Kasetsart J. (Nat. Sci.) 39 : 226 - 234 (2005)

# Short-Term Stressor Effects of Water Deprivation Prior to the Onset of Lay on Subsequent Reproductive Performance of ISA Brown Pullets

**Nirat Gongruttananun and Ratana Chotesangasa**


## ABSTRACT

The influence of water deprivation prior to the onset of production on sexual maturity and subsequent reproductive performance was investigated in commercial pullets. Three hundred 16-week-old ISA Brown pullets were used in this study. The birds were housed in cages (100 cages of 3 birds/cage) situated in an open sided poultry shed and randomly divided into three treatment groups. The 3 groups were 1) control (received feed with water at all times) 2) dehydrated and 3) dehydrated+$NH_4Cl$. Following an acclimatization period of 4 weeks, the birds in all groups were placed on a commercial layer ration and the treatments began. In the dehydrated and dehydrated+$NH_4Cl$ groups, the drinking water was removed completely from the pullets for 48 hours of dehydration but feed available at all times. The water was then returned to the birds following the water withdrawal period, and thereafter until the end of the trial. The pullets in the dehydrated+$NH_4Cl$ group were fed on the layer diet supplemented with 1%$NH_4Cl$ throughout the experimental period. It was found that feed intake dropped rapidly, by approximately 50%, when the birds were subjected to water deprivation. Neither age at first egg nor at 50-60 % of production was influenced by the interruption of drinking water supply. Little difference in egg weight at first egg was noted between the dehydrated birds and the normally hydrated hens. Pullets deprived of water were slower coming into egg production especially during the first 2 weeks of production period, however, thereafter egg production was similar among all treatment groups with numerical advantages for the normally hydrated hens. There were no carryover effects of water deprivation on subsequent egg weight, albumen weight, Haugh units, yolk weight or yolk color throughout the entire 12 weeks of the test. Body weights were not different either before the treatment or at the end of the study ($P>0.05$).

The results might be interpreted as indicating that an interruption of drinking water supply for 2 days prior to the commencement of egg production induced transient effects of nutrient deficiency resulted in retardation of reproductive development. The acidified layer ration failed to show any positive effects on reproductive performance for the first 12 weeks of lay. The pullets, however, appeared to overcome the detrimental effects as they approached sexual maturity.


**Key words:** water deprivation, sexual maturity, ammonium chloride, onset of lay, egg weight, egg production

Department of Animal Science, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand.

Received date : 18/10/04 Accepted date : 27/04/05

## INTRODUCTION

Water is recognized as a vitally important nutrient for chickens. Lacking of drinking water can have a disastrous effect on the performance of the birds. Interruptions of the water supply could happen as a result of failure of watering systems. As cited by Marsden *et al*. (1965), there was an occurrence of water deprivation at a turkey breeding flock station due to the weekend attendant failing to refill the water in the water supply. A mortality rate of 13% was observed in older poults and 28% in young birds deprived of drinking water for 2 days. Generally, the birds were weak, unsteady on their feet, and some were dead. Symptoms were ataxia and convulsions including violent flopping, head retraction, gasping, falling over backwards, and lying on their side or back, kicking and died. The removal of drinking water in laying hens even for a few days appears to have a long-term effect on subsequent egg production. Sunde (1962) reported that lack of drinking water for 36 hours had a long term detrimental effect on body weight and egg production. This author indicated that the lowest egg production occurred on the fifth day following water deprivation. Bierer *et al*. (1965) found reduced feed intake and a sudden drop in egg production in White Leghorn hens deprived of water for 48 hours on Days 3 and 4 following water deprivation. Adams (1973) deprived White Leghorn laying hens of water for 48 to 72 hours and found an 8-week period of declined egg production. This finding was consistent with that reported by Sunde (1962) who observed adverse effects on egg production for 10 weeks in water-deprived hens. Prolonged water deprivation has been reported that severely affected the avian kidneys resulting in renal failure and sudden death (Siller, 1981). Glahn *et al*. (1988) demonstrated that adding ammonium chloride in the diet could reduce the incidence of kidney damages without deleterious effects on egg production. However, the many works reported have been conducted with hens that had been in production for some time and, thus, little or no information is available on the influence of the dehydration mentioned on sexual maturity.

