The effect of magnesium on thyroid hormone levels and growth of broilers

W. Rattanatayarom [1], K. Angkanaporn [2], H.G. Classen [3]

Zusammenfassung

30 weiße Arbor-Acre Broiler (15 männliche, 15 weibliche) im Alter von ca. 4 Wochen und mit einem Durchschnittsgewicht von 778 ± 74 Gramm (Mittelwert und Standardabweichung) wurden unter kontrollierten Bedingungen gehalten: Hell-Dunkel-Zyklus: 16 : 8 Stunden; Temperatur: 28°C; Luftfeuchtigkeit: 70%. Die Tiere wurden zufällig auf 3 Gruppen verteilt. Die Kontrollen (G1, n=10) erhielten Standardfutter (66 mmol Mg/kg TG, 19% Protein, 4% Fett) und Leitungswasser (0,29 mmol Mg/L) als Trinkwasser ad libitum. Zwei weitere Gruppen (G2; G3) erhielten dasselbe Futter aber Trinkwasser, welches mit Magnesium-L-Aspartat HCl (MAH) so angereichert war, daß Mg-Konzentrationen von 16 mmol/L (= G2) und 32 mmol/L (= G3) resultierten. Die Behandlung dauerte 3 Wochen; viermal wurde mit heparinisierten Spritzen Blut aus den Flügelvenen entnommen, und zwar zu Beginn (W0), nach 1 Woche (W1), 2 Wochen (W2) und nach 3 Wochen (W3). Die Blutproben wurden sofort während 10 Minuten bei 4500 U/min zentrifugiert, das Plasma wurde bei -20°C bis zu den Messungen aufbewahrt. Mg und Ca wurden mittels AAS gemessen (Shimadzu, Model 680), Trijodthyronin (T3) und Thyroxin (T4) mit dem Enzymun-Test System ES 700 (Boehringer, Mannheim). Die statistische Auswertung erfolgte mit dem SPSS-Programm, Version 6.1.3.
In der 3. Versuchswoche hatten die G3-Broiler stärker zugenommen als die Tiere aus den Gruppen G1 und G2. Die Futterverwertung war ebenfalls besser in G3 als in G1. Verglichen mit den Kontrollen stieg das Plasma-Mg in G2 und G3 signifikant an ($p < 0,05$); Plasma-Ca veränderte sich nicht. Die T3-Spiegel waren in der ersten Woche signifikant erhöht ($p < 0,05$) im Vergleich zu G1. Während der 2. und 3. Woche fielen die Konzentrationen ab, waren aber in G2 und G3 weiterhin höher als in G1. Die T4-Spiegel waren höher in G2 und G3 als in G1 ($p < 0,05$); sie stiegen bis zur 2. Woche an und fielen in der 3. Woche in allen Gruppen ab. Die Ergebnisse zeigen, daß Mg-Supplementation einen Anstieg von Trijodthyronin und Thyroxin bei Broilern verursachen kann; die erhöhten Schilddrüsenhormonspiegel könnten ein Faktor sein, der das Wachstum in den mit Mg supplementierten Gruppen verbessert.

