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Effect of Corn Silage Combined with Prebiotic or Probiotic on Performance, Immune Response, Blood Parameters, and Bone Indices in Molted Layer Hens

Research Article

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ABSTRACT

This experiment was conducted to evaluate the effects of corn silage with prebiotic or probiotic on performance, immune response, and bone indices in layer hens during the molting period. Sixty 89-weeks old hens after the acclimatization period was used in a completely randomized design with five treatments including production diet (PD), feed withdrawal (FW), 20% PD plus 80% corn silage (CS80), 80% corn silage with 20% PD and probiotic at a level of 0.5 g/kg (CS80+Pro); 80% corn silage with 20% PD and prebiotic at a level of 1 g/kg (CS80+Pre). Four replicates of three birds were allocated to each treatment and the experiment lasted for 14 days. Results indicated that hens fed corn silage alone or combined with prebiotic or probiotic had significantly lesser feed intake than the PD group (P<0.05). Hens subjected to feed withdrawal treatment or corn silage feeding loss of roughly 22 to 30 percent of their body weight which was significantly lesser than hens fed PD (P<0.05). The FW and the use of corn silage alone or with prebiotic or probiotic compared to PD, resulted in a significant decrease of the ovary and liver weights (P<0.05). Blood phosphorus concentration was significantly higher in corn silage with prebiotic or probiotic treatments than in FW and PD treatments (P<0.05). This experiment showed that corn silage could be used as an effective method for force molting instead of feed withdrawal.

KEY WORDS corn silage, laying hen, molt, prebiotic, probiotic.

INTRODUCTION

Molt induction is a management tool used to prolong hen lay. This physiological process is associated with severe loss of feed intake, weight loss, productive tract reflux, and ovulation pause (Glatz and Tilbrook, 2020). Molt induction at the end of the laying phase will improve the egg quality of aged hens, more eggs produced, prolong the reproductive period, and ultimately increase the economic efficiency of commercial laying hens (Ayasi *et al.* 2022). One of the most common methods is to remove the feed and change the length of the light to reduce the weight by 25% to 30%.

With the regression of the bird's reproductive system, the bird's production cessation and prepares for the next production period (Moghaddam *et al.* 2018). The hens that lost the most body weight showed the greatest improvement in egg shell quality and the highest egg production in the next laying cycle (Baker *et al.* 1983).

Starvation puts stress on the bird, and therefore molting causes to weaken the immune system (Landers *et al.* 2007). This situation makes birds involved with pathogenic bacteria such as *Salmonella* (Holt, 2003), and susceptible to bone problems such as osteoporosis and bone fragility in older hens in the post-molting period (Park *et al.* 2004). Therefore, feed withdrawal for several days is immoral and endangers birds' health.

High-fiber diets such as alfalfa meal may be used in weight-loss programs (Ayasi *et al.* 2022). Corn silage is a high-fiber feed obtained from the controlled fermentation of whole corn by anaerobic bacteria (lactic acid-producing bacteria) and is widely used as animal feed. In addition, corn silage and some feed additives such as prebiotics and probiotics increase the production of short-chain fatty acids and decrease the pH of the cecum by expanding the population of lactic acid-producing bacteria (Khan *et al.* 2020).

Probiotics are live microorganisms that provide health benefits to the host when administered in sufficient amounts (Hill *et al.* 2014). Prebiotics are a substrate that is selectively used by host microorganisms and has beneficial effects on health (Gibson *et al.* 2017). Prebiotics and probiotics increase the production of short-chain fatty acids and decrease the pH of the cecum by expanding the population of lactic acid-producing bacteria, which reduces the population of pathogenic microorganisms in the gastrointestinal tract (Donalson *et al.* 2008; Neal-McKinney *et al.* 2012). On the other hand, improving the function of the immune system (Pourabedin and Zhao, 2015) and improving the morphological characteristics of the intestine have also been well-proven (Zhao *et al.* 2019). Egg weight and egg mass improved in laying hens fed with probiotic (Protexin) supplementing the diet (Khan *et al.* 2011). The use of Protexin probiotic in laying hens can improve performance and egg traits (Nobakht and Fard, 2016).

This study aimed to evaluate the immune response and some blood parameters during the molting period and the performance in molting hens fed by corn silage with and without multi-species probiotics (Protexin) or prebiotics (Bio-Mos®) in comparison with feed-deprived birds.

MATERIALS AND METHODS

Birds and experimental design

The animal trial was conducted following the Animal Care and Use Review Committee guidelines of Gorgan University of Agricultural Sciences and Natural Resources, Iran (Approval No. MAD 1392A/116/40).

