J Vet Clin 28(6) : 581-585 (2011)

Journal of Veterinary Clinics
www.ksvc.or.kr

Changes of Blood Mg^{2+} and K^{+} after Starvation during Molting in Laying Hens

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(Accepted: Dec 18, 2011)

Abstract : Either the fasting during natural molting or the starvation in induced molting would be a severe metabolic stress to laying hens. The metabolic stress during starvation and subsequent refeeding syndrome could lead to unbalance of mineral homeostasis, including Mg^{2+}, K^{+} and P required by ATP synthesis. Since Mg^{2+} is a fundamental ion for normal metabolic processes and stress may not only increase in demands of Mg^{2+} but also produce consequence of Mg^{2+} deficiency, we investigated the changes of blood ionized and total ions related to starvation during molting in laying hens. We founded the significant decrease in blood Mg^{2+} and K^{+} accompanied by the changes of biochemical parameters relating to increased metabolic stress after molting. These results suggested that appropriate Mg^{2+} and K^{+} supplements to laying hens could have beneficial effects during molting and subsequent refeeding that could produce a severe hypomagnesemia and hypokalcemia.

Key words **:** laying hen, molting, starvation, hypermagnesemia, hypokalcemia.

Introduction

In nature, most adult avian species undergo annual natural molting through voluntary fasting to renew their feathers (14). Domesticated birds will naturally molt once per year, triggered by the declining day lengths of the fall (2). During this time, feed consumption is reduced and feathers are lost. New feathers grow to form a full plumage for the bird's protection in the colder weather. Otherwise, in commercial layer industry, older hens can be artificially forced to molting before the end of a first laying cycle resulted from economic concerns of significant decline significant decline of the laying cycle, egg production and quality (21). The induced molting results in body weight (BW) losses up to 40% of their mass and a pause in oviposition due to regression of the reproductive tract. After the molting period, egg production and quality improve significantly compared to the premolt period. Several procedures have been used to initiate molting. These include feed withdrawal up to 14 days (6), water withdrawal for 2 days (1), photoperiodic reduction (11) and nutrient restriction including low Ca^{2+} (5), low Na^{+} (4), high dietary zinc plus low Ca^{2+} (4) or high aluminium (12). Each method can be used alone or combined with other methods. All molting programs necessitate severe BW losses and cessation of egg production since hens with greater BW losses can produce more eggs during the postmolt cycle compared to those with lower BW losses (2).

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Either the fasting during natural molting or the starvation in induced molting would be a severe metabolic stress to the animals. The metabolic stress during starvation and the subsequent refeeding could lead to unbalance of mineral homeostasis, particulary Mg^{2+}, K^{+} and phosphorous (P) (7). Also, the circulating Mg^{2+} showed a critical inverse relationship between stress (13) because the body reaction to stress may increase in demands of Mg^{2+} and produce consequence of Mg^{2+} deficiency (18). In the blood, the ionized Mg^{2+} is the most interesting form with respect to biological properties and movable nature so that the measurement of the ionized Mg^{2+} can give more reliable information than total Mg^{2+} that can not move into/from cells (15). The decrease in circulating ionized Mg^{2+} through Mg^{2+} loss from body or Mg^{2+} depletion of reservoir in body may lead not only to develop diseases but also to exacerbate the damage caused by the disease (17). Therefore, we investigated the changes of blood ionized and total Mg^{2+} and Ca^{2+} related to starvation during molting in laying hens.

Materials and Methods

Animals

Because the concentration of blood ions depend on diets, drinking water and environment and can be changed with time (3), we moved hens from a farm into controlled facility with a temperature ($23 \pm 2^{o}C$) and humidity ($50 \pm 5\%$). 20 commercial strain (DeKalb) single comb white Leghorn hens, 80 wk of age were used in the present study as a concept of pilot

study. The hens were housed in individual cages (80 × 60 × 60 cm) with 16 hr light/8 hr darkness, feed and water ad libitum for 2 weeks prior to the beginning of the experiment.

In order to induce molting, we used the modified on-again, off-again (ON-OFF) method (1,2,20). In the ON-OFF method, feed and water were removed for the first 3 days. Every other day from Day 4 to 7, hens were fed the control ration at 45 g per hen and water was provided ad libitum. From Day 8 to 14, feed was removed but water was provided ad libitum. Light was reduced to 8 hr a day.

