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Effect of Probiotic *Lactobacillus* Species Supplementation on Productive Traits of White Leghorn Chicken

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ABSTRACT

Probiotics are live microbial food ingredients that have a beneficial effect on human health. Intake of probiotics improves feed intake, egg production and egg quality in laying breeds. The objective of this study was to evaluate the effect of the probiotic *lactobacillus* species supplementation on productive traits of White Leghorn chicken. For this purpose, 30 samples of cow milk were collected from Haramaya university dairy farm during the period from May to August 2015. The probiotic properties of each isolates were confirmed by simulating gastrointestinal tract conditions. Based on physiological and biochemical tests *Lactobacillus acidophilus* and *Lactobacillus plantarum* were isolated. The experimental design used in this experiment was single-factor Completely Randomized Design (CRD) with treatments basal feed (control), supplementation of *L. acidophilus* (T2), *L. plantarum* (T3) and their combination (T4) and a 5% ($P<0.05$) level significance was used. Supplementation of Lactobacillus species improved the Feed Intake (FI), Hen Day Egg production (HDEP) and egg weight. The FI recorded were 98.9 g/day/hen, 99.8 g/day/hen, 101.8 g/day/hen and 105.0 g/day/hen in control, T1, T2 and T3 respectively. HDEP of 0.31%, 0.33%, 0.33% and 0.34% were recorded at control, T1, T2 and T3 respectively. The egg weight of the control treatment, T1, T2 and T3 were 50.8g, 51.4 g, 51.4g and 51.9g respectively. Probiotic *Lactobacillus* species (*L. acidophilus and L. plantarum*) improves the productive traits of the laying flock. Chicken received the combination of probiotic *lactobacillus* species significantly perform best in FI, HDEP and egg weight.

Key words: Chicken, *Lactobacillus*, Probiotic, Productive trait, Supplement

INTRODUCTION

Probiotics are defined as live microbial food/feed a supplement which beneficially affects the host animal by improving its intestinal balance that prevent from the growth of pathogenic bacteria, help the growth, multiplication and establishment of beneficial microflora in the intestinal environment (Fuller, 1989). Feeding viable *Lactobacillus* improves feed consumption, size of egg, and mineral retentions and decreases intestinal length from 7 to 59 weeks of age (Nahanshon et al., 1996).

Probiotics supplementation into poultry diets improves feed intake and growth performance in poultry breeds (Sarangi et al., 2016). Similarly, inclusion of probiotics significantly influences feed conversion ratio, egg production performance and egg quality in laying strains (Lei et al., 2013;Inatomi, 2016).Commonly used microorganisms as probiotics in animal feed are mainly bacteria strains belonging to different genera, e.g. *Lactobacillus, Enterococcus, Pediococcus, Bacillus* and microcopic fungi, including *Saccharomyces* yeasts (Guillot, 2009). Feeding viable *Lactobacillus* species increased daily feed consumption, egg size, and nitrogen and calcium retentions in laying breeds (Nahashon et al., 1996). Probiotics improve feed intake and body weight gain in chicken fed with probiotics compared with that in control group fed basal diet (Zhang and Kim, 2014).

Moreover, probiotics have several beneficial impacts, including stimulating appetite, improving intestinal microbial balance, stimulating the immune system, producing digestive enzymes and utilizing indigestible carbohydrates (Prins, 1977; Nahanshon et al., 1992; Nahanshon et al., 1993; Fuller, 1989; toms and Powrie, 2001; Gilliland and Kim, 1984; Saarela et al., 2000). The objective of this study was to evaluate the effect of the probiotic *lactobacillus* species supplementation on productive traits of White Leghorn chicken.

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MATERIALS AND METHODS

Study area and sample collection

The experiment was conducted at Haramaya university poultry farm, Ethiopia (Effect of probiotic supplementation) and microbiology laboratory (isolation, characterization and testing *Lactobacillus* species). A total of 30 samples of raw cow milk were collected from Haramaya university dairy farm during the study period May to August 2015. The raw cow milk samples were collected using sterile bottles and transported to the microbiology laboratory in icebox for analysis. Aseptic sampling was followed as described by the Health Protection Agency (HPA, 2014) and the Food and Drug Administration (FDA, 2003). After arrival at the laboratory, samples were kept at temperatures below 4^{o}C and were analyzed within 48 hours of collection.

Ethical approval

This research did not involve feeding of birds with pathogenic microorganisms, introduction of any intervention in/on birds, or direct collection of cells, tissues or any material from birds.