A total of 60 Lohmann Selected Leghorn (LSL) 89-weeks old were obtained from a local commercial layer and then placed in the wire cage system (dimensions of 40×45 cm) under controlled climate conditions. Further, the hens were divided into five treatment groups with four replicates in each treatment (three birds per replicate). Before the experiment, laying hens were kept for ten days for the proper terminology adjustment period. Daily 110 g feed was offered to each hen and a light period of 16 hours of light and 8 hours (hr) of dark (16L:8D) was adopted.

The mean temperature of the house was 27 ± 1.5 ˚C, and the average light intensity was 4.5 ± 0.5 lx. One week before the molting period, the lighting program was changed to 8 hr of light and 16 hr of darkness per day (8L:16D). The five dietary treatments including production diet (PD); total feed withdrawal (FW); 80% corn silage with 20% production diet (CS80); 80% corn silage with 20% production diet and probiotic at a level of 0.5 g/kg (CS80+Pro); 80% corn silage with 20% production diet and prebiotic at a level of 1 g/kg (CS80+Pre) was offered. Corn silage had 30% dry matter, 7% crude protein, 0.29% calcium, and 0.24% phosphorus. Protexin was used based on catalog recommendations. The production diet was based on the LSL recommendation guide and prepared in mash form (Table 1). All groups had free access to water and feed during the molting period for 14 days, except the FW group, which was deprived of feed during the experiment. The mean room temperature was 28 ± 1 ˚C during the molting period, and the light intensity was approximately 15.5 ± 1.5 lx.

Table 1 The ingredients and chemical composition of basal diet

Ingredients	%
Corn	63.56
Soybean meal (44% CP)	23.88
Soy oil	0.66
Dicalcium phosphate	1.09
$CaCO_3$	9.93
Salt	0.29
DL-Met.	0.08
L-Lys.	0.01
Vitamin supplement[1]	0.25
Mineral Supplement[2]	0.25
Calculated composition	
AME, kcal/kg	2720
CP, %	15.3
Met. %	0.3
Met+Cys. %	0.54
Lys. %	0.59
Thr. %	0.41
Ca, %	4.09
Available P, %	0.35
Na, %	0.15

[1] Provided the following per kilogram of diets: vitamin A (trans retinyl acetate): 3500000 IU; vitamin D_3 (cholecalciferol): 1000000 IU; vitamin E (DL-a-tocopheryl acetate): 9000 IU; vitamin K_3: 1000 mg; Thiamine: 900 mg; Riboflavin: 3300 mg; vitamin B_3 (niacin): 5000 mg; vitamin B_5 (pantothenic acid): 15000 mg; vitamin B_6: 150 mg; vitamin B_9: 500 mg; vitamin B_{12}: 7.5 mg and Choline chloride: 250000 mg.

[2] Provided the following per kilogram of diets: Mn (from $MnSO_4$, H_2O): 50000 mg; Zn (from ZnO): 50000 mg; Fe (from $FeSO_4.7H_2O$): 25000 mg; Cu (from $CuSO_4$, $5H_2O$): 5000 mg; I 9from Ca $(IO_3)_2H_2O0$: 500 mg and Se (from sodium selenite): 100 mg.

Multi-species probiotic and prebiotic

Protexin is one of the commercial multi-strain probiotics used in poultry production (Probiotics International Limited, UK manufacture). Protexin contains different natural bacteria, mostly of Lactobacillus serotypes, including *Lac-*

tiplantibacillus plantarum, *Lacticaseibacillus rhamnosus*, *Lactobacillus delbrueckii subsp. bulgaricus*, *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Streptococcus thermophilus and Enterococcus faecium.*

Bio-Mos® is the outer cell wall from a selected strain of *Saccharomyces cerevisiae* yeast rich in β-glucans and mannan oligosaccharides which is a prebiotic-supplemented feed for animal and poultry nutrition.

Data collection

The feed was withdrawn at the start of the experimentand hens received their respective molting diets for 14 days. At the beginning and end of the experiment, all birds were weighed and the percentage of body weight loss (BWL) was calculated. At the end of the experiment, one hen was selected from each pen, weighed, slaughtered, and then their oviduct and ovary were weighed by a digital scale, and data were expressed as a percentage of live body weight. After slaughtering, the right leg bones of hens were collected. Cleaned bones were weighed and then placed in a muffle furnace and ashed at 600 ˚C for eight hours (Muszyński *et al.* 2017).