Histology

The tissue were fixed in 10% neutral buffered formalin solution and embedded in paraffin blocks. A 6 μm section from each slide was stained with hematoxylin-eosin (H&E). Slides were observed under a light microscope (Leica DMRBE; × 400).

Measurement of blood ions and metabolites

Before and after starvation, blood samples were collected from wing vein with a lithium heparin syringe and immediately analyzed whole blood ions using Nova Stat Profile 8 CRT (NOVA Biomedical Corp, Waltham, MA, USA) including the plasma pH, blood gas compositions and the concentrations of ionized Na^+, Cl^-, K^+, Ca^{2+}, Mg^{2+} and lactate$^-$. Hematocrit (Hct) was measured by conductivity. The concentration of HCO_3^- was calculated using the Henderson-Hasselbach equation. The anion gap values were calculated by the formula; $[Na^+ - (Cl^- + HCO_3^-)]$.

Sera were separated by centrifugation at 3000 rpm after 1 hr incubation at room temperature and stored at −20°C until the analysis by Olympus AU5200 (Olympus America, Melveille, NY, USA) for alkaline phosphatase (ALP), glutamyl transpeptidase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), amylase, creatine kinase (CK), total bilirubin, total protein (TP), globulin, albumin, cholesterol, uric acid, urea nitrogen, creatinine, total Ca^{2+} and P. Total Mg^{2+} in serum was measured using an atomic absorption spectrophotometer (Analab 9200, Seoul, Korea) at wavelength of 285.2 nM.

Statistical analysis

The results were expressed as the means ± standard error of the mean (SEM). The data was analyzed via analysis of paired student's *t*-test using Prism 5.03 (GraphPad Software Inc, San Diego, CA). A p value of < 0.05 was considered to significant.

Results

Effects of starvation during molting on body weight, egg follicles and liver

During study period, body weight was found decreased from a baseline value of 2117 ± 62 to 1744 ± 65 (17.6%) and 1389 ± 56 g (34.4%) at 7th and 21st day accordingly after molting (compared with 0 day measurement).

Left panel of Fig 1 shows existence of different size of egg follicle in the sac before start of forced molting. After molting, egg follicle disappeared and egg sac was shrunk. Right panel of Fig 1 shows the portal tract in this section includes a branch of the hepatic portal vein and hepatic artery, central vein. The hepatocytes from molted animals were loosely arranged in wide sinusoid compared to controls.

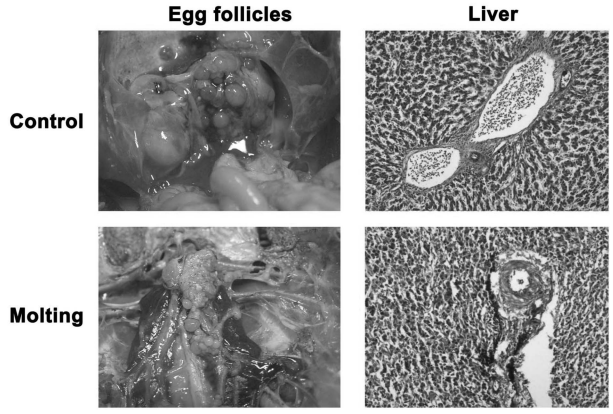

Fig 1. Effects of forced molting on egg follicles and liver of laying hens. After molting, egg follicle disappeared and egg sac was shrunk. The hepatocytes of molted hens were loosely arranged in wide sinusoid compared to control hens.

Effects of starvation during molting on blood pH, glucose, hemoglobinin, hematocrit, osmolarity and gas composition

After molting, the hens lost body mass (24.1%) and paused in the oviposition. Molting increased in blood glucose, hemoglobin, Hct and osmolality. However, PO_2 and O_2sat were decreased after molting. There was no significance of change in PCO_2.

Effect of starvation during molting on blood electrolytes

Molting decreased in K^+. Mg^{2+}, tMg^{2+}, tCa^{2+}, Ca^{2+}/Mg^{2+}, tCa^{2+}/tMg^{2+} and HCO_3^-. However, anion gap was decreased after molting. There were no significances of change in Na^+, P, Cl^- and tCa^{2+}.

