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EFFECT OF BEE POLLEN AND PROBIOTICS ON GROWTH PERFORMANCE, ORGANS, AND THIGH MEAT pH OF BROILER COBB 500

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ABSTRACT

Slightly more than one third of the global meat consumed comes from chickens. Various studies have been carried out on the use of bee pollen or probiotics in broiler feed; however, very limited studies have been carried out about their combined effect on broiler production. The objective of the present study was to investigate the effect of feeding by pollen and probiotics on broiler chicken Cobb 500. The effect of different concentrations of commercial bee pollen and probiotics was evaluated on growth performance, organs, and thigh meat pH of 45 days old chickens. The rate of body weight gain on day 21, 33, and 39 were significantly different among treatment and control groups and on day 33 d, all the experimental groups showed significant increase in weight gain as compared to control. T_3 treatment had highest weight gain (380 g) on day 45. Some of the organs such as lung, digestive system, and intestine length were also significantly affected by the application of bee pollen and probiotics. The broilers fed with bee pollen and probiotics were found to have increased but more stable pH of thigh meat as compared to that of control at 24 hours of low temperature (-20°C) storage. At 24 h, T_2 treatment showed highest pH value(6.52) for thigh meat Results of the present study indicated that the meat quality of broiler could be improved with the use of bee pollen and probiotics as they could increase and keep the pH stable during storage.

Keywords: Bee pollen, broiler Cobb 500, meat pH, probiotic, weight gain

INTRODUCTION

Chicken meat is the second most widely eaten meat in the world which is about 35% of the world meat consumption and represents approximately 88% of global poultry meat output (**FAO, 2014**). Broiler, a chicken of meat type stain, is bred and raised for meat production (**USDA, 2002**) and is characterised by rapid growth and low behavioral activity (**Estevez, 2007**).
Antibiotics have been included in poultry feeds to improve feed conversion ratio, growth and diseases prevention **(Gadde *et al.*,2017)**. However, a huge amount of antibiotics use in poultry feed as growth promoter and as drug against diseases has drawn serious attention. Public health advocates have suggested nullifying this practice as it can spread more antibiotic-resistant bacteria. In the US alone, around 23000 people die and 2 million people get infected annually because of antibiotic resistant bacteria (**CDC, 2014**). Due to rising concerns on food safety, many firms are removing the use of antibiotics in the feed of broilers and exploring natural options to control enteric pathogens which severely affect animal welfare and food safety (**USDA, 2005**). Therefore, to raise chickens without any antibiotics would be better option from human and environment health perspectives.
Bee pollen has promising therapeutic and nutritional value with higher content of essential amino acids, minerals, and the sugar mannitol (**Nogueira *et al.*, 2012; da Silva *et al.*, 2014**). It has high antioxidant and free radical scavenging potential due to polyphenol substances (**Kroyer and Hegedus, 2001; Almaraz-Abarca *et al.*, 2004**). It has the antimicrobial activity against pathogenic fungi and gram positive and negative bacteria (**Melliou and Chinou, 2011**). In broiler bee pollen could be potential feed supplement by promoting early development of digestive system (**Wang *et al.*, 2007**). It has positive effect on meat pH value, shelf-life and oxidative stability (**Haščík *et al.*, 2011; *Hašèík et al.*, 2012; Šulcerová *et al.*, 2014**). **Guarner and Schaafsma (1998)** define probiotics as living microorganism when consumed in adequate amounts provides specific health benefits on the host. Probiotics are microorganism living in the gastrointestinal tract and can be potential in health, nutrition, and growth performance of chicken (**Wallace and Chesson, 1995; Gong *et al.*, 2002**). Probiotics has significant effect on meat pH value and improve the meat quality (**Aksu *et al.*, 2005; Karaoğlu *et al.*, 2006**). Different evidences and research suggest that probiotics would be good alternative for replacing the antibiotics (**Edens, 2003**). Pollen and probiotics can improve metabolism and develop resistance against pathogens and thus enhance growth performance in broiler (**Haščík *et al.*, 2015**).
Considerable number of reports on use of bee pollen or probiotics in broiler feed has been published; however, very limited study has been carried out about their combined effect on broiler production. **Hascík *et al.*** (2015) has supplied bee pollen or propolis as feed additive and probiotics via drinking water to the broiler Ross 308 and investigated the pH value of breast and thigh muscles. In the present study, the effect of bee pollen and probiotics as feed additives on pH value of thigh meat and growth performance of broilers Cobb 500 was investigated.