At the beginning and end of the experiment, one hen in each replicate was selected for measuring blood parameters. The blood samples (5 mL) were taken from the brachial vein and collected in heparin tubes. 3 ml of the blood was used for measuring differential counts of white blood cells.

For this purpose, 100 cells were counted, once on each slide, using a light microscope at ×1000 magnification (Olympus CX31; Olympus Company, Tokyo, Japan). The heterophils (H) and lymphocytes (L) were counted and then the H/L ratio was determined. The remaining blood samples (2 mL) were centrifuged at 3000 × g for 12 min. and then plasma levels of glucose, calcium, and phosphorus were determined spectrophotometrically with commercial kits (Pars Azmun, Tehran, Iran).

The day before applying the treatments, one bird from each replicate was randomly selected and 0.3 mL of 5% sheep red blood cells (SRBC) was injected into the breast muscle of the hens to determine the humoral immune response. The blood samples (3 mL) were taken from the brachial vein of injected hens on days 1, 7, and 14 of the experiment. Serum samples were then used to measure antibody titer against SRBC using a direct hemagglutination assay (He *et al.* 2020).

Statistical analysis

All data were analyzed in a completely randomized designusing the General Linear Model (GLM) procedure of SAS software (SAS, 2005). Duncan's multiple range test was used to separate the statistical significance among the means, and significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

The experiment results on heterophile, lymphocyte, and H/L ratio at the beginning and 14 days of the experiment are presented in Table 2. At the beginning of the experiment, there was no significant difference in heterophile, lymphocyte, and H/L ratio. On day 14 molting, the heterophile and H/L ratio was significantly higher in FW than in PD and CS80+Pretreatments. Also, the lymphocyte in the FW group was significantly lower than in PD and CS80+Pre treatments. Table 3 shows no significant difference among any treatments in antibody production against SRBCatany time period.

The results of the effect molt period on feed intake, body weight, and relative organ weights are presented in Table 4. The greatest feed intake was seen in the PD group, and feed intake was similar among other treatments except for the FW group ($P<0.05$). The greatest BWL belonged to the FW group while the lesser was for the PD group, in which the differences were statistically significant compared to the other groups ($P<0.05$). Birds in the PD group had higher liver and ovary weights than birds in other groups ($P<0.05$). The treatments had no significant differences in heart, spleen, and oviduct weight ($P<0.05$).

There was no significant difference in glucose, calcium (Ca), and phosphorus (P) at the beginning of the experiment (Table 5). On day 14 of the molting period, the phosphorus was significantly higher in CS80 + Pro and CS80 + Pre than PD and FW groups.

Table 6 shows no significant difference between treatments in the ash of the tibia and femur but tibia and femur weight were significantly higher in the FW group than in other treatments ($P<0.05$).

The use of probiotics and prebiotics stimulates the immune system and increases resistance to pathogens (Tang *et al.* 2017). Probiotics and prebiotics can improve immune system function (Hassan and Ragab, 2007; Alaqil *et al.* 2020). Heterophils are cells that have bactericidal properties and play an important role in the inflammation of the body because these cells are one of the most abundant granulocytes in the body of most avian species (Mitchell and Johns, 2008). The H/L ratio in laying hens under stress conditions increases (Davis *et al.* 2000). Increasing the number of heterophile indicates the presence of pathogens and stress in the body. Further, the H/L ratio also indicates immunity status, which lower ratio indicates greater immunity and the possibility of resistance to pathogens and stress (Sturkie, 1995; Price *et al.* 2018). Similar findings were reported by Khan *et al.* (2011) and Tang *et al.* (2017) who find the addition of prebiotics and probiotics caused a decrease in the H/L ratio. An increase in the percentage of lymphocytes indicates an immune-stimulating effect.

Table 2 Effects of treatments on heterophile (H), lymphocyte (L), and heterophile-lymphocyte (H/L) ratio of laying hens at 1 and 14 days of the experiment

Treatment	Day-1			Day-14		
	Heterophile (%)	Lymphocyte (%)	H/L ratio	Heterophile (%)	Lymphocyte (%)	H/L ratio
PD	23.75	72.25	0.313	21.50^{c}	78.50^{a}	0.275^{c}
FW	25.00	75.00	0.336	29.75^{a}	70.25^{c}	0.424^{a}
CS80	24.25	75.75	0.322	29.00^{ab}	71.00^{bc}	0.411^{ab}
CS80 + Pre	22.50	77.50	0.291	25.75^{b}	74.25^{b}	0.340^{b}
CS80 + Pro	24.25	75.75	0.321	28.00^{ab}	72.00^{bc}	0.390^{ab}
SEM	1.43	1.30	0.025	1.20	1.20	0.02
P-value	0.79	0.79	0.79	0.001	0.001	0.002

