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Probiotics in Drinking Water Alleviate Stress of Induced Molting in Feed-deprived Laying Hens

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ABSTRACT : An experiment was conducted to evaluate the physiological changes of laying hens subjected to feed removal during induced molting while received probiotics in the drinking water. Post-molt performance and egg quality criteria were also studied. Ninety 78-week-old Hy-line W36 laying hens were divided into two treatment groups according to equal body weight and subjected to induced molting by continuous feed removal until around 30% BW reduction. The experiment lasted 12 wks consisting of 4-wk molting and 8-wk post-molt periods. Treatment 1 received no probiotics and was considered as the control. Treatment 2 was similar to the control except that hens received probiotics in the drinking water at 400 mg/L during feed deprivation. The results indicated that hens in both groups went out of production by Day 5. However, hens received probiotics reached 5 and 50% egg production sooner than the control (30 and 52 days vs. 31 and 54 days). Starvation during molting increased heterophil to lymphocyte (H/L) ratio, hematocrit and plasma T4 and Na^+ levels while plasma T3 and Cl^- levels were decreased. Probiotics had no significant impact on BW reduction during molt. Post-molt egg production and egg mass were higher in hens which previously received probiotics, but these responses were not significant. However, feed conversion ratio was significantly better in hens which received probiotics. Hematocrit, plasma thyroid hormone concentrations (T3 and T4) and plasma Na^+, K^+ and Cl^- levels during molting were not significantly influenced by supplementation of probiotics. However, H/L ratio showed a significant ($p<0.05$) reduction in birds which received probiotics suggesting beneficial effects of this product for feed-deprived laying hens. No significant difference was observed in post-molt egg quality criteria. (**Key Words :** Feed Deprivation, Laying Hens, Molting, Probiotics)

INTRODUCTION

Molting in avian species is defined as periodic shedding and replacement of feathers which is accompanied by involution of reproductive organs (Berry, 2003). Natural molting of laying hens generally takes four months (North and Bell, 1990), which raises economic concerns as the hens continue to be fed during non-production times (McDaniel and Aske, 2000). The molting process can be sped up by management practice called induced molting. Induced molting uniformly rests all hens and returns them to a more consistent high rate of lay for an extended period (McDaniel and Aske, 2000). Conventional induced molting program usually involves a period of fasting for 10 to 15 days or up to a 30% body weight reduction achieved (Ruszler, 1998). Hens subjected to continuous fasting experience stress and are highly susceptible to infection by salmonella (Holt, 2003). Egg industry, therefore, should seek for alternative molting programs or ways alleviating the stress of induced molting.

Probiotics define as microbial cell preparations or components of microbial cells that have a beneficial effect on health (Fuller, 2001). Probiotics have the ability to alleviate the stress and improve the immunocompetence (Revolledo et al., 2006). Moreover, probiotics have been showed to reduce colonization of Salmonella in poultry (Revolledo et al., 2006). Most recent report has suggested stopping the use of antibiotics and emphasized application of suitable alternatives (Dibner et al., 2007). In the present study, the efficacy of probiotics provided in the drinking water was evaluated for molted hens subjected to continuous feed removal and physiological parameters as well as postmolt laying performance were examined.

MATERIALS AND METHODS

Ninety 78-week old Hy-line W36 laying hens were kept

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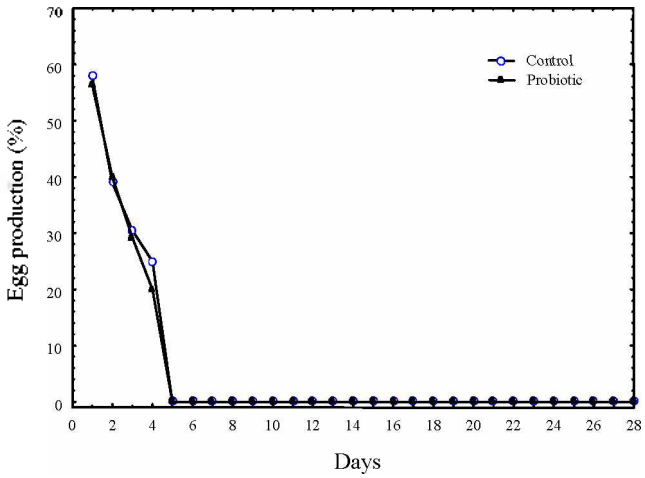

Figure 1. Trend of reduction in egg production during molting period.