Summary

30 white Arbor-Acre broilers (15 males; 15 females) aged 4 weeks and weighing 778 ± 74 g (mean ± SD) were maintained under identical field conditions, they were exposed to a light: dark cycle of 16 h light: 8 h dark, temperature of 28 °C, humidity of 70%. The broilers were randomly divided into 3 groups: The control group (G1, n = 10) was fed standard feed (Mg content 66 mmol/kg dry weight, measured by analysis, protein 19 %, and fat 4 %) and tap water (Mg content 0.29 mmol/L measured by analysis) as drinking water ad libitum. The Mg-normal group (G2, n = 10) and Mg-high group (G3, n = 10) received the same food, but drinking water was enriched with Mg in form of magnesium-L-aspartate hydrochloride (MAH). MAH was added in 2 concentrations, 4 g/L and 8 g/L respectively, yielding approximately 16 and 32 mmol Mg/L. Water was offered ad libitum for 3 weeks. Mg was supplemented in drinking water in G2,G3 until the end of the experiment. Blood collection was done 4 times with heparinized syringes from wingvein of unfasted animals at the starting week (W0), at one week (W1), two weeks (W2), and three weeks (W3) after Mg supplementation. Totally 116 heparinized blood samples were immediately centrifuged at 4,500 rpm for 10 minutes, plasma samples were separated and stored at –20 °C until measurements. All samples were analyzed for Mg and Ca with atomic absorption spectrophotometer from Shimadzu Model 680 from Japan, triiodothyronine (T3), and thyroxine (T4) were analyzed by enzymun-test® T3 and enzymun-test® T4 by using the equipment named Enzymun-Test® System ES 700 from Boehringer Mannheim. Data were analyzed statistically using SPSS for window version 6.1.3. At week three of the experiment, broilers in G3 had higher weight gain than G1 and G2. Feed conversion rate was also better in G3 than in G1. Plasma Mg of G2, G3 significantly increased ($P < 0.05$) during week 1 to 3 as compared to G1 on the same week while there was no change in plasma calcium. Hormone T3 was significantly increased and higher ($P < 0.05$) in G3 than G1 at week 1 of the experiment. Plasma T3 level declined during week 2 to week 3 but level of T3 in G2, G3 still were higher than in G1. Plasma T4 was higher in G2 and G3 than G1 ($P > 0.05$) and the T4 level gradually was increased and was highest at week 2 and decreased at week 3 of the experiment in all groups of broilers.
The results suggest that Mg supplementation can increase the level of T3 and T4 in broilers. This increase thyroid hormone may be one of the factors that improve growth in Mg-treated groups.

Introduction

Magnesium (Mg) is an essential activator of about 300 different enzymes, among others enzyme systems using ATP for substrate. Thus Mg is important in several energy-demanding processes such as cell membrane permeability, neuromuscular excitability, protein, nucleic acid and fat synthesis [3]. *Attech* et al. in 1983 [1], Grashorn et al. in 1988 [6] and *Rattanatayarom* et al. in 1996 [12] found that Mg supplements have a tendency towards increased growth rate of broilers probably by improving feed conver-

[1] Department of Pharmacology, Faculty of Medicine, Srinakharinwirot University, Bangkok (Thailand)
[2] Department of Physiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok (Thailand)
[3] Department of Pharmacology and Toxicology of Nutrition, University of Hohenheim, Stuttgart (Germany)