Effects of starvation during molting on blood metabolites

Molting increased in ALP, GOT, GPT, CK and cholesterol. However, globulin and TP were decreased after molting. There were no significances of change in GGT, amylase and albumin.

Discussion

As expected, the hens lost significant their body mass after the starvation during molting in the present study. Molting is a natural avian behavior which their bodies undergo a rejuvenation process in order to maintain a healthy productive condition (2). However, the fasting during natural or induced

Table 1. Effects of starvation during molting on blood pH, glucose, hemoglobinin, hematocrit, osmolarity and gas composition

	Molting	
	Day 0	Day 14
pH	7.46 ± 0.01	7.44 ± 0.01
Glucose, mM/L	11.3 ± 0.1	12.6 ± 0.2***
Hb, mM/L	9.5 ± 0.2	10.8 ± 0.3***
Hct, %	29 ± 1	33 ± 1***
Osmolality, mM/kg	314 ± 1	320 ± 1*
PCO_2, %	37.7 ± 1.4	35.6 ± 1.1
PO_2, %	60.4 ± 1.1	57.8 ± 1.1***
O_2sat, %	91.8 ± 0.5	86.4 ± 1.3***

Hb, hemoglobin;Hct, hematocrit; PCO_2, partial CO_2 tension; PO_2, partial O_2 tension; O_2sat, O_2 saturation. The data are reported as the mean ± SEM. *$p < 0.05$ and ***$p < 0.001$; paired student's *t*-test versus Day 0.

Table 2. Effects of starvation during molting on blood electrolytes

	Molting	
	Day 0	Day 14
Na^+, mM/L	155 ± 1	157 ± 1
K^+, mM/L	3.8 ± 0.1	3.4 ± 0.1*
Ca^{2+}, mM/L	1.54 ± 0.03	1.63 ± 0.04
Mg^{2+}, mM/L	0.68 ± 0.01	0.54 ± 0.01***
Ca^{2+}/Mg^{2+}	2.28 ± 0.03	3.00 ± 0.08***
P, mM/L	1.89 ± 0.12	1.79 ± 0.07
HCO_3^-, mM/L	26.7 ± 0.6	24.9 ± 0.5*
Cl^-, mM/L	115 ± 1	115 ± 1
Anion gap	12.8 ± 0.5	17.0 ± 0.8***
tCa^{2+}, mM/L	3.56 ± 0.06	3.41 ± 0.07
tMg^{2+}, mM/L	1.45 ± 0.03	0.95 ± 0.02***
tCa^{2+}/tMg^{2+}	2.46 ± 0.03	3.52 ± 0.08***

Ca^{2+}, ionized Ca^{2+} normalized to pH; Mg^{2+}, ionized Mg^{2+} normalized to pH; Ca^{2+}/Mg^{2+}, the ration of Ca^{2+} per Mg^{2+}; anion gap, $[Na^+ - (Cl^- + HCO_3^-)]$ tCa^{2+}, total Ca^{2+}; tMg^{2+}, total Mg^{2+}; tCa^{2+}/tMg^{2+}, the ration of tCa^{2+} per tMg^{2+}. The data are reported as the mean ± SEM. The data are reported as the mean ± SEM. *$p < 0.05$ and ***$p < 0.001$; paired student's *t*-test versus Day 0.

molting should demand an increased metabolic stress, resulted in unbalance of mineral homeostasis in body (7). Decreased nutrient intake and poor nutritional status may lead to depleted mineral status including Mg^{2+}, K^+ and P required by ATP synthesis (16). We founded the significant decrease in blood Mg^{2+} and K^+ accompanied by the changes of biochemical parameters relating to increased metabolic stress after molting. Blood ionized and total Ca^{2+} did show no significant change, resulted in the significant increase in the ratio of Ca^{2+}/Mg^{2+}. Regarding the change in the ratio, a possible explanation will be differences of bioavaility between Ca^{2+} and Mg^{2+} (15). Ca^{2+} is stored mainly in bone (99%), whereas body Mg^{2+} is distributed through out different compartments (53% in bone, 27% in muscle, 20% in others such as soft tissues and blood) as reservoirs (17). Binding kinetics and releasing rate