Isolation of lactic acid bacteria lactic acid bacteria

Lactic acid bacteria (LAB) were isolated from raw cow milk. A 0.1 ml of different dilution (10^{-2} to 10^{-8}) of samples was inoculated on De Man Ragosa Sharpe (MRS) agar medium (pH 6.2) plates and incubated at 37^{o}C for 24-36 hours anaerobically. The presence of acetate, citrate and tween-80 in MRS agar allows selective isolation of LAB, at the same time ensuring the removal of most fastidious organisms.

Physiological and biochemical characterization of lactic acid bacteria

Phenotypic properties of LAB such as cell morphology of all isolates were determined using a microscope by Gram staining (Bergey et al., 1989). Isolates were further tested for different tests including catalase test, CO_2 production form glucose, growth at different temperatures (15, 37 and 45^{o}C) as well as the ability to grow in different concentrations of sodium chloride, antibiotic resistance and pH in MRS agar. Sugar fermentation patterns of LAB isolates were determined using different sugars.

Feasibility tests of *Lactobacillus* probiotics

Feasibility tests of *Lactobacillus* was carried out using Gastrointestinal Tract (GIT) conditions of chicken including, antibiotic resistance, resistance to low pH, resistance to bile salt, bile salt hydrolysis and antimicrobial activity against pathogens were done using standard procedures.

Experimental animal management and design

A total of 120 White leghorn layers were used for the study. The feed ingredients used in the experiment were according to standard layers diet (basal diet) and probiotic bacteria were supplemented. Before the commencement of the actual experiment and placing the experimental animals in the pen, watering troughs, feeding troughs, laying nests and the pen itself were cleaned thoroughly, disinfected and sprayed. The birds were vaccinated for the common diseases.

The chickens were randomly distributed into the pens each having the capacity of 10 hens. The birds were fed in a group providing feed twice a day at 8:00 and 16:00 hours. Each pen was provided with laying nest, feeders and watering point. A regular 16 hours light was provided throughout the experimental period of 84 days (12 weeks). The birds were acclimatized for one week for the new feed treatment.

A completely randomized design with four treatments was used as in table 1. T1 was control without probiotic bacteria supplementation, T2 was supplementation of *Lactobacillus acidophilus* in the diet, T3 was supplementation of *Lactobacillus plantarum* in the diet and T4 was supplementation of both *Lactobacillus acidophilus* and *Lactobacillus plantarum* in the ration. Each treatment was replicated three times having 10 layers each replica. The probiotic bacteria used for the study were the isolated, characterized and cultivated probiotic bacteria in the Haramaya University, microbiology laboratory.

Response criteria

The parameters employed in this experiment were: Feed Intake (FI), Hen Day Egg Production (HDEP), egg weight and egg size. FI was calculated by subtracting the amount of feed refusal from the amount of feed offered/day. HDEP was calculated as the ratio of the number of eggs collected/day with the number of birds in the pen. Eggs collected during the experiment categorized as jumbo, extra-large, large, medium, small and pee wee based their size (table 2).

Data analysis

Collected data were analyzed using of SAS 9.1.3 and data on production and egg quality parameters were stratified into the main factor (probiotics). A 5% ($P<0.05$) level of significance was used to determine statistical significance.

RESULTS AND DISCUSSION

Isolation, testing and characterization of *lactobacillus* probiotic

Probiotic *Lactobacilli* species including *lactobacillus acidophilus* (hudf8) and *lactobacillus*

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plantarum (hudf20) were the candidates of LAB species from raw unpasteurized cow milk samples (Table 3, 4 and 5).

Effects of probiotic *lactobacillus* species on productive traits

The effects of probiotic *L. acidophilus* and *L. plantarum* on FI, HDEP, egg weight and egg size are presented in (Table 6). Supplementation of probiotic *lactobacillus* species improved the FI, HDEP and Egg weight. However, there was no significant effect on egg size in layers supplemented with probiotic. Significantly higher FI, HDEP and egg weight was recorded at chicken supplemented the combination of the *lactobacillus* species (*L. acidophilus* and *L. plantarum*).