Dedicated to the 50th Anniversary of VERLA-PHARM Comp., July 13th, 1999

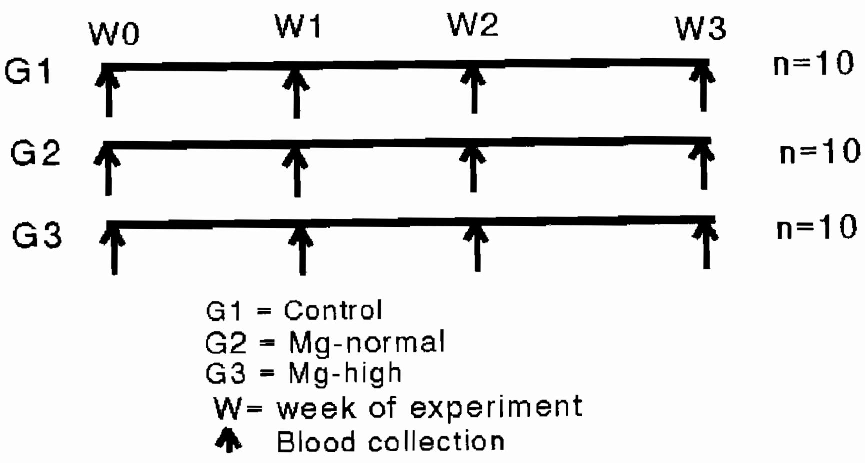
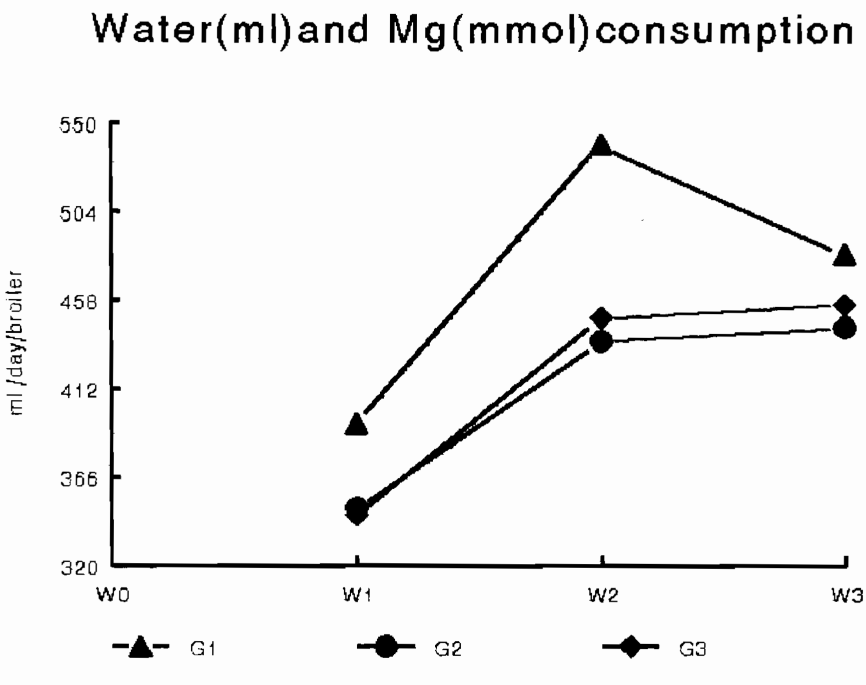


Fig. 1: Flow chart of the experiment G1= control G2 = drinking water yielding 16 mmol Mg/L and G3 = drinking water yielding 32 mmol Mg/L (Mg as MAH).



Group	W1		W2		W3	
	ml	mmolMg	ml	mmolMg	ml	mmolMg
G1	394	0.11	539	0.16	482	0.14
G2	350	6	437	7	444	7
G3	346	11	449	14	456	15

Fig. 2: Mean daily water consumption and Mg uptake of broilers in different groups. Numbers of data in table represent for water consumption (ml/broiler/day) and Mg intake via drinking water (mmol/broiler/day).

sion rate (FCR). However it is still unknown by what mechanisms Mg supplements could affect FCR in broilers. Thyroid hormones have been reported to be involved and may function as tissue growth and metabolism regulators [17]. But little is known about thyroid function following Mg supplements in broilers.

Chicken have been frequently used as experimental animals for studying thyroid function since both, mammalian and avian spp have the same histological cellular composition of thyroid glands and are highly specific for thyrotropin [15]. Chicken of the obese strain (OS) were used as an animal model of spontaneous autoimmune thyroiditis or Hashimoto's thyroiditis of man [5, 18].

In view of this situation the following study was conducted in broiler chicken to investigate the influence of Mg supplemented drinking water on these points of view:

1. growth as measured by weight gain, feed and water intake and food conversion rate (FCR),
2. thyroid function as measured by plasma T3 and T4,
3. blood electrolytes as measured by plasma levels of Mg and Ca.