The objective of the study was to determine the effects of water deprivation for 48 hours, at the point of lay, on sexual maturity and subsequent reproduction performance in commercial pullets. In addition, the use of a diet acidified with $NH_4Cl$ in dehydrated birds was also examined, to determine if dietary acidification could be effective in preventing performance damages.

## MATERIALS AND METHODS

Three hundred ISA Brown pullets, 16 weeks of age, were used in this study. The birds were randomly divided and assigned into three treatment groups as follows: 1) control, 2) dehydrated, and 3) dehydrated+$NH_4Cl$. The pullets were allotted to cages (100 cages of 3 birds/cage) placed in an open-sided layer house, and received 16 hours of light per day. An acclimatization period of 4 weeks was allowed. In the control group, the pullets were provided food and water *ad libitum* throughout the experimental period. In the dehydrated and dehydrated+$NH_4Cl$ groups, the drinking water was withdrawn completely for 48 hours of dehydration but feed was available at all times. The water was then returned (rehydration) to the dehydrated and dehydrated+$NH_4Cl$ groups following the water withdrawal period. The pullets in both control and dehydrated groups were fed a layer diet, the birds in the dehydrated+$NH_4Cl$ group received the layer diet supplemented with 1%$NH_4Cl$ throughout the study. The layer ration used in this study was a mash commercial corn-soybean ration, formulated to have a calculated analysis of 3.25% Ca, 0.5% available phosphorous (aP), and an ME value of 2,851 cal/kg. The acidified layer diet was made by adding 0.45 kg of $NH_4Cl$ to 44.9 kg of the layer diet (Glahn *et al*., 1988). Observations of sexual maturity and subsequent production performance of the experimental birds were made and recorded. Daily egg production was recorded and presented as the numbers of egg per hen per 100 days (percentage of hen-day), and feed intakes were determined during the 2 days of water deprival period, and then biweekly thereafter until 32 weeks of age. All eggs obtained from the last day of each two weeks were assessed individually for egg weight, albumen weight, albumen height (Haugh units), yolk weight and yolk color. Mean body weights of the experimental bird were recorded at the beginning and the termination of the trial.

The experiment was commenced in July, 2003 and terminated in December, 2003. The average of minimum and maximum ambient temperatures were 26.04 ± 1.98 °C and 33.29 ± 0.66 °C, respectively. The statistical evaluation of the data was performed by analysis of variance. Repeated measures ANOVA was used where appropriate. Mean values were compared using Duncan's multiple range test to determine significance (Snedecor and Cochran, 1980). Significance was assumed if $P<0.05$.

## RESULTS

Generally, no deaths occurred during the experimental period. The birds remained seemingly in good health. During the water withdrawal period, aggressive behavior was observed in the birds in the water deprived groups, such as head movement and nonnutritive pecking. On Day 2 of water withdrawal, the dehydrated pullets appeared to increase their alertness. The degree of aggression, however, disappeared gradually as the drinking water was reintroduced. Birds deprived of water drank rapidly and avidly after the water was returned.