MATERIALS AND METHODS

Experiment location and chicken breed

The experiment was carried out at Institute of Agriculture and Animal Science, Lamjung, Nepal (28°7'32.5"N 84°24' 58.6"E) from April to June in 2015. The location is a humid sub-tropical zone with an average maximum and minimum temperature of 28-30ºC and 20-22ºC, respectively. One-day old chickens of breed 'Cobb 500' were purchased from a hatchery (Trivedi Sangam Hatchery, Chitwan, Nepal). The breed Cobb 500 is one of the widely raised breeds of broilers in the study area.

Feeding

The 1-d old chickens were provided with standard broiler feed (Pancharatna feeds, Chitwan, Nepal) and water on an *ad libitum* basis until 14 d. After 15 d they were allowed free access to a standard pellet feed (Pancharatna feeds, Chitwan, Nepal) and water ad libitum throughout the experiment period of 45 d. Birds were divided into six treatment groups based on the amounts of supplimentary bee pollen and probiotics fed from 15 d afterwards. Treatments were named as T1, broilers fed with 500 mg of pollen and 2000 mg of probiotics; T2, broiler fed with 1000 mg of pollen and 3000 mg of probiotics; T3, broilers fed with 1500 mg of pollen and 4000 mg of probiotics; T4, broiler fed with 2000 mg of pollen and 5000 mg of probiotics; T5, broilers fed with 2500 mg of pollen and 6000 mg of probiotics; and control, broiler fed only with the commercial pellet feed as other treatment groups but without any supplementary pollen or probiotics. There were 15 birds in each treatment. Nutrient composition of the feeds has been shown in Tab 1. The feeds did not contain any antibiotics or coccidiostats.

Table 1 Nutrient content in the standard broiler feed and standard pellet feed

Nutrient	Standard broiler feed	Standard pellet feed
Protein (%)	18-20	14-17
Metabolizable energy kcal/lb	1425	1400
Lysine (%)	1.21	0.85
Methionine +Crystine (%)	0.92	0.6
Calcium (%)	0.95	0.8
Available phosphorus (%)	0.48	0.40
Sodium (%)	0.2	0.12
Potassium (%)	0.83	0.3
Chlorine (%)	0.25	0.12

Formulation of probiotics and bee pollen

Each 500 g of probiotics (Vetoquinol SA, Lure Cedex, France) contained *Saccharomyces cerevisiae* SC-47 (150000 million CFU), *Saccharomyces boulardii* (25000 million CFU), *Lactobacillus acidophilus* (22500 million CFU), *Propionibacterium freudenrlichii* (25000 million CFU), and Sea weed powder (50 g). Commercially available bee pollen (Gorkha honeybee, Chitwan, Nepal) was used to feed the birds. The pollen contained 42.5, 25, 12.5, 4.5, 0.65, 7.5, and 8% of carbohydrate, protein, free amino acids, fat, vitamins, moisture, and other constituents, respectively.

Measurement of different parameters of birds

.Live weight of each bird was taken before commencing the treatments on 15 d and on every six days until 45 d. A randomly selected 3 birds from each treatment group were slaughtered on 45 d in the morning. The chicken meat was kept in refrigerator (-20°C) until analyses.