Material and method

30 white Arbor-Acre broilers (15males; 15 females) aged e.g. 4 weeks weighing between 660 to 930 g were maintained under identical field conditions, they were exposed to a light: dark cycle of 16 h light: 8 h dark, temperature of 28 °C, humidity of 70 %. The broilers were randomly divided into 3 goups: The control group (G1, n = 10) received standard food (Mg content 66 mmol/kg dry weight measured by analysis, protein 19 %, and fat 4 %) and tap water (Mg content 0.29 mmol/l measured by analysis) as drinking water ad libitum. The Mg-normal group (G2, n = 10) and Mg-high group (G3, n = 10) received the same food but drinking water was enriched with Mg in form of magnesium-L-aspartate hydrochloride (MAH). MAH was added

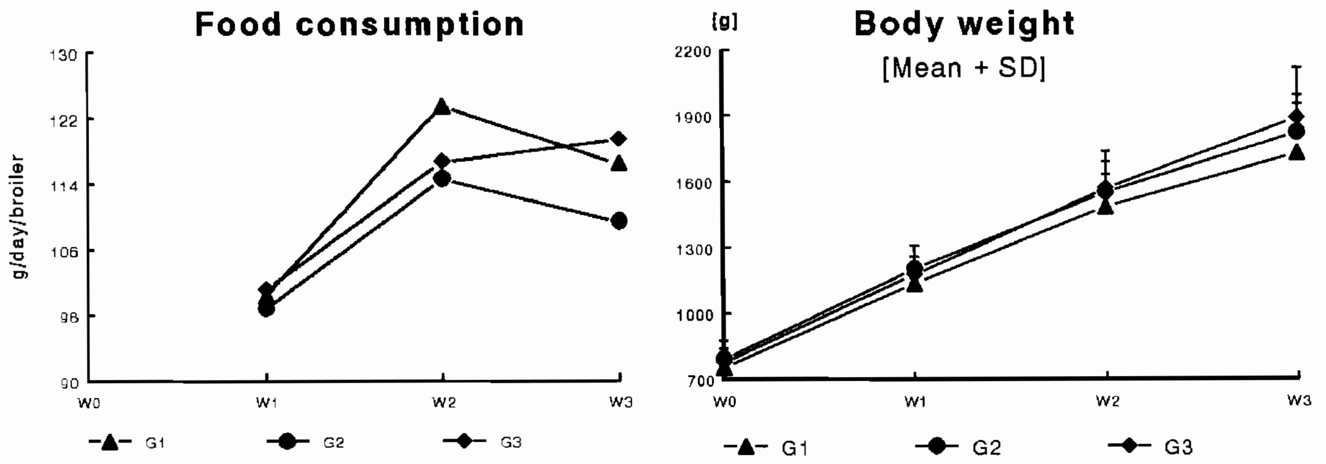


Fig. 3: Mean daily food consumption (top) and increase of body weight of broilers in different groups (bottom)

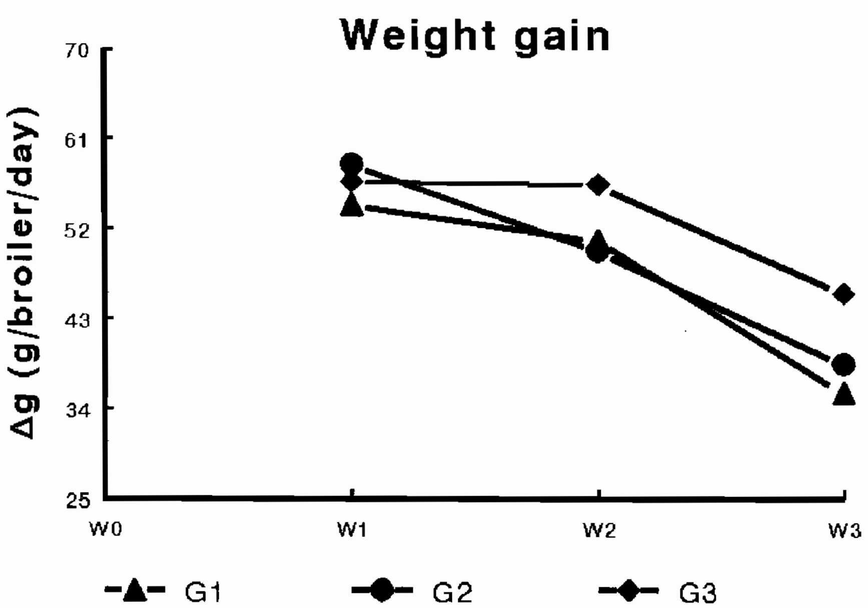


Fig. 4: Mean daily weight gain of broilers in different groups. W0: 778 ± 74 g

Tab. 1: Feed conversion rate (g/g) of broilers in each group supplementing with MAH. Overall FCR (W1-W3) means of feed intake during W1 to W3 divided by sum of weight gain during W1 to W3.