Table 1 shows mean values of feed intake of all treatment groups during and after water deprivation. Feed intakes of the pullet in the two groups of dehydration reduced sharply when the drinking water was removed for 48 hours ($P<0.05$). However, feed consumption of the dehydrated hens caught up, and was similar to that of the normally hydrated birds after 2 weeks of replenishment of the drinking water and remained so until the end of the experimental period ($P>0.05$). Effects of water deprivation on ages at the onset of lay and at 50% of production are illustrated in Figure 1. Although the significant difference was not noticed, the first egg of the control hens (144.7 days) was laid earlier than that of the dehydrated birds (150 days) and dehydrated+$NH_4Cl$ hens (148 days). However, the birds in all groups came to 50% of egg production at the same age ($P>0.05$). The size of the first egg was significantly affected by the treatments as shown in Figure 2. It was apparent that egg weight of the first egg of the

**Table 1** Feed consumption during water deprivation and rehydration in the different treatment groups.

| Group | 48 hours-of dehydration | Rehydration (weeks) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2 | 4 | 6 | 8 | 10 | 12 |
| | Feed intake (g/bird/2days) | Feed intake (g/bird/d) | | | | | |
| Control | 155.0±12.5$^a$ | 72.7±4.1 | 80.0±3.3 | 89.6±3.8 | 96.5±4.4 | 100.5±5.0 | 107.0±3.3 |
| Dehydrated | 77.0±8.3$^b$ | 73.7±2.9 | 81.2±4.1 | 91.5±3.9 | 94.1±2.0 | 101.2±6.1 | 108.5±6.2 |
| Dehydrated +$NH_4Cl$ | 62.5±7.9$^c$ | 72.9±3.8 | 80.3±2.4 | 88.9±2.7 | 92.4±3.5 | 100.9±3.6 | 106.6±2.7 |

$^{a-c}$ Means with no common superscript differ significantly among groups ($P<0.05$).

control pullets (43.9 g) was similar to that of the dehydrated birds (42.7 g), which was significantly heavier than that of the dehydrated+$NH_4Cl$ hens (39.3 g) ($P<0.05$). Influences of water deprivation for 48 hours at the point of lay on subsequent egg production are represented in Table 2. Obviously, withdrawal of drinking water adversely affected hen-day egg production during the initial state of the production period. The dehydrated pullets laid at a lower rate than did the normally hydrated birds' interval 20-22 weeks of age ($P<0.05$) whereas egg production of the birds in the dehydrated+$NH_4Cl$ group was not significantly different from that of the birds in the control group. However, thereafter egg production did not significantly differ for all groups until the end of the study. Details of egg quality measurement of the different treatment groups for the first 12 weeks of production are summarized in Table 3. Deprival of drinking water did not significantly affect subsequent egg weight, or any aspect of egg quality parameters throughout the experimental period ($P>0.05$). Figure 4 presents mean values of body weight of the different treatment hens at the