PD: production diet; FW: feed withdrawal; CS80: 80% corn silage + 20% production diet; CS80 + Pre: 80% corn silage + 20% production diet + prebiotic; CS80 + Pro: 80% corn silage + 20% production diet + probiotic.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 Effects of treatments on antibody production against ship red blood cells (SRBC) of laying hens at 1, 7, and 14 days of the experiment (means of log2 of the reciprocal of the last dilution exhibiting agglutination)

Treatment	Day-1	Day-7			Day-14		
	IgT	IgT	IgG	IgM	IgT	IgG	IgM
PD	1.75	3.66	2.66	1.00	4.12	3.25	0.87
FW	2.12	3.00	1.66	1.33	2.75	1.75	1.00
CS80	1.5	4.00	3.33	0.66	3.25	3.00	0.25
CS80 + Pre	2.25	5.00	4.00	1.00	5.00	4.25	1.25
CS80 + Pro	1.5	3.75	3.25	0.50	5.00	4.25	0.75
SEM	0.46	1.05	0.71	0.47	0.82	0.66	0.27
P-value	0.64	0.83	0.39	0.82	0.24	0.08	0.46

PD: production diet; FW: feed withdrawal; CS80: 80% corn silage + 20% production diet; CS80 + Pre: 80% corn silage + 20% production diet + prebiotic; CS80 + Pro: 80% corn silage + 20% production diet + probiotic.
SEM: standard error of the means.

Table 4 Effects of treatments on feed intake, body weight, and relative organ weightafter 14- days molting period

Treatment	Feed intake, g per bird	Initial BW (g)	BW loss (%)	Ovary (% BW)	Oviduct (% BW)	Liver (% BW)	Spleen (% BW)	Heart (% BW)
PD	684.71^{a}	1690.83	8.81^{c}	1.35^{a}	0.37	1.96^{a}	0.099	0.41
FW	-	1587.08	30.22^{a}	0.43^{b}	0.35	1.41^{b}	0.107	0.40
CS80	488.84^{b}	1584.17	23.72^{b}	0.29^{b}	0.23	1.38^{b}	0.109	0.39
CS80 + Pre	504.60^{b}	1605.00	22.20^{b}	0.48^{b}	0.21	1.45^{b}	0.100	0.37
CS80 + Pro	389.95^{b}	1630.83	25.67^{b}	0.59^{b}	0.35	1.41^{b}	0.102	0.38
SEM	38.10	27.98	1.71	0.122	0.075	0.10	0.007	0.031
P-value	0.0023	0.08	0.0001	0.0004	0.505	0.008	0.85	0.91

PD: production diet; FW: feed withdrawal; CS80: 80% corn silage + 20% production diet; CS80 + Pre: 80% corn silage + 20% production diet + prebiotic; CS80 + Pro: 80% corn silage + 20% production diet + probiotic.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Effects of treatments on some serum parameters after 14- days molting period (mg/dL)

Treatment	1 day			14 day		
	Ca	Glucose	P	Ca	Glucose	P
PD	9.75	194.26	4.11	13.29	180.96	3.11^{b}
FW	7.30	190.64	4.81	9.33	161.36	2.66^{b}
CS80	13.92	163.90	4.91	11.48	165.97	4.35^{ab}
CS80 + Pre	14.99	142.92	6.57	18.24	156.60	7.01^{a}
CS80 + Pro	9.43	179.56	5.65	17.76	190.29	6.33^{a}
SEM	3.31	24.03	0.82	23.46	20.73	0.74
P-value	0.51	0.67	0.38	0.30	0.93	0.01

PD: production diet; FW: feed withdrawal; CS80: 80% corn silage + 20% production diet; CS80 + Pre: 80% corn silage + 20% production diet + prebiotic; CS80 + Pro: 80% corn silage + 20% production diet + probiotic.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 Effects of treatments on the tibia and femur

Treatment	Tibia (g)		Femur (g)	
	Dry weight	Ash	Dry weight	Ash
PD	4.59[b]	2.42	6.05	3.28
FW	5.48[ab]	3.23	6.38	3.47
CS80	5.60[ab]	3.13	6.90	2.70
CS80 + Pre	6.28[ab]	3.12	6.61	3.01
CS80 + Pro	6.95[a]	2.97	7.72	2.79
SEM	0.66	0.5	0.6	0.5
P-value	0.18	0.8	0.38	0.8