	Week (s) after starting the experiment			
Group	W1	W2	W3	Overall FCR (W1-W3)
G1	1.85	2.43	3.46	2.42
G2	1.69	2.31	2.85	2.20
G3	1.78	2.08	2.65	2.13

in 2 concentrations, 4 g/L and 8 g/L respectively, yielding approximately 16 and 32 mmol Mg/L. Water was offered ad libitum for 3 weeks. Blood collecting was done 4 times with heparinized syringes from wing-vein of unfasted animals at the starting week (W0), at one week (W1), two weeks (W2), and three weeks (W3) after Mg supplementation (see Fig. 1). Totally 116 heparinized blood samples were immediately centrifuged at 4500 rpm for 10 minutes, then plasma samples of each week (W0, W1, W2, W3) were separated and stored at –20 °C until measurements. All samples were analyzed for Mg and Ca with atomic absorption spectrophotometer, Shimadzu Model 680, T3 and T4 were analyzed by enzymun-test® T3 and enzymun-test® T4 by using the equipment named Enzymun-Test® System ES 700 from Boehringer Mannheim.

Statistics

Data were analyzed with SPSS for window version 6.1.3 . All data were first analyzed for normal distribution, homogeneity of variances and then either subjected to parametric ANOVA plus Scheffe-test, or to non parametric ANOVA (Kruskal-Wallis test) followed by Mann-Whitney U-Wilcoxon Rank Sum test.The level of significance was set at 5 %. Plasma calcium, food and water consumption, weight gain, FCR

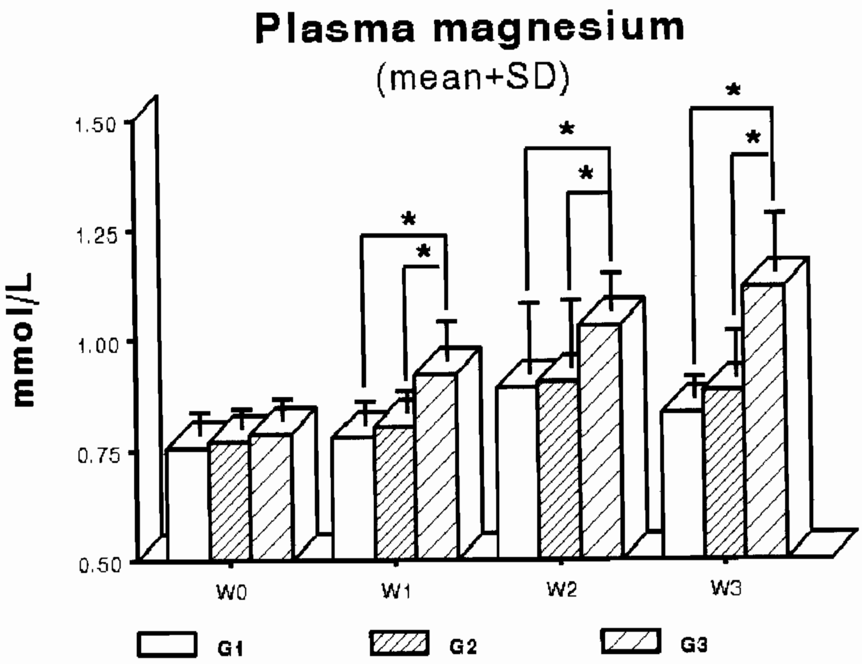


Fig. 5: Magnesium concentration (mmol/L) in plasma of broilers. Plasma Mg in Mg-treated groups (G2 and G3) significantly increased (P<0.05) during W1 to W3. G1 = control, G2 = Mg-normal and G3 = Mg-high.

were analyzed with parametric, while plasma magnesium and thyroid hormone were analyzed with non parametric tests.