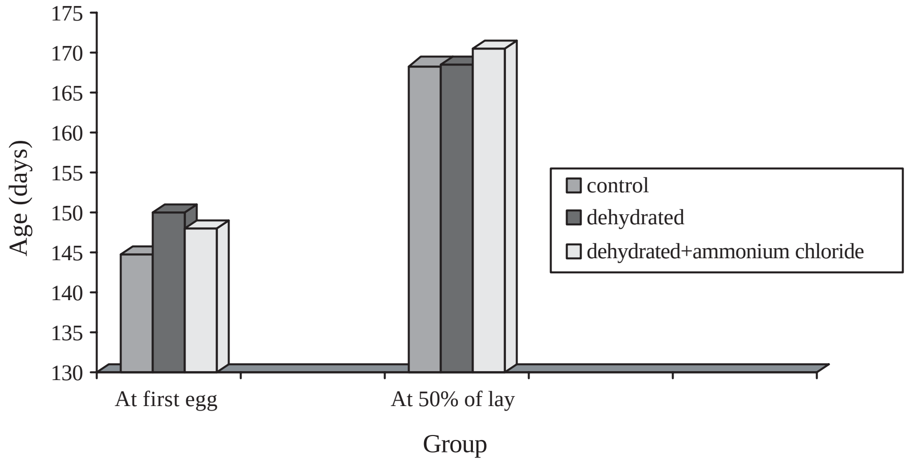


**Figure 1** Averages of age at the onset of lay and 50% of production of the different treatment hens.

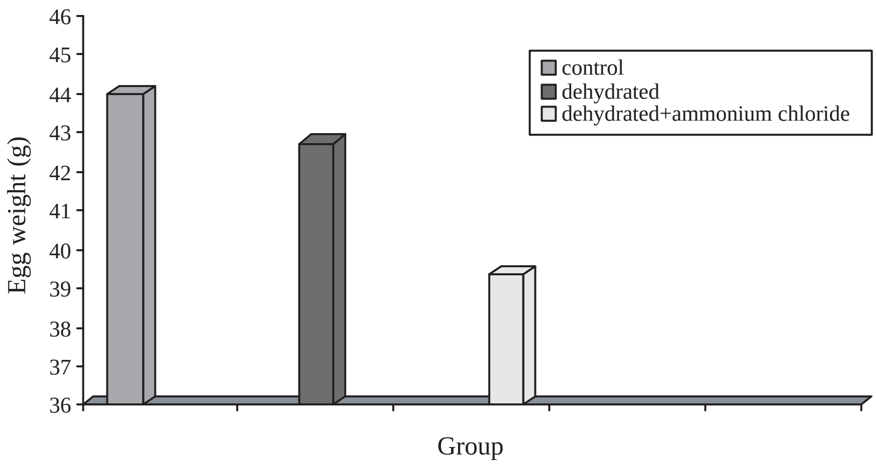


**Figure 2** Averages of egg weight at first egg of the different treatment hens.

**Table 2** Effects of water deprivation for 2 days during the pullet-laying transition period on subsequent egg production in the different treatment groups.

| Group | Age interval (weeks) | | | | | |
|---|---|---|---|---|---|---|
| | 20-22 | 22-24 | 24-26 | 26-28 | 28-30 | 30-32 |
| | Egg production (%) | | | | | |
| Control | 11.4±6.5[a] | 34.8±8.5 | 58.8±7.8 | 74.9±4.2 | 74.6±6.3 | 82.8±3.5 |
| Dehydrated | 3.8±1.1[b] | 32.3±10.6 | 54.2±8.9 | 71.9±7.3 | 73.0±6.3 | 82.2±3.8 |
| Dehydrated+$NH_4Cl$ | 6.8±4.8[ab] | 24.4±14.0 | 49.5±13.5 | 74.6±3.8 | 73.7±7.2 | 80.2±6.0 |

[a-b] Means with no common superscript differ significantly among groups (P<0.05).

**Table 3** Influences of interruption of drinking water supply for 48 hours prior to the onset of lay on subsequent egg quality parameters in the different treatment groups.

