeds of the dogs are at increased risk of developing DM as compared to the other breeds with Samoyeds, Miniature Schnauzers, Miniature Poodles, Pugs, and Toy Poodles being at greater risk of developing DM, while lower risk was associated with certain breeds like German Shepherd Dog, Golden Retriever, and American Pit Bull Terrier (Hess *et al*, 2000)

Not only breed predisposition, but also gender and the age group affected help in elucidating the cause of the disease. Intact female dogs and neutered males were at increased risk of developing DM as compared

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to the intact males (Marmor *et al*, 1982; Heeley *et al*, 2020). Dogs more than 8 yrs of age have increased odds of developing the disease (Heeley *et al*, 2020). Among the intact females, insulin resistance serves as a major cause of DM, particularly due to the effects of the diestrus phase. This, in turn, is proved by the fact that ovariohysterectomy removes the source of progesterone and improves the tissue responsiveness to insulin. The classification system is similar to the one used among the human population. Type 1 insulin-dependent DM is the most common among dogs. Type 2 Insulin-independent DM is not clinically recognized among the dogs.

Despite a multifactorial etiology, the clinical signs in the vast majority of the dogs with DM include polyuria, polydipsia, polyphagia, and weight loss. Increased fat mobilization leads to hepatic lipidosis and hepatomegaly. If the dog is not treated for the primary causes, a protracted course of the disease will lead to ketonemia, ketonuria, and in severe cases, Diabetic ketoacidosis. Treatment involves lifelong administration of Insulin as the vast majority of the dogs are suffering from type 1 DM. Apart from Insulin administration, reducing insulin resistance in obese animals and in intact females has an important role in the management of DM. Mean survival time after the onset of diagnosis in dogs is up to 2 years of age, but with adequate glycemic control and dietary management, a good quality of life can be sustained up to 5 years of age (Sinha *et al,* 2024; Fall *et al*, 2007). The present study was conducted to determine the prevalence of diabetes mellitus among dogs presented with polyuria and polydipsia and to evaluate the associated demographic factors, clinical signs, complications, and therapeutic response following insulin therapy and management as per AAHA guidelines.

MATERIALS AND METHODS

This study was conducted over a period of 2 yrs at the Veterinary Polyclinic, Faridkot, on dogs presented with the history of polyuria and polydipsia. A total of 2500 dogs were presented over a period of 2 yrs. Among these animals, 254 animals were presented with the history of polyuria and polydipsia. Anamnesis, physical examination, and routine workup, including CBC, Liver function test (LFT), Kidney function test (KFT), Blood glucose, routine urinalysis, along with urine sediment examination and radiography, were done to elucidate the cause of the disease. Complete physical examination, along with the signalment recording, was performed in all these animals to determine the exact cause of the disease. Routine blood workup, urinalysis, and radiography were done using the recommended methods from the local laboratories. The majority of these dogs were diagnosed with chronic kidney disease (n=148), followed by hepatic disease (n=62), pyometra (n=23), and DM (n=07). In the rest of the cases (n=14), etiology was not established. Apart from the dogs diagnosed with DM, the rest were treated using the appropriate guidelines provided. Dogs in which the etiology was not established were referred to the referral hospital to determine the cause of the disease.

Dogs diagnosed with DM were selected, and various parameters, including age, sex, breed, clinical signs such as polyuria, polydipsia, polyphagia, weight loss were noted. Apart from the associated clinical signs, complications such as cataract, urinary tract infection and iatrogenic hypoglycemia, if developed, were noted. Diagnosis of DM was confirmed based on persistent hyperglycemia and glucosuria; because the more reliable test, *i.e.,* serum fructosamine, was not available at the local laboratories. Blood glucose estimation was done by collecting venous blood from restrained animal and submitted to the local laboratory using appropriate methods. Urine in these animals was collected on free catch basis and in animals suspected for lower urinary tract disease urine collection was done by cystocentesis. These urine samples were then subjected to dipstick analysis and sediment examination. Treatment in the affected animals was done using the recommended guidelines (Behrend *et al*, 2022) and response to treatment, along with remission time, was recorded. Owners were trained for subcutaneous administration of Insulin and blood glucose estimation using the routinely available glucometer (Accu-check Instant). Remission was said to occur when the polyuria and polydipsia have improved, and the dog has started to gain weight. All the above findings were noted down in a Microsoft Excel worksheet, and the descriptive analysis was performed.

RESULTS AND DISCUSSION

A total of 2500 dogs were examined during a period of two years, and out of these animals, 254 were screened because of the primary complaint of polyuria and polydipsia. A vast majority of these cases were diagnosed to be of chronic kidney disease (58.2%) based on the laboratory findings, such as elevated BUN and Creatinine values, along with anemia, hyperphosphatemia, and urinalysis findings. These findings were later confirmed using the ultrasonographic findings, which in the majority of the dogs revealed loss of cortico-medullary differentiation.