After slaughtering weight of breast, liver, heart, lung, spleen, digestive system, drumstick, leg, gall bladder, and caeca; dressing percentage; and length of large intestine were measured.

For pH measurements, **Dutson (1983)** was followed with slight modifications. Meat samples taken from thigh were cut into small pieces. The meat sample (50 g) was put into blender cup, distilled deionized water (500 mL) was added to the cup, and blended for 30 s at high speed. Samples were transferred into clean beaker and the pH value was measured. The pH of thigh meat was measured at 2 and 24 h after slaughtering.

Statistical analysis

Data were analyzed using SAS 9.4 (SAS Institute, Cary NC, USA). The average values among treatments were separated using least significant difference (LSD) tests at P< 0.05. Values were expressed as mean±SE.

RESULTS AND DISCUSSION

Body weight gain of broiler

Supplementation of bee pollen and probiotics significantly affected the body weight gain of broiler on 21, 33, and 39 d (Tab 2). However, it was not significantly different on 27 d as well as on the day of slaughter (45 d). Feeding with bee pollen and probiotics was found to increase the weight gain on 21 and 39 d significantly although it was not consistent for all treatment groups. However, on 33 d, all the experimental groups showed significant increase in weight gain as compared to control. Moreover, feeding bee pollen and probiotics from 15 d caused intermittent increase in the weight gain among different treatments up to 45 d. The mortality rates of chickens among the different treated and untreated groups were not significantly (P> 0.05) different (data not shown).

Table 2 Weight gain (g) of broiler Cobb 500 fed with different amounts of bee pollen and probiotics from 15 to 45 days

Treatment	Day				
	21	27	33	39	45
Control	243±6.5b	413±26.3a	363±53.1b	450±32ab	337±19.8a
T1	247±6.7b	370±25.7a	452±62.5a	372±72b	330±19.2a
T2	232±6.4bc	426±26.1a	540±34.4a	505±56a	248±19.5a
T3	268±6.8a	340±25.8a	453±87.8a	403±48ab	380±19.9a
T4	226±6.3c	380±25.4a	482±62.5a	561±64a	351±19.3a
T5	261±6.9a	358±25.6a	445±62.5a	503±80a	282±20.1a

Legend: Control – without any supplemental bee pollen or probiotics, T1 – 500 mg of pollen and 2000 mg of probiotics, T2 – 1000 mg of pollen and 3000 mg of probiotics, T3 – 1500 mg of pollen and 4000 mg of probiotics, T4 – 2000 mg of pollen and 5000 mg of probiotics, T5 – 2500 mg of pollen and 6000 mg of probiotics. Results are expressed as mean ±SD (n=3) and significantly different at a P<0.05. Means sharing similar letter in a column are statistically non-significant (P>0.05).

The efficiency of pollen and probiotics on weight gain was investigated on 21, 33 and 39 d (Tab 2). Body weight of broiler was significantly different after 27 d and experimental groups fed with probiotics have more body weight than control group which is also confirmed by **Khan *et al*. (2007)**. **Eckert *et al.* (2010)** had also found probiotics fed broilers intermittently gained more body weight as compared to control. In another study **Giannenas *et al.* (2014)** had found higher weight gain on 35 and 42 d in probiotics fed broiler than that in control. **Fazayeli-Rad *et al.* (2015)** found to continuously increase the weight gain until 6 weeks of broiler chickens fed with bee pollen. Although the reason of intermittent weight gain of broilers in the present study was not well understood, the improved weight gain might be due to nutrient composition of bee pollen along with other different phenolic compounds, natural substances of antioxidant activities, and health protection **(Szczêsna, 2007; Šarić *et al.*, 2009)** and probiotics by maintaining and improving intestinal microbial balance and health of gastrointestinal tract as well as increasing digestive enzyme activity which help enhance production performance **(Tortuero and Fernandez, 1995; Jin *et al.*, 1997)**.