Table 3. Effects of starvation during molting on blood metabolites

	Molting	
	Day 0	Day 14
ALP, U/L	624 ± 53	361 ± 44***
GGT, U/L	37.4 ± 1.5	37.2 ± 1.3
GOT, U/L	164 ± 5	243 ± 22***
GPT, U/L	3.3 ± 0.5	5.6 ± 0.5***
Amylase, U/L	301 ± 20	278 ± 15
CK, U/L	651 ± 58	861 ± 67***
Albumin, g/L	22.8 ± 0.8	22.3 ± 0.5
Globulin,g/L	45.8 ± 2.1	40.9 ± 1.7***
Cholesterol, mM/L	3.98 ± 0.33	5.48 ± 0.28***
TP, g/L	68.6 ± 2.7	63.3 ± 1.9*

ALP, alkaline phosphatase; GGT, glutamyl transpeptidase; GOT, glutamic oxalacetic transaminase; GPT, glutamic pyruvic transaminase; CK, creatine kinase; TP, total protein. The data are reported as the mean ± SEM. The data are reported as the mean ± SEM. *$p < 0.05$ and ***$p < 0.001$; paired student's *t*-test versus Day 0.

are depend on the reservoirs; slow release from bone and more rapid release from muscle or soft tissues (ATP^2-Mg^{2+} degradation) (15).

In animal body, Mg^{2+} is one of the elements known to be necessary for normal metabolic processes (8). It is an essential nutrient for growth, production and reproduction of animal (19). Animal breeding, particularly of the egg-producing breeds requires attention to demands for minerals and vitamins derived from feeding and unnatural housing. Several factors influence Mg^{2+} requirements of laying hens such as species, age, performance or stress of the animals (9,10). Mg^{2+} deficiency could have detrimental effect of ovarian growth and ovulation in younger hens (9). It had reported that Mg^{2+} supplements to laying hens increased egg production, average egg weight and hatching of eggs. Circulating Mg^{2+} and egg shell thickness were severely reduced in hens given diets containing low Mg^{2+} (20).

It has been reported that the refeeding syndrome could aggravate the acute electrolyte derangements that occur during nutritional repletion in human (16). Therefore, the Mg^{2+} depletion during starvation may be further exacerbated as consequences of refeeding when nutrition will be provided to starved hens. The driving factor for the refeeding sequelae is insulin secretion (7). Insulin promotes glucose uptake, along with Mg^{2+}, K^+ and P into the cells. The intracellular shift, along with an already depleted electrolyte pool, can lead to dangerously low levels of Mg^{2+}, K^+ and P in blood. Serious respiratory, cardiac and neurological complication could occur, in the most severe cases, the complications may be fatal. It has been well documented Mg^{2+} supplement on therapeutic effects of repletion on Mg^{2+} deficiency (20). The interaction of minerals should be considered also as an important factor because of the close interaction between Mg^{2+} and Ca^{2+} and P in hens (8,9). The Mg^{2+} demand was found to be increased,

especially when additional Ca^{2+} and P was fed (10).

In view of the above arguments and the new data presented herein, we strongly propose that appropriate Mg^{2+} and K^{+} supplements to laying hens are needed during and after molting that could produce a significant decrease in circulating ionized Mg^{2+} and K^{+}.

Acknowledgements

This research was supported by research funds of Chonbuk National University in 2010 and Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2011-0013872).