in a house equipped with cages (45×50 cm) and exposed to 16 h daily photoperiod. Prior to beginning the experiment, all hens were weighted and distributed among 18 cages so that 5 hens were allotted to each cage with equal mean body weight in each cage. Each cage was considered as a replicate and nine such cages were assigned to each treatment. Each cage was equipped with separate feed trough and nipple drinker. There were two treatments consisted of a group deprived of feed at the start of experiment (day 1) until around 30% body weight loss relative to initial body weight (T1) and a group similar to T1 except that received probiotics in the drinking water during feed deprivation. The first two days following termination of fasting, hens were fed with limited amount of feed (45 g/day) and then fed *ad libitum* according to Hy-line W36 commercial management guide manual (2005). Probiotics (Protexin®) consisted of seven bacterial strains (*L. acidophilus*, *L. delbrueckii*, *L. plantarum*, *L. rhamnosus*, *Entococcus faecium*, *Streptococcus thermophilus* and *Bifidiobacterium bifidum*) and two fungus strains (*Aspergillus oryzae*, and *Candida pintolopesii*) and added to the tap water at the concentration of 400 mg/L. The solution was daily made and stored in a plastic tank linked to a pipe equipped with nipple drinkers for each cage. Addition of probiotics to the tap water was conducted on daily basis to ensure maximum survivability of microorganisms. The survival rate of microorganisms is sustained for 12 h when administered as liquid suspensions (recommended by Nikootec Co. Tehran, Iran). The tap water analysis showed trace contamination of electrolytes: Na^+ (0.2 mg/L), K^+ (0.1 mg/L) and Cl^- (0.2 mg/L).

The house was a thermostatically-controlled environment and temperature was maintained at 18±1°C throughout the experiment. The experiment lasted 12 weeks (Four weeks for the molt period and 8 weeks for the post molt lay period). On Day 1 (the initiation of feed withdrawal), the daily photoperiod was decreased to 8h. On Day 24 and 30, the photoperiod was increased to 10 and 12 h respectively, then, increased 30 min per week until a photoperiod of 16 h was reached at peak production. Records of BW were kept before induction of molt (Day 1), Day 12 when around 30% BW reduction occurred and Day 28 which was end of molt period. Blood samples were taken from the brachial vein of one hen per replicate (9 hens/treatment) on Day 1 (before molt), Day 14 (mid-molt) and Day 28 (end of molt). Blood samples were used for determination of hematocrit and differential leukocyte counts (Gross and Siegel, 1983). Plasma was discarded by centrifugation in 10,000 g for 15 min and plasma samples were used to measure electrolyte levels (Na^+, K^+ and Cl^-) using a flame photometer (Corning 480 model) and plasma T3 and T4 hormone concentrations by radioimmunoassay using a commercial kit (REF KT2CT, Barcelona, Spain). Records of egg production were kept daily for 8 weeks post molts. Records of egg weight were kept weekly. The results of laying performance have been presented on a monthly basis. Egg quality measures were recorded on two eggs randomly collected weekly from each replicate during the 8 weeks of postmolt period (week 83 through week 95). The experimental animals were kept, maintained and treated in accepted standards for the human treatments of animals.

Table 1. Effect of probiotics provided in the drinking water during feed deprivation of molt on time to 5 and 50% egg production

Treatment	Days to 5% egg production	Days to 50% egg production
Control	31	54
Probiotic	30	52

Data were subjected to a complete randomized design and analyzed by GLM procedure of SAS software (1997). In case where the data comprised among the days of molt, they were subjected to a nested design.

RESULTS

The reduction in hen-day egg production during 28d molt period is shown in Figure 1. The figure indicates that hens on both treatments went out of production by Day 5. Table 1 depicts time to 5 and 50% egg production in both treatments. As depicted in Table 1, hens received probiotics returned to egg production sooner than the control and had higher egg production and egg mass, even insignificant (Table 5). However, feed conversion ratio was significantly improved in hens received probiotics during molting (Table 5).

Data analysis showed a significant reduction in BW during molt period (Table 2). Trends in body weight loss did not differ between treatments as summarized in Table 3. Hens in the control and probiotic groups lost 27.0 and 25.5% of their body weight by Day 12. Again, both group

Table 2. Changes in parameters of hens measured at different times during molting period

Time in molting	BW (g)	Hematocrit (%)	H/L (%)	T3 (ng/dl)	T4 (µg/dl)	Na (mM/L)	K (mM/L)	Cl (mg/L)
Before molt	1,620.7a	37.8c	0.37b	250.6ab	1.03c	129.2b	5.55a	1.56a
Day 7 in molt	1,222.5c	42.1a	0.75a	188.1c	2.10a	170.3a	5.16a	0.54c
Day 14 in molt	1,192.2c	40.7ab	0.65a	227.2b	1.03c	181.9a	5.31a	1.14b
Day 28 in molt	1,368.8b	38.5bc	0.37b	279.4a	1.43b	172.9a	3.79b	0.95b
SEM	17.29	0.93	0.06	12.56	0.09	7.91	0.25	0.12

Means within each column with uncommon superscript have significant difference ($p<0.05$).