Measurement of different parts

There were no significant difference in live weight, slaughter weight, dressing percentage, and weights of breast, liver, heart, spleen, drumstick, leg, gall bladder, and caeca; however, weight of lung, digestive system, gizzard, and length of intestine were significantly (P< 0.05) different among treatments (Tab 3).

Table 3 Measurement of different parts on 45 days of broiler Cobb 500 fed with different amounts of bee pollen and probiotics

Treatment	Control	T1	T2	T3	T4	T5
Live Wt. (g)	2740±200a	2427±92.62a	2507±185.23a	2593±56.96a	2613±217.97a	2433±180.49a
Slaughter Wt. (g)	1837±95a	1620±87.19a	1505±264.32a	1786±76.51a	1682±174.82a	1652±158.39a
Breast (g)	622.17±42.43a	523.52±46.50a	502.88±62.15a	636.12±27.14a	614.62±67.19a	514.52±70.97a
Liver (g)	59.37±5.85a	52.4±2.17a	53.2±2.76a	48.0±2.98a	59.17±7.27a	55.6±4.30a
Heart (g)	13.51±0.26a	13.01±0.61a	11.77±1.11a	11.57±1.37a	12.10±1.80a	12.75±1.75a
Lung (g)	13.54±1.27a	10.85±0.98b	10.83±0.54b	10.59±0.64b	10.98±0.44b	11.18±0.49ab
Spleen (g)	3.05±0.27a	3.06±0.82a	2.67±0.50a	2.64±0.60a	2.61±0.75a	2.40±0.60a
Digestive (g)	322.55±31.22a	280.27±11.63abc	246.02±11.15bc	224.39±15.60c	293.4±22.83ab	276.89±17.87abc
Drumstick (g)	133.23±14.75a	123.67±3.58a	118.23±23.96a	119.81±11.65a	108.62±11.84a	135.85±16.32a
Leg (g)	276.86±15.44a	251.64±24.11a	216.24±38.25a	242.91±31.34a	264.04±34.91a	196.88±5.73a
Gall Bladder (g)	1.92±0.18a	3.13±1.01a	2.11±0.30a	2.54±0.32a	2.60±0.32a	2.85±0.08a
Dressing (%)	67.15±1.43a	66.69±1.06a	66.22±7.04a	68.80±1.48a	64.21±2.03a	67.66±1.66a
Intestine length (cm)	163.67±1.86ab	187.33±7.22a	171±4ab	185.67±13.38a	181.67±19.95ab	149±5.51b
Caeca (g)	9.33±0.67a	11.33±1.86a	9.0±3a	8.5±1.5a	12.33±1.86a	7.67±0.88a

Treatments are defined in Table 2. Results are expressed as mean ±SD (n=3) and significantly different at a P<0.05. Means sharing similar letter in a row are statistically non-significant (P>0.05).

Results of **Haščík *et al.* (2015)** agreed with the present study for non-significant difference on live and slaughter weights and dressing percentage of chicken fed with bee pollen. Final weight of broiler fed with probiotics was not also significantly different on 42 d **(Nunes *et al.*, 2016)**. Similar results were also found by **Panda *et al.* (2003)** in layer feeding with probiotics had no significant affect in body weight. Although live and slaughter weights of birds among different treatments were not significantly different, improved meat quality could be expected with the use of probiotics **(Ko *et al.*, 2008; Zhou *et al*., 2010)**. Use of probiotics in chicken feed did not cause significant difference in spleen (Tab 3), however **Chen *et al.* (2013)** found increased spleen. However, in the same study they found no significant effect of probiotics on weight of liver and breast meat which agreed with the present study. Liver and spleen weight are not significantly affected by feeding bee pollen to broiler chickens **(Farag and El-Rayes, 2016)**. **Oliveira *et al.* (2013)** did not find significant effect of bee pollen on spleen weight of broiler. **Wang *et al.* (2007)** showed the effect of bee pollen on small intestine in broiler chickens and found the small intestine villi are longer and thicker from the duodenum, jejunum, and ileum in the pollen group. Their findings also suggested that bee pollen could promote the early development of the digestive system. The results of the present study did not completely agree with that of **Wang *et al.* (2007)** as the effect of bee pollen was not consistent in all the treatment groups although length of intestine in T5 was smaller than control group (Tab 3). **Fazayeli-Rad *et al.* (2015)** found relative pancreas, liver, intestine, and spleen weights were significantly increased with inclusion of bee pollen in broiler chicken feed. The significant difference on live and slaughter weights as well as different organs might be due to probiotic and pollen which act as a bio-regulator in intestine and strengthen the host natural ability which was supported by **Endo *et al.* (1999)**. Reports showed that effect of probiotics in chicken depends on different factors such as bird age, farm hygiene, environmental stress, composition of microbial species, dosage, methods of using as well as the frequency of application **(Torshizi *et al.*, 2008; Awad *et al.*, 2009; Flint and Garner, 2009; Mountzouris *et al.*, 2010)**.