| Group | Age (weeks) | | | | | |
|---|---|---|---|---|---|---|
| | 22 | 24 | 26 | 28 | 30 | 32 |
| | Egg weight (g) | | | | | |
| Control | 48.7±2.6 | 50.8±1.0 | 54.9±1.3 | 56.4±2.6 | 55.6±1.9 | 56.1±3.3 |
| Dehydrated | 47.4±3.6 | 51.9±1.5 | 53.7±1.5 | 55.3±1.7 | 54.8±1.4 | 54.4±1.0 |
| Dehydrated+$NH_4Cl$ | 46.4±2.7 | 49.9±2.6 | 52.9±2.0 | 56.4±1.9 | 55.4±2.5 | 55.6±3.3 |
| | Haugh units | | | | | |
| Control | 93.6±4.5 | 96.8±5.2 | 91.8±2.4 | 88.7±4.2 | 90.0±2.2 | 87.1±6.0 |
| Dehydrated | 94.3±6.4 | 98.0±2.2 | 94.3±2.1 | 90.2±0.7 | 91.3±2.4 | 89.0±1.0 |
| Dehydrated+$NH_4Cl$ | 95.2±4.1 | 95.9±2.7 | 94.0±3.7 | 92.1±3.2 | 88.7±4.2 | 86.5±4.7 |
| | Yolk color | | | | | |
| Control | 10.1±0.8 | 11.8±0.3 | 11.9±0.3 | 12.5±0.2 | 11.8±0.3 | 12.1±0.1 |
| Dehydrated | 10.4±0.4 | 12.2±0.3 | 12.4±0.3 | 12.2±0.2 | 12.2±0.1 | 12.1±0.2 |
| Dehydrated+$NH_4Cl$ | 10.3±0.4 | 12.3±0.4 | 12.2±0.2 | 12.2±0.2 | 11.8±0.3 | 12.1±0.2 |
| | Yolk weight (%) | | | | | |
| Control | 22.5±1.2 | 22.5±0.8 | 22.7±0.4 | 22.9±0.4 | 23.2±1.2 | 22.6±0.3 |
| Dehydrated | 21.3±1.2 | 22.1±0.9 | 22.2±0.2 | 23.4±0.2 | 22.6±0.6 | 23.1±0.6 |
| Dehydrated+$NH_4Cl$ | 23.3±1.9 | 22.6±0.9 | 23.0±0.9 | 23.0±0.2 | 23.1±0.2 | 23.6±1.5 |
| | Albumen weight (%) | | | | | |
| Control | 67.8±1.4 | 68.1±0.6 | 67.8±0.7 | 67.7±0.8 | 67.5±0.8 | 67.3±0.7 |
| Dehydrated | 69.4±1.5 | 68.2±0.8 | 68.3±0.7 | 67.1±0.6 | 68.1±0.7 | 67.1±0.6 |
| Dehydrated+$NH_4Cl$ | 67.2±2.1 | 68.5±0.8 | 67.9±0.7 | 67.5±0.3 | 67.4±0.2 | 66.6±1.7 |

There were no significant differences between groups (P>0.05).
All data in the tables are given as mean ± standard deviation.

beginning and the end of the investigation. No significant differences were observed in body weight among groups before the treatment or at the end of the experiment.

## DISCUSSION

No adverse effects of water deprivation for 2 days at the onset of lay were observed on body weights of the bird as illustrated in Figure 4. However, the hens in the study were not weighed during the water removal. The water deprival period of 48 hours caused no apparent behavioral ill effects on the hens, except for an aggressive behavior. The pullets in both dehydration groups showed an increase in aggressive pecks during the water withdrawal period, especially on Day 2 of water deprivation. The increased degree of aggression might arise from frustration (Duncan and Wood-Gush, 1971) resulting from hens expecting the availability of drinking water but not finding it. The finding of aggressive behavior in the dehydrated hens in the study was similar to that observed in White Leghorn laying hens deprived of food (Simonsen, 1979). It is well documented that deprivation of food or water enhances levels of plasma corticosterone in avian species (Freeman *et al*., 1980; Arnason *et al*., 1986). Corticosterone hormone is secreted from the adrenal glands, located anterior and medial to the cephalic lobe of the avian kidneys (Ringer and Meyer, 1976). Freeman *et al*. (1980) indicated that starvation was a strong stimulus for the releasing of corticosterone in domestic birds.

The findings of this study, that removal of drinking water for 48 hours prior to the onset of lay reduces feed intake, were consistent with those reported by several workers (Sunde, 1962; Adams, 1973). Korr (1939) reported that the birds responded to water deprivation by reducing glomerular filtration rates (GFR) and urine flow rates, and increasing the osmotic pressure of blood and urine. Later, dehydration was reported to induce an increase in plasma osmotic concentrations (Koike *et al*., 1983). These findings were consistent with those reported by Roberts (1991).