Table 3. Effect of probiotic on body weight changes, hematocrit, and heterophil to lymphocyte ratio in molted hens subjected to continuous fasting

Criteria	Control	Probiotic	p-value
Body weight (g)			
Before molt	1,616.1±24.7	1,625.3±26.9	0.805
Day 7 in molt	1,212.8±23.6	1,232.2±35.8	0.656
Day 14 in molt	1,177.0±26.8	1,207±27.3	0.441
Day 28 in molt	1,365.6±25.1	1,371.8±22.2	0.855
Hematocrit (%)			
Before molt	37.0±1.97	38.6±1.53	0.514
Day 7 in molt	40.9±1.74	43.2±0.89	0.249
Day 14 in molt	39.4±1.86	42.0±1.24	0.270
Day 28 in molt	38.8±0.68	38.2±1.15	0.684
H/L ratio			
Before molt	0.38±0.04	0.36±0.05	0.737
Day 7 in molt	0.81±0.13	0.68±0.13	0.512
Day 14 in molt	0.80±0.11	0.51±0.06	0.039
Day 28 in molt	0.49±0.04	0.45±0.08	0.730

gained similarly so that they had equal body weight on Day 28.

Hematocrit value was significantly ($p<0.05$) higher when measured on Day 7 compared to before molt indicating that fasting elevated this variable. Nevertheless, there was no significant difference with respect to this variable between Day 28 and before molt suggesting that refeeding restored the changes of this variable (Table 2). Hematocrit did not significantly changed by Probiotics (Table 3).

Feed deprivation during molt significantly enhanced H/L ratio so that the difference between before molt and 7 or 14 d after molt was significant (Table 2). The ratio of H/L was reduced by giving Probiotics so that the difference between treatments was significant on Day 14 ($p = 0.039$) (Table 3).

Starvation during molting significantly enhanced plasma T4 level with a concomitant reduction in plasma T3 level (before molt vs. Day 7). Refeeding restored the changes in plasma T3 and T4 concentrations (before molt vs. Day 14 or 28) (Table 2). There were no significant differences between treatments with respect to circulatory T3 and T4 levels (Table 4).

Molting significantly enhanced Na^+ level whereas dropped Cl^- level regardless of treatment (Table 2). Providing probiotics in the drinking water during fasting period of molting did not significantly change the plasma Na, K and Cl concentrations (Table 4).

No significant difference was found between the treatments in terms of egg quality criteria post molt.

DISCUSSION

Probiotics did not influence the reducing trend of egg production rate during molt period. However, hens received Probiotics attained 5 and 50% egg production earlier than the control and had higher egg production and egg mass post molt. Although the difference between the control and probiotic-received group with respect to aforementioned parameters was close to being significant, these responses were not statistically significant. Feed conversion ratio, however, was significantly improved in hens received probiotics. This observation is consistent with report of Yoruk et al. (2004) who showed that supplementation of Probiotics composed of Lactobacillus, Bifidobacterium, Streptococcus and Enterococcus in laying hen diets increased egg production and egg mass and improved feed conversion ratio. Research with broilers has also indicated a significant improvement in feed conversion ratio as a result of feeding probiotics to chickens (Yu et al., 2007). It can be explained that probiotics helped colonization of beneficial microbial flora in the gastrointestinal tract and avoided

Table 4. Effect of probiotic on plasma thyroid hormones and electrolyte levels in molted hens subjected to continuous fasting

Criteria	Control	Probiotic	p-value
T3 (ng/dl)			
Before molt	233.8±13.79	267.4±17.15	0.782
Day 7 in molt	193.1±18.44	183.0±30.92	0.146
Day 14 in molt	242.5±20.10	211.9±15.65	0.245
Day 28 in molt	275.5±17.53	283.2±10.96	0.716
T4 (μg/dl)			
Before molt	1.00±0.05	1.06±0.09	0.593
Day 7 in molt	2.14±0.21	2.06±0.16	0.749
Day 14 in molt	0.99±0.05	1.07±0.04	0.244
Day 28 in molt	1.52±0.14	1.34±0.13	0.356
Na (mM/L)			
Before molt	140.0±9.92	129.0±7.01	0.403
Day 7 in molt	177.3±5.57	163.3±13.84	0.362
Day 14 in molt	191.1±14.09	172.7±14.61	0.377
Day 28 in molt	179.1±6.79	166.7±11.03	0.351
K (mM/L)			
Before molt	6.19±0.61	5.46±0.45	0.349
Day 7 in molt	5.47±0.21	4.84±0.39	0.182
Day 14 in molt	5.53±0.40	5.08±0.53	0.505
Day 28 in molt	3.84±0.22	3.73±0.27	0.754
Cl (mg/L)			
Before molt	1.78±0.23	1.33±0.25	0.216
Day 7 in molt	0.48±0.13	0.60±0.10	0.476
Day 14 in molt	0.89±0.11	1.39±0.07	0.002
Day 28 in molt	0.92±0.16	0.98±0.13	0.795

colonization of pathogenic bacteria. Subsequently, hens received probiotics had healthier gut and consequently better performance.