The second most common etiology for the presenting clinical signs was hepatic disease (24.4%), which was suspected based on the clinical signs and the findings of the liver function test and later confirmed using the USG findings from the referral hospitals. Pyometra (09%) is the other cause in dogs presented with polyuria and polydipsia. Pyometra in dogs was confirmed primarily based on the findings of CBC, such as leucocytosis and radiography. In a dog in which radiographic findings were not evident, USG was advised to confirm the diagnosis.

Among the 2500 dogs examined, a total of 7 dogs were diagnosed with DM, with a prevalence rate of 0.28%. These findings were in accordance with the previously done studies of Wilkinson (1960) which stated prevalence in the range of 0·0005 and 1·5 per cent. Among the dogs presented, majority were females (71.4%), and the rest were males (28.6%). These findings were in agreement with the previously done studies of Heeley *et al*, (2020) according to which 48.2% of the animals presented were females. As far as the age is concerned, the majority of the dogs presented were 04 to 05 years of age (n=05), and the rest of the dogs (n=02) were 09 and 11 years respectively. All the dogs within the age group of 4-5 years of age were females, and the rest of the dogs, which were more than 09 years of age, were male. These findings were in contrast to the previous studies of Heeley *et al*, 2020 which showed that increased odds were associated with animals more than 8 years of age. The possible reason in these dogs might be due to the intact nature of females, in which the diestrus phase leads to insulin resistance (Nelson and Cuoto, 2019). Whereas in males, the findings were in association with the previous studies (Heeley *et al*, 2020).

The most commonly presented breeds during this study were the Dachshund (57.1%), followed by the Labrador retriever (28.5%), Pugand Shih-tzu (14.2% each) respectively. These findings were in contrast to studies of Hess *et al*, (2000) in which Samoyeds, Miniature Schnauzers, Miniature Poodles, Pugs, and Toy Poodles were at greater risk of developing DM. The possible reason may be due to the overpresentation of Dachshund at clinic. Clinical signs observed in these dogs were polyuria, polydipsia, polyphagia, and weight loss. Polyuria, polydipsia, and weight loss were present in all seven dogs presented, whereas polyphagia was seen in only two dogs (28.57%), and the rest had inappetence (71.42%). These findings were in accordance with the previously done studies of Plotnick and Greco (1995) which stated presence of polydipsia in 93% of dogs, polyuria in 77%, weight loss in 44% and polyphagia in only 19% of the dogs. The low prevalence of polyphagia in these animals may be due to concurrent ketoacidosis (Plotnick and Greco, 1995).

Cataract formation was seen in only two dogs (28.57%), and the possible reason for this might be due to the advanced nature of the disease. Whereas vomiting at the time of presentation was seen in two dogs (28.57%). The presence of Gastrointestinal signs, such as inappetence and vomiting, may be associated with concurrent pancreatitis and cholangio-hepatitis (Greco, 2001). Diagnosis in these animals was based on persistent hyperglycemia along with glucosuria. The more reliable test, i.e., serum fructosamine, cannot be evaluated due to the unavailability of this test in the local area. The average blood glucose concentration in these dogs was 390.71 mg/dL, whereas in the majority of the dogs, the urinalysis findings suggested a 3+ concentration of glucose in the urine. These findings of concurrent glucosuria and hyperglycemia have been used for a long time in the diagnosis of DM and are also the test of choice in field conditions, where the availability of newer and more reliable tests is sparse. The possible reason for glucosuria suggests the presence of hyperglycemia, which exceeds the renal tubular resorption threshold.

Urine sediment examination was also performed in addition to routine urinalysis in all the dogs to know whether concurrent lower UTI disease is present or not. Only two dogs (28.57%) were having lower urinary tract disease. These animals in addition to lower urinary tract disease signs such as stranguria, hematuria and pollakiuria were also having elevated pus cell count/hpf in the sediment. Most of these dogs, particularly during the early phase of treatment, also developed the other complication of DM i.e., iatrogenic hypoglycemia which was already explained to the owner and was managed at home by the owner using the application of sugar solution/honey to the gums. The possible reason could be the higher dosage of Insulin, particularly during the initial phases of treatment when the dosage of insulin is not completely set (Idowu and Heading, 2018). Dogs, upon diagnosis, were treated based on the guidelines provided by AAHA. Treatment involves exogenous administration of insulin along with dietary modification and incorporation of exercise in the routine schedule of dogs to overcome obesity and any type of insulin resistance if present (Behrend *et al,* 2022). All the dogs were initially treated using intermediate-acting Insulin preparation (NPH) @ 0.5 IU/kg body weight bid.