References

1. Alodan MA, Mashaly MM. Effect of induced molting in laying hens on production and immune parameters. Poult Sci 1999; 78: 171-177.
2. Berry WD. The physiology of induced molting. Poult Sci 2003; 82: 971-980.
3. Ben Rayana MC, Burnett RW, Covington AK, D'Orazio P, Fogh-Andersen N, Jacobs E, Külpmann WR, Kuwa K, Larsson L, Lewenstam A, Maas AH, Mager G, Naskalski JW, Okorodudu AO, Ritter C, St John A; International Federation of Clinical Chemistry and Laboratory Medicine (IFCC); IFCC Scientific Division Committee on Point of Care Testing. IFCC guideline for sampling, measuring and reporting ionized magnesium in plasma. Clin Chem Lab Med 2008; 46: 21-26.
4. Berry WD, Brake J. Postmolt performance of laying hens molted by high dietary zinc, low dietary sodium, and fasting: egg production and eggshell quality. Poult Sci 1987; 66: 218-226.
5. Breeding SW, Brake J, Garlich JD, Johnson AL. Molt induced by dietary zinc in a low-calcium diet. Poult Sci 1992; 71: 168-180.
6. Damron BL, Christmas RB. Final-week performance of straight-run broilers as affected by early coccidiostat withdrawal followed by increased dietary salt. Poult Sci 1997; 76: 1637-1640.
7. Fuentebella J, Kerner JA. Refeeding syndrome. Pediatr Clin North Am 2009; 56: 1201-1210.
8. Gaál KK, Sáfár O, Gulyás L, Stadler P. Magnesium in animal nutrition. J Am Coll Nutr 2004; 23: 754-757.
9. Hajj RN, Sell JL. Magnesium requirement of the laying hen for reproduction. J Nutr 1969; 97: 441-448.
10. Hess JB, Britton WM. Effects of dietary magnesium excess in White Leghorn hens. Poult Sci 1997; 76: 703-710.
11. Hurwitz S, Wax E, Nisenbaum Y, Plavnik I. Responses of laying hens to forced molt procedures of variable length with or without light restriction. Poult Sci 1995; 74: 1745-753.
12. Hussein AS, Cantor AH, Johnson TH. Effect of dietary aluminum on calcium and phosphorus metabolism and performance of laying hens. Poult Sci 1989; 68: 706-714.
13. Ising H, Bertschat F, The K, Stoboy V, Goossen C, Hengst G: Stress-induced Ca/Mg shifts and vascular response in animals and men: Comparison to electrolyte alterations in myocardial infarction patients. Magnes Bull 1986; 8: 95-103.
14. Le Ninan F, Cherel Y, Robin JP, Leloup J, Le Maho Y. Early changes in plasma hormones and metabolites during fasting in king penguin chicks. J Comp Physiol B 1988; 158: 395-401.
15. Maguire ME, Cowan JA. Magnesium chemistry and biochemistry. BioMetals 2002; 15: 203-210.
16. Mehanna HM, Moledina J, Travis J. Refeeding syndrome: what it is, and how to prevent and treat it. BMJ 2008; 336: 1495-1498.
17. Saris NE, Mervaala E, Karppanen H, Khawaja JA, Lewenstam A. Magnesium. An update on physiological, clinical and analytical aspects. Clin Chim Acta 2000; 294: 1-26.
18. Seelig MS. Consequences of magnesium deficiency on the enhancement of stress reactions; preventive and therapeutic implications (a review). J Am Coll Nutr 1994; 13: 429-446.
19. Stolkowski J. Magnesium in animal and human reproduction. Rev Can Biol 1977; 36: 135-177.
20. Waddell AL, Board RG, Scott VD, Tullett SG. Role of magnesium in egg shell formation in the domestic hen. Br Poult Sci 1991; 32: 853-864.
21. Webster AB. Physiology and behavior of the hen during induced molt. Poult Sci 2003; 82: 992-1002.

환우(換羽, molting)에 의한 절식 후 산란계의 혈액 Mg^{2+}과 K^{+} 변동

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요 약 : 자연적 혹은 인위적 환우 기간의 절식은 산란계에서 심각한 대사성 스트레스가 될 수 있다. 절식 그리고 필수적으로 수반되는 사료 재급여 증후군의 대사성 스트레스는 ATP 생성과 밀접한 Mg^{2+}, K^{+}과 P 등의 무기염류 불균형을 야기할 수 있다. Mg^{2+}은 생체 대상과정에서 필수적인 무기염류이며 스트레스는 생체 Mg^{2+} 요구량을 증가시킬 뿐만 아니라 Mg^{2+} 결핍을 야기할 수 있기 때문에 산란계에서 환우 기간의 절식에 관련된 혈액내 이온의 이온화 농도 및 결합형을 포함한 총농도의 변동을 관찰하였다. 환우 후에 대사성 스트레스와 관련된 생화학 인자의 변화와 수반하여 혈액내 Mg^{2+}과 K^{+}의 감소가 관찰되었다. 따라서 환우 기간의 절식 및 사료 재급여 증후군은 심각한 저마그네슘혈증 및 저칼륨혈증을 야기할 수 있으므로 환우 그리고 재급여 과정에서 Mg^{2+}과 K^{+}의 투여가 권장된다.

주요어 : 산란계, 환우, 절식, 저마그슘혈증, 저칼륨혈증