Birds received Probiotics lost 23.6 and 25.5% of their initial body weight after 7 and 12d respectively, and birds on the control lost 25.1 and 27.0% after the respective days. Although these differences were not significant, probiotics tended to prevent vigorous weight loss. By Day 28, birds on both groups regained their lost weight as equal as each other.

Starvation during molting resulted in elevated Hematocrit and refeeding caused it to return to the level similar to that of before molting. There are reports suggesting that starvation during molting increases packed cell volume (Keshavarz and Quimby, 2002). Molting causes a remarkable regression in ovary and oviduct weight (Khajali et al., 2007) which is associated with the loss of estrogenic activity. According to Sturki (1982), the loss of estrogenic activity could result in increased erythropoiesis and accounts for enhanced hematocrit. Probiotics did not have any significant impact on hemetocrit.

The heterophil to lymphocyte ratio is commonly used as an indicator of stress. Under stress conditions the ratio is tended to increase (Davis et al., 2000). Davis et al. (2000) showed that H/L ratio was significantly higher during a forced molt compared to other times of the year. As depicted in Table 2, fasting resulted in higher rate of H/L (before molt vs. Day 7) and refeeding restored the rate to the normal range just like before molting (before molting vs. 28 d molt). These observations showed that fasting during molting is a vigorous stress to the birds. Enhanced H/L ratio as a result of fasting was previously reported (Khajali et al., 2007). Probiotics, however, could be able to alleviate the stress of fasting so that H/L ratio was significantly reduced compared to the control.

Feed deprivation during molting led to decreased plasma T3 concentration and increased plasma T4

Table 5. Effect of probiotic on postmolt laying hen performance (83-95 wk of age)

variable	Control	Probiotic	SEM	p-value
HDEP* (%)	56.0	60.5	2.68	0.102
Egg mass* (g/b/d)	35.9	38.8	1.71	0.099
Feed conversion ratio*	2.88[a]	2.60[b]	0.15	0.046
Albumin height (mm)	4.58	5.22	0.309	0.289
Shell thickness (mm)	0.404	0.398	0.015	0.658
Shell strength (kg/cm^2)	3.76	3.47	0.18	0.370

* Data from week 83 through week 95.

concentration. Refeeding restored normal plasma T3 and T4 levels (Table 2). This is consistent with previous reports (Reyns et al., 2002; Decuypere et al., 2005). The decrease in plasma T3 level in feed deprived hens is likely to be the result of a shift in the balance between deiodination of T4 by hepatic deiodinase enzyme type I (D1) and T3 degradation by hepatic deiodinase enzyme Type III (D3) (Decuypere et al., 2005). Decuypere et al. (2005) reported increased hepatic deiodinase III mRNA levels at the first day of starvation which was dropped after refeeding. Probiotics did not influence the circulatory T3 and T4 levels (Table 4).

Plasma Na^+ level was significantly increased as a result of starvation during fasting whereas plasma Cl^- showed a significant reduction. Plasma K level was not significantly influenced by feed deprivation (Table 2). Probiotics did not have a significant impact on plasma electrolyte levels except for plasma Cl^- level on Day 14 (Table 4). Changes in electrolyte levels may be related to hormonal changes during molting other than fasting. Nevertheless, there is no report supporting these findings.

No significant difference was found between the treatments in terms of egg quality criteria post molt. Nevertheless, probiotic-received group had numerically higher albumin height than the control. This observation agrees those of Hayirli et al. (2005). Likewise, shell strength against breaking was numerically lower in probiotic-received group than the control. This may not be a consistent observation as there is no report supporting this finding.

CONCLUSIONS

Feed deprivation during molting is associated with increased plasma T4, H/L ratio, hematocrit and Na^+, while plasma T3 and Cl^- are reduced. Application of probiotics had a beneficial effect during feed deprivation of molt due to alleviating the stress as measured through decreased H/L ratio. Moreover, use of probiotics caused egg production to start earlier and results in increased postmolt egg production and improved feed conversion ratio.

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