Thigh meat pH

pH values of thigh meat of different treatments at 2 and 24 h were significantly affected by bee pollen and probiotics supplementation (Tab 4). The pH of thigh meat at 2 h was significantly different; however the effect of bee pollen and probiotics was not consistent on all the treatment groups. The pH at 2 h was found to be the highest for T1 followed by T4. When moved to 24 h, all the treatment groups except T1 showed significantly high pH value for thigh meat as compared to control group (Tab 4).

Table 4 Measurement of thigh meat p^H on 45 days of broiler Cobb 500 fed with different amounts of bee pollen and probiotics

Treatment	2 hours	24 hours
Control	6.48±0.17bc	6.01±0.13c
T1	6.93±0.17a	5.99±0.07bc
T2	6.27±0.13cd	6.52±0.1a
T3	6.28±0.1d	6.25±0.27ab
T4	6.59±0.13b	6.34±0.23ab
T5	6.26±0.2d	6.28±0.08ab

Treatments are defined in Table 2. Results are expressed as mean±SD (n=3) and significantly different at a P<0.05. Means sharing similar letter in a column are statistically non-significant (P>0.05).

Results of this experiment were similar to that of **Karaoğlu *et al.* (2006)** in which the meat pH of broiler was high in the probiotics fed groups. The results of higher meat pH in treatment groups were also in agreement with that of **Haščík *et al.* (2011)** who studied the impact of probiotic on broiler meat pH value. Similar results were found in the broiler fed with bee pollen and had high pH of breast and thigh meat **(Haščík *et al.*, 2012)**. **Sulcerová *et al.* (2014)** also found similar results when fed broiler with bee pollen and propolis. The effect of bee pollen and probiotics on increased pH value of meat was also found by **Haščík *et. al.* (2015)**. The possible reason for improving the meat pH level was due to effect of bee pollen and probiotics in reducing the oxidative stability of meat and further inhibiting the meat oxidation **(Marcinčák *et al.*, 2005)**. Bee pollen and probiotics fed broiler meat showed high pH at 24 h of storage which might be good for preserving meat shelf-life as **Newton and Gill (1981)** reported that high pH (> 6.0) did not accelerate growth of spoilage organisms.

CONCLUSION

The effect of different concentrations of commercial bee pollen and probiotics as feed supplement was evaluated on growth performance, various organs, and pH of thigh meat in broiler chickens Cobb 500. The body weight gain on 21, 33, and 39 d were found to be significantly affected by the bee pollen and probiotics. There were significant differences for some of the organs such as lung, digestive system, and intestine length. The treatment groups were found to have increased and more stable pH value of thigh meat as compared to control at 24 hours of cold storage. Results of the present study showed the possible improvement in the meat quality of broiler chicken by feeding bee pollen and probiotics as it influenced the pH value during storage.

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