Sexual maturity of the experimental pullets, as estimated from age at first egg and at 50% production, was not significantly affected by interruption of drinking water which would be a reflection of a similar body weight of the experimental birds (Figure 4). Several reports from previous studies (Summers *et al*., 1987; Summers and Leeson, 1994) have shown that there is a specific body weight threshold for the onset of production of birds. The investigators suggested that pullets must achieve the certain body weight in order to trigger the onset of production. It was well documented from previous several studies (Brody *et al*., 1980; Leeson and Caston, 1991) that regardless of feed or management regimen, if the pullet had the chance to consume diets at all times, the animal could quickly catch up in body weight so that a uniform weight was obtained at the onset of lay. The results from the study indicated that, as the drinking water was withheld, feed intake decreased markedly by approximately 50-60 % comparing with that consumed by the normally hydrated hens (Table 1). This might be comparable to the circumstance of feed restriction for 2 days. However, it was of interest to notice that feed intake in the two dehydration groups increased rapidly and returned to the normal value within 2 weeks after the birds were allowed access to the drinking water again. This demonstrated the ability of the pullets to increase feed consumption in an attempt to achieve a mature body weight as they approached sexual maturity.

The results from this study revealed that the interruption of drinking water for 48 hours at the point of lay had minor effects on subsequent reproductive performance due to feed restriction. If one compared feed intake of the experimental birds during water deprivation (Table 1) with the average of egg weight at first egg (Figure 2), it was likely that the amount of nutrient intake supported egg size at the initial phase of lay. In addition, the dehydrated pullets were coming into production slowly than the conventionally reared birds as evidenced by hen-day egg production during the first 2 weeks of production period (Table 2), and age at first egg. It did appear to be a trend in the dehydrated birds for requirement of much more times to be ready-to-lay pullets as compared with the control pullets (Figure 1). Likewise, the trend for slightly lower production was evident for the hens in the two groups of water withdrawal as depicted in Figure 3, confirming by the experiences of others (Isaacks *et al*., 1960; Brake *et al*., 1985; Summers *et al*., 1991) that feed restriction during the prelaying phase had only a slightly adverse effect on subsequent egg production. Summers *et al*. (1991) deprived White Leghorn pullets of food for 6 days at 17 weeks of age, they found that the 6-day feed withdrawal period delayed sexual maturity and reduced egg weight only during the first 2 weeks of production. Brake *et al*. (1985) stated that nutrient intake during the prelay period was a main factor influencing performance of hens. These authors suggested that protein intake