In most of the dogs (85.7%), remission was seen by using this product, whereas in one dog, Lente insulin was used due to the non-remission using the NPH insulin. Remission time in dogs after using Insulin was 15 days, and the average dosage was 0.63 IU/kg. This remission was achieved after constant glucose monitoring at home using the commercially available glucometers (Accu-check instant). Apart from exogenous insulin administration, owners were advised for dietary modification, which was entirely based on AAHA guidelines. It involves either feeding the commercially available feeds for diabetic dogs, and those who can't handle the expenses of the commercially available diet were advised to restrict caloric intake and increase expenditure through exercise. Increasing the fibre content of the feed serves as a central point in managing DM (Behrend *et al*, 2022). All of these animals are followed up on since the onset of diagnosis. Despite the proper treatment, two of these animals succumbed to the disease with a case fatality rate of 28.57%. Both of these dogs died within 1.5 years of diagnosis of the disease. These findings were in agreement with the previous studies done of Fall *et al* (2007) which stated median survival time of two years after the onset of diagnosis. This study was done to check the prevalence of DM over a period of two years, but the sample size over a period of two years was quite small, necessitating the need for further studies to be conducted to know the prevalence and the causal factors associated with the onset of the disease.

CONCLUSION

Diabetes mellitus in dogs is a common endocrine disorder characterised by persistent hyperglycemia and glucosuria, with decreased production or decreased response to insulin or both being the major causes of the disease. Treatment entirely focuses on exogenous administration of insulin along with dietary management and introduction of exercise. The present study necessitates the pet owner education about the etiopathogenesis of the disease, along with the management strategies to curb the disease.

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Effect of Dietary Supplementation of *Bacillus Subtilis* and *Bacillus Clausii* based Probiotics alone or in combination on Performance in Jabalpur's Colour Birds

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ABSTRACT

The study was conducted to investigate the effect of dietary supplementation of *Bacillus subtilis* and *Bacillus clausii* based probiotics alone or in combination on performance in Jabalpur colour birds. A total of two hundred forty, 30 weeks age laying hens Jabalpur colour bird were selected from the poultry farm, adhartal Jabalpur. These layers were randomly distributed to five replicates of six layers each and allotted to 8 dietary treatments. The standard layer diet (T0) was formulated as per ICAR (2013) specification. T1: Control diet + antibiotic (avilamycin@100g/ ton of feed) T2: Control diet + *Bacillus subtilis* (2× 10^9cfu/g) @250g / ton of feed) T3: Control diet + *Bacillus subtilis* (2× 10^9cfu/g) @500g / ton of feed)T4: Control diet + *Bacillus clausii* (2× 10^9cfu/ g) @250g /ton of feed) T5: Control diet + *Bacillus clausii* (2× 10^9cfu/ g) @500g / ton of feed) T6: Control diet + *Bacillus subtilis* (125g) + *Bacillus clausii* (125g) (2× 10^9cfu/ g) @250g/ ton of feed) T7: Control diet +*Bacillus subtilis* (250g) + *Bacillus clausii* (250g) (2× 10^9cfu/ g) @500g/ ton of feed) respectively for 12 weeks of experimental period. Daily egg production recorded, there by hen day production was calculated. The result of 12 weeks (30^{th} to 42^{nd} weeks) of present study indicated that supplementation of *Bacillus subtilis* in combination with *Bacillus clausii* in layer diet significantly ($p<0.05$) improved overall production performance egg production and hen day production in Jabalpur colour birds.

Keywords: *Bacillus clausii, Bacillus subtilis,* Birds, Dietary supplementation, Probiotics

INTRODUCTION

Currently, the total Poultry population in our country is 851.81 million (as per 20^{th} Livestock Census and egg production is around 129.60 billion during 2021-22. The per capita availability during 2021-22 was around 95 eggs per annum. The Egg production has shown positive growth as 6.19% during 2021-22. For enhancing the productivity of laying hens and lower the incidence of disease commercially, a huge number of antibiotic growth promoters (AGPs) are given. Overuse of antibiotics at doses below therapeutic levels resulted in the development of superbugs, germs that are resistant to antibiotics and persists in both human and animal food chains (Xiang *et al,* 2019). Consequently, the European Union has restricted the use of AGPs in feed since 2006.Therefore; alternatives to antibiotics are urgently needed.

Probiotics of various commercial preparations are being considered worldwide for poultry as performance enhancers and suitable alternatives to antibiotics. The probiotics are considered as "direct-fed microbial" and they affect the host positively by balancing the intestinal microbial populations.