In this experiment, improvement in FI was recorded as a result of probiotic supplementation. Raka et al. (2014) reported a rise in feed and water consumption in laying hens fed with Liquid Probiotics Mixed Culture (LPMC) containing two type microorganisms, *Lactobacillus* and *Bacillus* species which is in agreement with the current study. Similarly, Nahashon et al. 1996, feeding viable *Lactobacillus* at 1100 mg kg^{-1}(4.4 $\times 10^{7}$ colony forming unit kg^{-1}) increased daily feed consumption, egg size, nitrogen and calcium retentions. Another study by Zhang and Kim (2014) reported an increase body in FI in chicken fed with multi-strain probiotics compared with that in control group fed basal diet. Similar results were observed with studies by Lei et al. (2013), Inatomi (2016) and Sarangi et al. (2016) in that Probiotics supplementation into poultry diets improves feed intake and growth performance in laying flocks. However, Inclusion of probiotic caused no significant increase in feed consumption, egg production and egg weight (P>0.05) (Mahdavi et al., 2005). Another study, Saadia and Nagla (2010) reported FI values of different treated groups were approximately similar and lacked significance with layer flock that fed with probiotics.

The study shows an increase in HDEP and average egg weight due to probiotic supplementation. Raka et al. (2014) reported the highest HDP and egg weight in layers supplemented with LPMC containing two type microorganisms, *Lactobacillus* and *Bacillus* species. Similarly, Yörük et al. (2004) reported that egg production in Hisex Brown layers fed with probiotics contained *L. plantarum* and *L. acidophilus*, showed greater egg production than the group fed with basal diet. Moreover, there were linear increases in egg production with increased supplemental probiotic. Moreover, significant improvement in egg production was observed in hens supplemented with a mixed culture of *L. acidophilus* and *L. casei* (Haddadin et al., 1996).

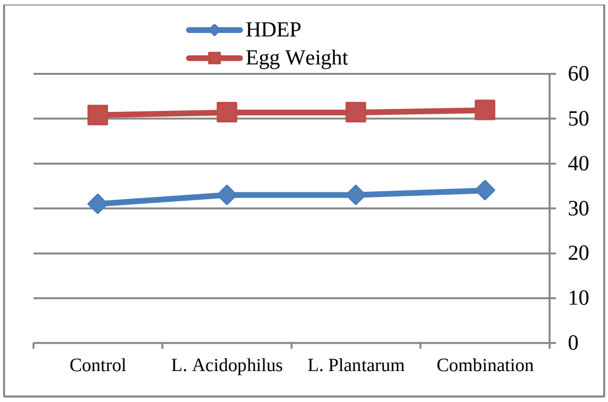

Figure 1.effect of *Lactobacillus acidophilus*, *Lactobacillus plantarum* and their combination on hen day egg production and egg weight in White Leghorn hens during the study period

A study by Davis and Anderson (2002) found no significant improvement in egg production of hens supplemented with Prima Lac, a commercial product containing *Lactobacillus* species. Similarly, Addition of probiotic had no significant effect (P>0.05) on shell hardness and shell thickness and these were expected which have already been reported (Haddadin et al., 1996 and Mohan et al., 1995). The same result was reported by Ramasamy et al. (2008) in which, supplementation of *Lactobacillus* cultures did not influence the egg production of hens throughout the experimental period and no significant difference in egg weight in hens fed with *L. acidophilus.*

Table1. Layout of the experiment on effect of probiotic *lactobacillus* species on productive traits in White Leghorn chicken during the study period

Treatments	Number of replication	Supplementation of lactic acid probiotic bacteria	Number of birds per replica	Total number of birds per treatment
T1	3	No probiotic bacteria (control)	10	30
T2	3	*Lactobacillus acidophilus*	10	30
T3	3	*Lactobacillus plantarum*	10	30
T4	3	*Lactobacillus acidophilus* and *Lactobacillus plantarum*	10	30

T1: treatment 1; T2: treatment 2; T3: treatment 3 and T4: treatment 4

Table 2. Modern egg size chart for adult laying chicken used from May to August 2015

Size	Minimum weight (g)
Jumbo	70
Extra-large	63
Large	56
Medium	49
Small	42
Pee wee	<42

Table 3. Physiological and biochemical characteristics of *Lactobacillus* strains isolated from fresh cow milk

Characteristic	Isolates	
	***Lactobacillus acidophilus* (hudf8)**	***Lactobacillus plantarum*(Hudf20)**
Gas from glucose	+	-
Cell shape	bacillus	bacillus
Ammonia from arginine	-	-
Motility	-	-
Catalase test	-	-
Aerobicity	f.a	f.a
Growth at different temperature		
10°C	-	-
15°C	+	-
45°C	v	+
Growth at different pH		
2.0	-	-
4.0	-	+
5.0	+	+
Growth in the presence of NaCl		
2%	+	-
4%	+	+
6.5%	-	-
Carbohydrate fermentation		
Lactose	+	+
Maltose	+	+
Glucose	+	+
Galactose	+	+
Mannose	+	+
Mannitol	+	-
Melezitose	+	-
Salicin	-	-
Melibiose	-	-
Cellulose	+	-
Rhamnose	-	-
Sucrose	-	+
Ribose	-	-

v=variable reaction; f.a=facultative anaerobic; n=2

Table 4. Probiotic feasibility test of *Lactobacillus* strains simulating under gastrointestinal tract conditions of adult layers