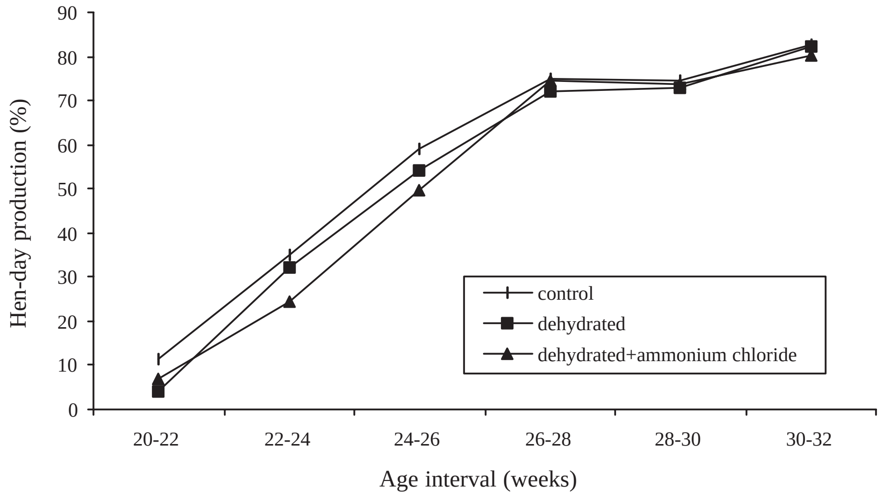


**Figure 3** Hen-day egg production of hens on the different treatments from 20 to 32 weeks of age.

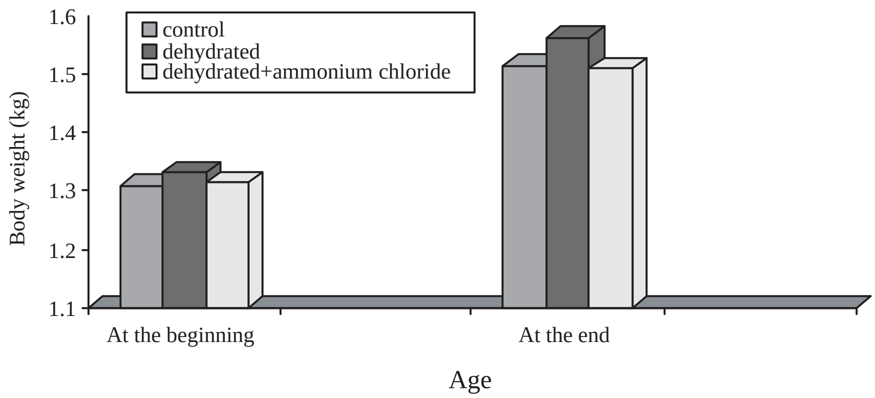


**Figure 4** Averages of body weight at the beginning and the end of the experiment of the different treatment birds.

was the major factor allowing accumulation of protein reserves in the body, which resulted in increased egg production and egg weight. Presumably, in this study, the dehydrated birds consumed inadequate amount of nutrients especially protein intakes, resulting from a reduction of feed intake during 2 days of water deprivation, in which the degrees of nutrient deficiency would be so much severely that affected reproductive development. This would indicate that such conditions adversely affect carrying reserves of body composition of the pullets at the point of lay, thereby poor production and small egg size at first egg were observed in the laying house. Unfortunately, the data of gonads of the birds in the current experiment were not determined. Additional studies are needed to conduct whether water deprivation influences the development of reproductive organs of the domestic fowl.

The results obtained from the study indicated that withdrawal of drinking water supply for 48 hours prior to the onset of egg production had transiently adverse effects on subsequent reproductive performance as evidenced by reducing of egg weight at first egg and egg production during the initial state of lay, suggesting that due to reduced feed intake during the dehydration period resulted in protein and/or nutrient deficiencies, therefore growth and reproductive development was retarded. However, the pullets deprived of water tended to overcome the nutrient restricted stress effect as they approached sexual maturity. This would be a reflection of the ability of the animals to compensate for such circumstance by increasing feed intake and body composition. Acidification diets with $NH_4Cl$ did not exhibit any beneficial effects on sexual performance for the 12 weeks of observation period. These results were in agreement with the results reported by Glahn *et al*. (1988). The workers demonstrated that dietary acidification with $NH_4Cl$ at a level of 1% did not affect production performance. They also indicated that a major advantage of the $NH_4Cl$ acidified diet was that it reduced the incidence of kidney lesions.

## CONCLUSION

Lacking of drinking water for 48 hours, prior to the onset of lay, did not affect sexual maturity, body weight, egg weight or subsequent reproductive performance. However, the pullets deprived of water commenced to lay slightly late during the initial period of production. Obviously, feed consumption of the birds dropped abruptly as the birds were subjected to water deprivation. It was possible that dehydration induced a short-term stressor, leading to a reduction in feed intake. Reduced feed consumption resulted in nutrient deficiencies and retardation of reproductive development. No beneficial effects on reproductive performance of adding 1%$NH_4Cl$ to feed were evident.

## ACKNOWLEDGEMENTS

The authors wish to acknowledge the financial support of the Kasetsart University Research and Development Institute (KURDI) in the carrying out of this work.

## LITERATURE CITED

Adams, A.W. 1973. Consequences of depriving laying hens of water a short time. **Poultry Sci.** 52: 1221-1223.

Arnason, S.S., G.E. Rice, A. Chadwick and E. Skadhauge. 1986. Plasma levels of arginine vasotocin, prolactin, aldosterone and corticosterone during prolonged dehydration in the domestic fowl: effect of dietary NaCl. **J. Comp. Physiol B**156:383-397.