It is well recognized that probiotics organisms are beneficial for the host animals (Mazanko *et al,* 2018; Neijat *et al,* 2019) and the most commonly used probiotics organisms are *Lactobacillus spp., Bacillus spp., Enterococcus spp., Streptococcus thermophillus, Bifidobacterium spp., Escherichia coli* and fungal species. Spore-forming probiotics, (SFPs) especially Bacillus species, (like *Bacillus Subtilis, Bacillus clausii and Bacillus lechiformis)* have promising approach over other probiotics. Due to their encapsulation sporulation ability SFPs can reach the specific part of the gastrointestinal (GI) tract easily which is associated with their survival and colonization in the digestive tract (Khalid *et al,* 2022).

Of particular interest is a study By Adangle *et al* (2025) in which the authors demonstrated that dietary supplementation probiotic into four treatment groups, T0 (Diet without probiotic (Control), T1 (diet + 500 g probiotic (*Bacillus subtilis*)/ton of

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Table 1. Ingredient composition (% DM basis) of experiment diets.

Feed ingredient	Experimental diets (%)					
	T_0	T_1	T_2	T_3	T_4	T_5
Maize (kg)	450	450	450	450	450	450
Rice Polish (kg)	250	250	250	250	250	250
Soyaben meal (kg)	221	221	221	221	221	221
Shell grit (kg)	40	40	40	40	40	40
Calcite/LSP(kg)	25	25	25	25	25	25
Dicalcium phosphate (kg)	2.5	2.5	2.5	2.5	2.5	2.5
Mineral premix(kg)	2.5	2.5	2.5	2.5	2.5	2.5
Vitamin premix (kg)	2.5	2.5	2.5	2.5	2.5	2.5
Salt (kg)	6.5	6.5	6.5	6.5	6.5	6.5
Total	1000	1000	1000	1000	1000	1000
Nutrient composition calculated (%)						
Crude protein	16.5	16.5	16.5	16.5	16.5	16.5
Ca	3.26	3.26	3.26	3.26	3.26	3.26
Total P	0.44	0.45	0.44	0.44	0.44	0.45
Energy (Kcal ME/kg diet)	2602	2599	2602	2602	2602	2599
Nutrient composition analysed (%)						
Crude protein	16.32	16.40	16.61	16.20	16.59	16.70
Ca	3.30	3.32	3.39	3.21	3.22	3.41
Total P	0.37	0.33	0.49	0.39	0.31	0.35

Table 2. Average weekly egg production (egg/bird) of Jabalpur colour birds in different treatment groups

	Treatment							
Age (weeks)	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7
31st	4.06±0.20	4.08±0.05	4.10±0.14	4.12±0.12	4.12±0.10	4.16±0.09	4.22±0.08	4.26±0.12
32nd	4.06±0.16	4.16±0.06	4.12±0.12	4.20±0.22	4.04±0.11	4.12±0.13	4.40±0.18	4.50±0.32
33rd	4.10±0.19	4.10±0.13	4.04±0.07	4.26±0.19	4.10±0.08	4.32±0.18	4.24±0.11	4.48±0.14
34th	4.18±0.26	4.62±0.36	4.28±0.09	4.26±0.19	4.34±0.18	4.30±0.23	4.16± 0.09	4.48±0.23
35th	4.14^{b}±0.16	4.80^{ab}±0.20	5.10^{ab}±0.43	4.70^{ab}±0.34	4.56^{ab}±0.30	4.58^{ab}±0.29	5.10^{ab}±0.29	5.38^{a}±0.31
36th	4.20^{b}±0.21	4.62^{ab}±0.24	5.02^{ab}±0.31	4.90^{ab}±0.37	4.66^{ab}±0.15	4.80^{ab}±0.29	5.26^{a}±0.36	5.14^{ab}±0.37
37th	4.22±0.33	4.90±0.33	4.92±0.28	4.40±0.29	4.66±0.26	4.94±0.26	5.04±0.33	5.04±0.24
38th	4.12^{b}±0.24	4.84^{ab}±0.22	4.84^{ab}±0.27	4.94^{ab}±0.26	4.50^{ab}±0.38	4.58^{ab}±0.25	5.12^{a}±0.38	4.92^{ab}±0.29
39th	4.54±0.33	4.54±0.21	4.70±0.20	5.14±0.30	4.42±0.18	4.50±0.45	4.94±0.26	4.62±0.39
40th	4.44±0.31	4.32±0.19	4.70±0.25	5.10±0.29	4.52±0.27	4.44±0.28	4.88±0.32	4.70±0.25
41st	4.50±0.31	4.44±0.23	4.52±0.16	4.88±0.29	4.50±0.27	4.50±0.16	4.72±0.24	4.70±0.41
42nd	4.94±0.34	4.52±0.13	4.48±0.16	4.90±0.29	4.30±0.20	4.30±0.44	4.70±0.12	4.74±0.38
Avg.	4.29^{c}±0.09	4.50^{abc}±0.08	4.57^{abc}±0.09	4.65^{ab}±0.12	4.39^{bc}±0.06	4.46^{abc}±0.05	4.73^{a}±0.11	4.75^{a}±0.13