Characteristics	Isolates	
	***Lactobacillus acidophilus* (hudf8)**	***Lactobacillus plantarum*(hudf20)**
Resistance to low pH		
2.0	$1.03{\pm}0.02^a$	$0.98{\pm}0.00^a$
3.0	$1.25{\pm}0.00^a$	$1.05{\pm}0.01^a$
4.0	$1.31{\pm}0.00^a$	$1.32{\pm}0.00^a$
Resistance to bile acids 0.3 % (w/v)		
0hr	$1.23{\pm}0.03^a$	$1.23{\pm}0.00^a$
1hr	$1.01{\pm}0.01^a$	$1.13{\pm}0.01^a$
2hr	$0.93{\pm}0.00^a$	$0.98{\pm}0.02^a$
3hr	$0.87{\pm}0.00^a$	$0.89{\pm}0.00^a$
Antibiotic resistance		
Streptomycin	R	R
Gentamycin	R	R
Tetracycline	R	R
Heamolytic test	-	-

[a]Means bearing similar superscripts in the same column differs insignificantly (p>0.05); R=resistant; -=negative reaction, n=2

Table 5. Antimicrobial activity of *Lactobacillus* isolates from fresh cow milk from May to August 2015

Lactobacillus isolates	Means zone of inhibition zone (mm)			
	Streptococcus aureus	***Klebsiella pneumonia***	***Escherichia coli***	***Pseudomonas aeruginosa***
*L.acidophilus*hudf1	8±0.00^{b}	11±0.00^{b}	12±0.00^{b}	8±0.00^{b}
*L. plantarum*hudf3	9±0.01^{b}	12±0.03^{b}	9±0.00^{b}	11±0.00^{b}
*L.acidophilus*hudf8	21±0.02^{a}	18±0.00^{a}	17±0.02^{a}	17±0.00^{a}
*L.acidophilus*hudf12	11±0.02^{b}	10±0.00^{b}	13±0.00^{b}	11±0.00^{b}
*L. plantarum*hudf5	12±0.00^{b}	10±0.01^{b}	8±0.00^{b}	9±0.00^{b}
*L.acidophilus*hudf6	11±0.00^{b}	11±0.03^{b}	6±0.03^{b}	10±0.00^{b}
*L.plantarum*hudf20	19±0.03^{a}	20±0.03^{a}	18±0.00^{a}	20±0.00^{a}

^{ab} Means bearing different superscripts in the same column differ significantly ($p<0.05$); n=2

Table 6.effect of *Lactobacillus acidophilus, Lactobacillus plantarum* and their combination on productive traits in White Leghorn hens during May to August 2015

Parameter	Control	*Lactobacillus acidophilus*	*Lactobacillus plantarum*	Combination
FI (g/day/hen)	98.9 ± 1.16	99.8 ± 0.47	101.8 ± 2.12	105.0 1.00
HDEP (%)	0.31 ± 0.01	0.33 ± 0.01	0.33 ± 0.01	0.34 0.01
Egg weight (G)	50.8 ± 0.40	51.4 ± 0.35	51.4 ± 0.25	51.9 0.15
Egg size (%)				
Jumbo	----	----	----	----
Extra-large	11.7 ± 0.76	12.8 ± 0.20	13.1 ± 0.25	13.5 ± 0.15
large	22.7 ± 1.06	23.3 ± 0.46	23.9 ± 0.25	24.5 ± 0.50
Medium	44.4 ± 1.24	44.8 ± 0.59	44.4 ± 0.40	44.7 ± 0.47
Small	17.0 ± 0.35	15.2 ± 0.96	14.8 ± 0.40	13.9 ± 0.36
Pee wee	4.0 ± 1.00	3.97 ± 0.15	3.8 ± 0.10	3.3 ± 0.21

CONCLUSION

Supplementation of probiotics into layers diet improves their production performance. In this study, supplementation of probiotics significantly improves FI, HDEP and egg weight. Mixture of probiotics (*L. acidophilus* and *L. plantarum*) is recommended as it significantly improves FI, HDEP and egg weight. However, there was no significant effect of probiotic supplementation on egg size. Despite the improvements in productive traits, further investigation is recommended to establish the optimum dosage and mode of inclusion for different classes of poultry.

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Competing Interests

The authors declare that they have no competing interests.

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