Bierer, B.W., T.H. Eleazer and D.E. Roebuck. 1965. Effect of feed and water deprivation on chickens of various ages. **Poultry Sci**. 44: 1351.

Brody, T., Y. Eitan, M. Soller, N. Nir and Z. Nitsan. 1980. Compensatory growth and sexual maturity in breeder females reared under severe food restriction from day of hatching. **Br. Poultry Sci**. 21: 437-446.

Brake, J., J.D. Garlich and E.D. Peebles. 1985. Effect of protein and energy intake by broiler breeders during the prebreeder transition period on subsequent reproductive performance. **Poultry Sci**. 64: 2335-2340.

Duncan, I.J.H. and D.G.M. Wood-Gush. 1971. Frustration and aggression in the domestic fowl. **Anim. Behav.** 19: 500-504.

Freeman, B.M., A.C.C. Manning and I.H. Flack. 1980. Short-term stressor effects of food withdrawal on the immature fowl. **Comp. Biochem. Physiol. 67A**: 569-571.

Glahn, R.P., R.F. Wideman, Jr. and B.S. Cowen. 1988. Effect of dietary acidification and alkalinization on urolith formation and renal function in single comb white leghorn laying hens. **Poultry Sci**. 67: 1694-1701.

Isaacks, R.E., B.L. Reid, R.E. Davies, J.H. Quisenberry and J.R. Couch. 1960. Restricted feeding of broiler type replacement stock. **Poultry Sci**. 39: 339-346.

Koike, T.I., L.R. Pryor and H.L. Neldon. 1983. Plasma volume and electrolytes during progressive water deprivation in chickens (*Gallus domesticus*). **Comp. Biochem. Physiol. 74A**: 83-87.

Korr, I.M. 1939. The osmotic function of the chicken kidney. **J. Cell. Comp. Physiol**. 13: 175-179.

Leeson, S. and L.J. Caston. 1991. Growth and development of Leghorn pullets subjected to abrupt changes in environment temperature and dietary energy level. **Poultry Sci**. 70: 1732-1738.

Marsden, S.J., G.S. McKee and M.L. Crandall. 1965. Water deprival and replenishment in poults. **Poultry Sci.** 44: 793-797.

Ringer, R.K. and D.C. Meyer. 1976. Parathyroids, Ultimobrachial Bodies, and the Pineal, pp. 359-371. In P.D. Sturkie (ed.). **Avian Physiology** 3rd ed. Springer-Verlag, New York.

Roberts, J.R. 1991. Effects of water deprivation on renal function and plasma arginine vasotocin in the feral chicken *Gallus gallus* (Phasianidae). **Aust. J. Zool**. 39:439-446.

Siller, W.G. 1981. Renal pathology of the fowl – A review. **Avian Patho**. 10: 187- 262.

Simonsen, H.B. 1979. Effect of feed withdrawal on behavior and egg production in white leghorns on litter and wire. **Br. Vet. J.** 135: 364-369.

Snedecor, G.W. and W.G. Cochran. 1980. **Statistical Methods** 7th ed. The Iowa State University Press, Ames, Iowa, USA.

Summers, J.D. and S. Leeson. 1994. Laying hen performance as influenced by protein intake to sixteen weeks of age and body weight at point of lay. **Poultry Sci**. 73: 495-501.

Summers, J.D., S. Leeson and D. Spratt. 1987. Rearing early maturing pullets. **Poultry Sci**. 66:1750-1757.

Summers, J.D., D. Spratt and J.L. Atkinson. 1991. Delaying sexual maturity of pullets by nutrient restriction at the onset of production. **Can. J. Anim. Sci**. 71: 1215-1221.

Sunde, M.L. 1962. Amino acids, proteins, and stuff. **Poultry Sci**. 41: 1688.