feed.), T2 (diet + 1000 g probiotic (*Bacillus subtilis*)/ton of feed) and T3 (Diet + 1500 g probiotic (*Bacillus subtilis*)/ton of feed) hens receiving *Bacillus subtilis* supplementation (especially T2) exhibited significantly higher feed intake and enhanced egg production, with HDEP. Supplementation of *Bacillus clausii* @ 0.03 ml/L as probiotic in drinking water significantly improved the liver function and humoral immunity of broiler chicks (Mushtaq *et al*, 2023).

Although a great deal of work has been done with probiotics, mostly with broiler chickens. However, studies examining the effect of *Bacillus clausii* on laying hens are limited and results of comparative effects of single strain- and multi-strain spore forming probiotics preparations on egg type chickens are scanty in the literature. Based on previous research, two types of probiotics bacteria were chosen to be administrated in a laying hen diet.

MATERIALS AND METHODS

A total of two hundred forty, 30 weeks age laying hens Jabalpur colour birds were selected from the poultry farm, adhartal Jabalpur. These layers were randomly distributed to five replicates of six layers each and allotted to 8 dietary treatments. The standard layer diet (T_0) was formulated as per ICAR (2013) specification. T_1: Control diet + antibiotic (avilamycin@100g/ ton of feed) T_2: Control diet + *Bacillus subtilis* (2× 10^9cfu/g) @250g / ton of feed) T_3:

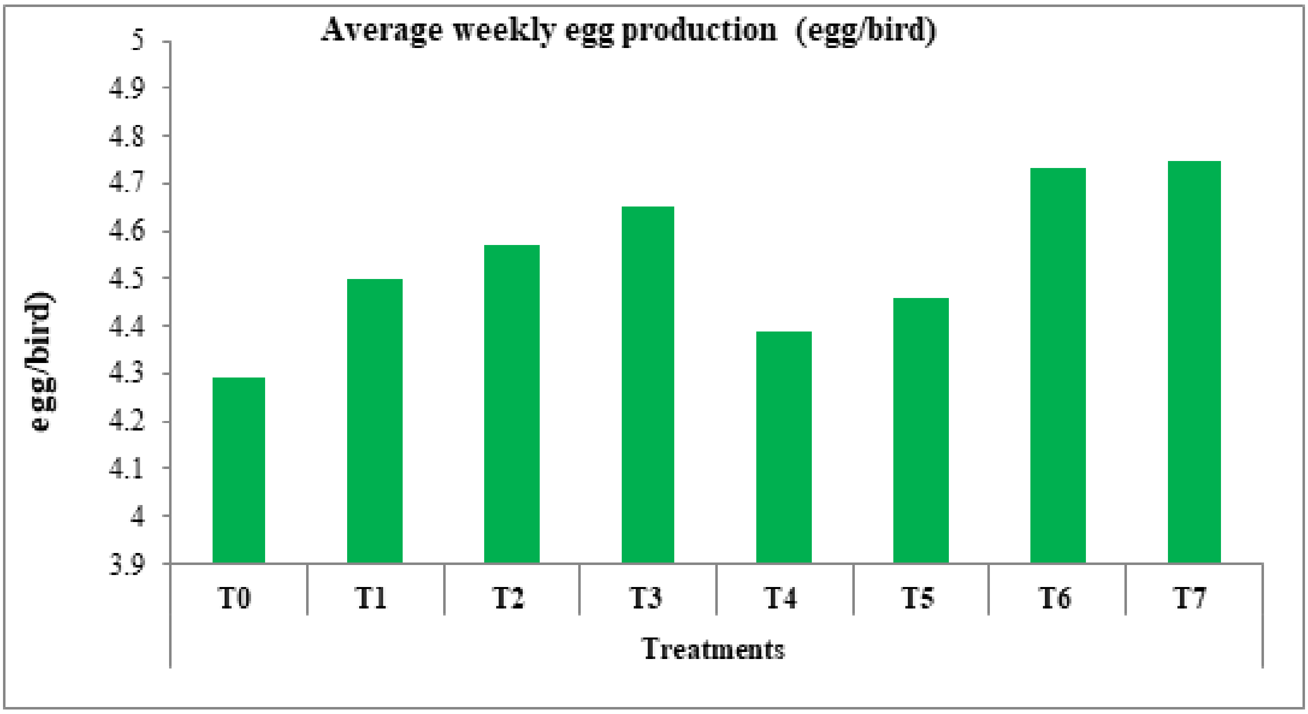

Fig 1.Average weekly egg production (egg/bird) of Jabalpur colour birds in different treatment groups