PD: production diet; FW: feed withdrawal; CS80: 80% corn silage + 20% production diet; CS80 + Pre: 80% corn silage + 20% production diet + prebiotic; CS80 + Pro: 80% corn silage + 20% production diet + probiotic.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

These cells play an important role in the production of humoral antibodies. Also, feeding with probiotics and prebiotics improves gastrointestinal tract (GIT) health, and decreases oxidative stress, thereby enhancing GIT microbiota and antioxidant capacity (Tayeri *et al.* 2018; Bayraktar and Tekce, 2020), and increasing immunity in laying hens during the molting period (Dastar *et al.* 2016). The results of antibody production against SRBC showed that the response of chickens to antibody production against SRBC in experiment treatments was not significantly different. These results are similar to other reports (Khodadadi *et al.* 2008; Bayraktar *et al.* 2021), which reported that injection of SRBC did not affect the production of antibody production against SRBC. They suggested that the H/L ratio was a better measure of stress conditions during molting. This study shows that the use of prebiotics and probiotics in stressful situations such as molting stimulates the immune response of laying hens and reduces the effects of stress.

Hens in the PD group had the highest feed intake (684.71 g) and had a significant difference with other treatments. Soe *et al.* (2009) reported that diluted diets of laying hens such as wheat bran and rice hull reduced feed intake during the molting period compared to the group fed the diet of laying. With its high fiber content and slow-down passage rate through the hen's digestive tract, corn silage causes the hen to feel satiety (Biggs *et al.* 2004; Ayasi *et al.* 2016). The optimal weight loss after the spotting period is 25% to 30%, and this amount of reduction can lead to increased egg production (Moghaddam *et al.* 2018). The body weight loss of molting treatments was between 22% to 30% in the present study. Bodyweight loss is due to weight loss in the liver, ovaries, and oviducts (Ayasi *et al.* 2022). Decreased photoperiod reduces the concentration of reproductive hormones by affecting the hypothalamic-pituitary-adrenal axis, leading to degeneration ovary and weight loss (Berry, 2003). Similar to Donalson *et al.* (2005), weight loss in the PD group could be due to reduced photoperiod during molting. Liver weight loss indicates a reduction in the liver's metabolizable energy sources such as glycogen and lipids (Donalson *et al.* 2005).

Birds' calcium stores are affected by estrogen (Wilson and Thorup, 1998), which is at its lowest during the molting period (Ashoori *et al.* 2021). Reducing feed intake and low amounts of minerals in corn silage reduced blood calcium and phosphorus levels (Gongruttananun *et al.* 2017).

Scholz-Ahrens *et al.* (2001) and Swiatkiewicz and Arczewska (2012) showed that prebiotics increases the absorption of minerals such as calcium. Align to this study, prebiotics increase the absorption of minerals such as calcium and phosphorus, leading to changes in serum phosphorus and increasing the reabsorption and storage of minerals (Rodehutscord and Rosenfelder, 2016). The availability of minerals such as phosphorus and calcium is very desirable for eggshell formation, skeletal structure improvement, and bone mineralization in laying hens (Pineda-Quiroga *et al.* 2019).

Stimulation of the molting by feed withdrawal is one of the factors increasing bone fracture in laying hens (Park *et al.* 2004). The present experiment results showed that the tibia's dry weight in the CS80 + Pro group was significantly higher than in other treatments. This result was similar to the report of Kim *et al.* (2006). Prebiotics and probiotics increase the population of lactic acid-producing bacteria such as lactobacillus, leading to increased short-chain production (Scholz-Ahrens *et al.* 2001). They also improve bone quality by increasing the absorption of some minerals such as calcium because of increasing solubility (Swiatkiewicz and Arczewska, 2012). Adding probiotics to the diet of laying hens can increase the mineral density in the tibia and femur and the mineral content of the femur (Yan *et al.* 2019). Current findings showed that probiotics positively affect the skeletal health of laying hens.

CONCLUSION

The results showed that corn silage with probiotic or prebiotic reduces body weight by 22% to 25% and the weight of internal organs during the molting period. Thus, corn silage is a method comparable to feed withdrawal for induced molt in laying hens. Therefore, instead of the feed withdrawal method, which is stressful in laying hens, it is recommended to use corn silage with probiotic or prebiotic as a molting method.

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