Control diet + *Bacillus subtilis* (2× 10+^{9}cfu/g) @500g / ton of feed) T_4: Control diet + *Bacillus clausii* (2× 10^{9}cfu/ g) @250g /ton of feed)T_5: Control diet + *Bacillus clausii* (2× 10^{9}cfu/ g) @500g / ton of feed) T_6: Control diet + *Bacillus subtilis* (125g) + *Bacillus clausii* (125g) (2× 10^{9}cfu/ g) @250g/ ton of feed) T_7: Control diet +*Bacillus subtilis* (250g) + *Bacillus clausii* (250g) (2× 10^{9}cfu/ g) @500g/ ton of feed) respectively.

The feed ingredients were procured from the market and analyzed for proximate composition before formulation of diets. The experimental diets were formulated as per layer feed (ICAR, 2013), (16.5% CP and 2600 kcal ME/kg diet). The analyzed protein and ME values of feed ingredients were used for computation of rations. Composition of experimental layer diets used in the study is given in Table 1. The experimental was conducted for 12 weeks were Jabalpur colour birds managed under same environment. The production performance of birds closely monitors throughout trail periods. Replicate-wise weekly egg production was recorded and hen day production was calculated. Statistical analysis of the data was done by using analysis of variance using complete randomized design as per Snedecor and Cochran (1994). Difference among the treatments was tested for significance by Duncan's Multiple Range Test (1995).

RESULTS AND DISCUSSION

Production performance

The average weekly egg production (eggs/bird) and hen day production of Jabalpur colour birds under different treatments from the 31st to 42nd week was recorded summarized in Table 2 and Figure 1. At the beginning (31st week), egg production ranged from 4.06±0.20 (T_0) to 4.26±0.12 (T_7), with no significant difference ($p>0.05$) among groups. During the experimental period, weekly egg production gradually increased across treatments, reaching the highest values in the 35th 36th and 38th weeks, The weekly egg production significantly ($p>0.05$) increases in spore forming probiotics (*Bacillus subtilis* and *Bacillus clausii*) combination diets T_6 and T_7 as compared to control (T_0) fed on basal diet at 35th 36th and 38th weeks. At the end of the experiment (42nd week), the egg production was 4.94±0.34, 4.52±0.13, 4.48±0.16, 4.90±0.29, 4.30±0.20, 4.30±0.44, 4.70±0.12, and 4.74±0.38 for groups T_0, T_1, T_2, T_3, T_4, T_5, T_6, and T_7, respectively.

The overall average weekly egg production (egg/bird) showed wide variation among all dietary treatments. The overall average weekly egg production (egg/bird) was significantly ($p<0.05$) higher in birds allotted T_1 diet (antibiotic supplemented or positive control diet) (4.50±0.08), as compared to birds fed on

Table 3. Average weekly hen day production (%) of Jabalpur colour birds in different treatment groups

	Treatments							
Age in weeks	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7
31st	67.67± 3.28	68.00± 0.82	68.33± 2.36	68.67± 1.93	68.67± 1.70	69.33± 1.45	70.33± 1.33	71.00± 1.94
32nd	67.67± 2.72	69.33± 1.00	68.67± 2.07	70.00± 3.61	67.33± 1.87	68.67± 2.20	73.33± 2.93	75.00± 5.38
33rd	68.33± 3.12	68.33± 2.17	67.33± 1.13	71.00± 3.10	68.33± 1.39	72.00± 2.95	70.67± 1.80	74.67± 2.32
34th	69.67± 4.33	77.00± 5.99	71.33± 1.43	71.00± 3.23	72.33± 3.05	71.67± 3.80	69.33± 1.55	74.67± 3.78
35th	69.00^{b}± 2.67	80.00^{ab}± 3.33	85.00^{ab}± 7.15	78.33^{ab}± 5.65	76.00^{ab}± 4.99	76.33^{ab}± 4.81	85.00^{ab}± 4.86	89.67^{a}± 5.12
36th	70.00^{b}± 3.50	77.00^{ab}± 3.96	83.67^{ab}± 5.15	81.67^{ab}± 6.12	77.67^{ab}± 2.45	80.00^{ab}± 4.89	87.67^{a}± 6.07	87.67^{a}± 6.23
37th	70.33± 5.46	81.67± 5.53	82.00± 4.61	73.33± 4.86	77.67± 4.37	82.33± 4.37	84.00± 5.44	84.00± 3.97
38th	68.67^{b}± 4.06	80.67^{ab}± 3.75	80.67^{ab}± 4.58	82.33^{ab}± 4.37	75.00^{ab}± 6.30	76.33^{ab}± 4.20	85.33^{a}± 6.35	82.00^{ab}± 4.75
39th	75.67± 5.54	75.67± 3.56	78.33± 3.33	85.67± 4.99	73.67± 2.95	75.00± 7.47	82.33± 4.30	77.00± 6.55
40th	74.00± 5.21	72.00± 3.22	78.33± 4.25	85.00± 4.86	75.33± 4.42	74.00± 4.61	81.33± 5.33	78.33± 4.25
41st	75.00± 5.24	74.00± 3.86	75.33± 2.66	81.33± 4.84	75.00± 4.56	75.00± 2.63	78.67± 4.03	78.33± 6.77
42nd	75.33± 5.74	75.33± 2.13	74.67± 2.66	81.67± 4.86	71.67± 3.33	71.67± 7.26	78.33± 2.04	79.00± 6.40
Avg.	71.53^{c}± 1.50	74.92^{abc}± 1.28	76.14^{abc}±1.44	77.50^{ab}± 1.93	73.22^{bc}± 1.05	74.36^{abc}±1.76	78.86^{a}± 1.79	79.11^{a}± 2.15

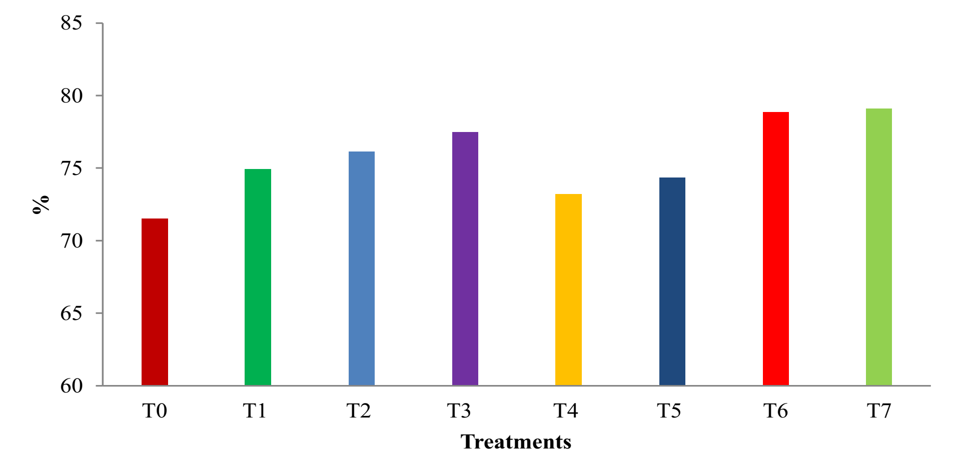

Fig 2. Average weekly hen day production (%) of Jabalpur colour birds in different treatment groups

control (T_0) or basal diet (4.29±0.09). Birds supplemented with different levels of *Bacillus subtilis and Bacillus clausii* strain alone or in combination preparations of spore forming probiotics caused significantly (P<0.05) improvement in the average weekly egg production (egg/bird). Among probiotic supplemented diets, maximum and significantly (P<0.05) higher average weekly egg production (egg/bird) were observed in birds allotted T_6 (4.73±0.11) and T_7 (4.75±0.13) diets. Probiotics combination diets (*Bacillus subtilis and Bacillus clausii* @125g and 250g/ton each). However, statistically it was comparable (P>0.05) to birds allotted T_2, T_3, T_5 and positive control T_1 diets. Minimum and significantly (P<0.05) lowest average weekly egg production (egg/bird) was found with those birds fed on T_0 and T_4 diets.

The average weekly hen-day production (%) of Jabalpur colour birds from the 31st to 42nd week of age under different dietary treatments (T_0–T_7) was recorded, summarized in Table 3. At the 31st week, the production percentage ranged from 67.67±3.28 in T_0 to 71.00±1.94 in T_7, with no significant differences among groups. A gradual increase was observed across treatments as the experiment progressed. By the 35th week, production was significantly higher (p<0.05) in supplemented groups, with values ranging from 76.00±4.99 in T_4 to 89.67±5.12 in T_7, compared to the control (T_0: 69.00±2.67). During the 36th to 38th weeks, production remained consistently higher in supplemented groups. The highest values were recorded in T_6 (87.67±6.07) and T_7 (87.67±6.23) at the 36th week and T_6 (85.33±6.35) at the 38th week, which were significantly higher (p<0.05) than the control (T_0: 68.67±4.06).

From the 39th to 42nd weeks, production levels stabilized across treatments, no marked differences (p>0.05) were observed among groups during this period, although the supplemented groups consistently maintained higher production compared to the control.

Birds supplemented with different levels of *Bacillus subtilis and Bacillus clausii* strain alone or in combination preparations of spore forming probiotics caused significantly ($P<0.05$) improvement in the HDP%. Among probiotic supplemented diets, maximum and significantly ($P<0.05$) higher HDP% were observed in birds allotted T_6 (78.86±1.79) and T_7 (79.11±2.15) diets (Probiotics combination diets (*Bacillus subtilis and Bacillus clausii* @125g and 250/ton each). However, statistically it was comparable ($P>0.05$) to birds allotted T_2, T_3, T_5 and positive control T_1 diets. Minimum and significantly ($P<0.05$) lowest HDP% were found with those birds fed on T_0 and T_4 diets.

The overall hen day egg production (%) showed wide variation among all dietary treatments. The overall HDP% was significantly ($p<0.05$) higher in birds allotted T_1 diet (antibiotic supplemented or positive control diet) (74.92±1.28), as compared to birds fed on control (T_0) or basal diet (71.53±1.50). Therefore, birds supplemented with different levels of *Bacillus subtilis* and *Bacillus clausii* strain alone or in combination preparations of spore forming probiotics improved HDP% of birds, significantly ($P<0.05$) when compared with non-supplemented groups and also statistically it was comparable ($P>0.05$) to birds allotted positive control (antibiotic supplemented) T_1 diets and thus suitable alternatives to antibiotics. Therefore, birds supplemented with different levels of *Bacillus subtilis* and *Bacillus clausii* strain alone or in combination preparations of spore forming probiotics can be considered as performance enhancers and suitable alternatives to antibiotics.

Average weekly egg production (egg/bird) of Jabalpur colour birds in different treatment groups was significantly higher in *Bacillus subtilis and Bacillus clausii* strain alone supplemented group as well as or in combination preparations of spore forming probiotics. In accordance with our findings, (Chen *et al*, 2020) reported the effect of dietary supplementation with *Bacillus subtilis* increased egg production and egg mass significantly compared to control group. (Liu *et al*, 2021) reported that laying hens fed diet that dietary *Bacillus subtilis* and essential oils (BSEO) supplementation on 900mg/kg (BSEO) significantly improve egg production. This improvement may be attributed to the action of several enzymes, including protease, amylase and cellulase, secreted in the gastrointestinal tract, whose activity can be enhanced by BS or EO supplementation (Li *et al,* 2018). Moreover, *Bacillus subtilis* has been reported to promote better gut morphology and stimulate the growth of beneficial intestinal microflora, which in turn enhances nutrient utilisation efficiency.

However, similarly (Ray *et al,* 2022) reported dietary supplementation of single-strain probiotics (SSP) containing *Bacillus Subtilis* (2×10^9cfu/g) and multi-strain probiotic (MSP) containing *Lactobacillus Acidophilus* $3 \times 10^7 - 10^8$ cfu/g, *Bacillus Subtilis* 3×10^7-10^8 cfu/g and *Saccharomyces Cerevisiae* $10^6 - 10^7$ cfu/g linearly increased egg production with dose increment in the MSP fed birds. The present results obtained are also in line with earlier reports (Darsi and Zhaghari, 2021) dietary *Bacillus subtilis* PB6 supplementation significantly improved egg production in broiler breeders at the late phase of production but, egg weight was not significantly influenced by probiotic supplementation. This effect could be attributed to the continuous administration of probiotics, which may have inhibited harmful microorganisms, thereby enhancing the overall health status.

In contast with our results (Sobczak and Kozłowski, 2015) suggested that there was no significant difference in egg production due to supplementation of *Bacillus subtilis* (1×10^8 CFU/kg) probiotics. Similarly, (Fathi *et al,* 2018) found that probiotics (*Bacillus subtilis*) supplementation in layer diets did not affect egg production traits compared with control group.

CONCLUSION

The overall production performance of the Jabalpur colour birds in terms of egg production and hen day egg production was improved by supplementation of with combination of *Bacillus subtilis* and *Bacillus clausii* probiotics (@125g each or @250g/ ton of feed, each) in comparison to those fed on control, positive control. Further investigations into optimal dosage levels of probiotics and synergistic combinations are essential for a more comprehensive understanding of their beneficial effects.

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