Results

General development: Drinking water enriched with different concentrations of Mg was well tolerated by the broilers. No softening of faeces occurred in animals of groups 2 and 3. All broilers survived in acceptable conditions until the end of the experiment. Water consumption increased during the second week; Mg uptake via drinking water amounted to approximately 7 mmol Mg/day in group 2, and 15 mmol Mg/day in group 3 (see Fig.2). Food consumption increased in G3 during W1 to W3 and body weight increased in all groups (see Fig.3). Mean daily weight gain was highest in G3 (see Fig 4). Food conversion rate, i.e. the amount of food necessary to increase one kilogram body weight gain, was the best in group 3 (2.65) compared to group 2 and 1 (2.85, respectively 3.46), as shown in Tab. 1.

Electrolyte distribution:
Depending on the Mg content of the drinking water, plasma Mg significantly increased during weeks 1 to 3, as compared to control group in the same week (see Fig. 5). There was no change in plasma calcium (data not shown).

Hormone levels:
Triiodothyronine (T3) in plasma was significantly increased and was higher in G3 than G1 at W1. Then plasma T3 level declined during W2 to W3 but level of T3 in G2, G3 still were higher than G1 (see Fig. 6). Hormone thyroxine (T4) in plasma was higher in G2 and G3 than G1 and the T4 level was increased and highest at W 2 and decreased at W 3 of the experiment in all groups of broilers (see Fig. 7).

Discussion

In 1996 *Rattanatayarom* and colleagues reported on the effect of Mg-supplemented drinking water on broilers . It was found that increased plasma-Mg levels tend to improve food conversion

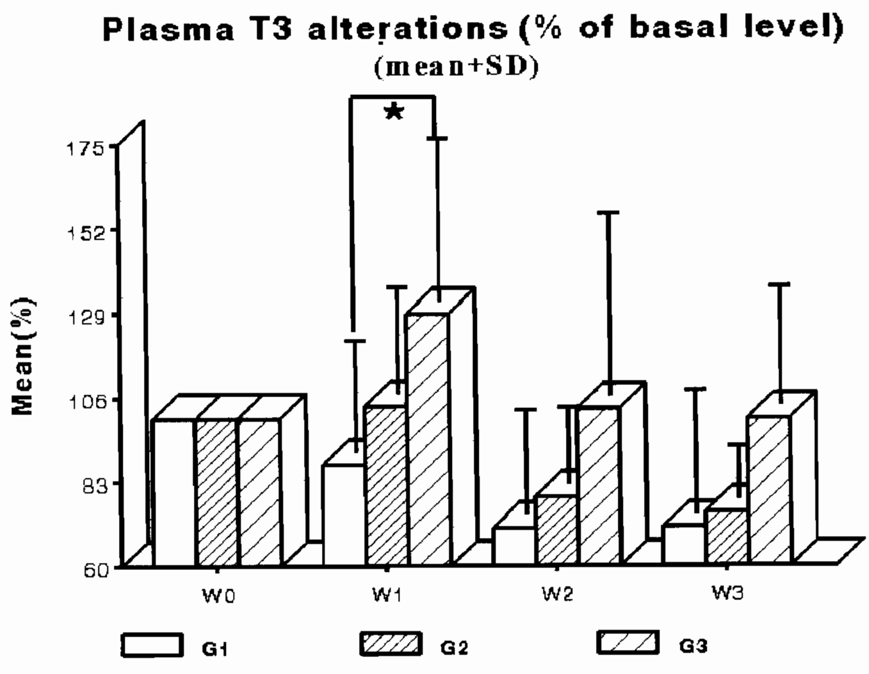


Fig. 6: Mean percental tri-iodothyronine changes (% T3) in plasma of broilers. Hormone T3 was significantly increased and higher (P < 0.05) in G3 than Gl at W1 of the experiment. G1 = control group and G3 = Mg-high group.

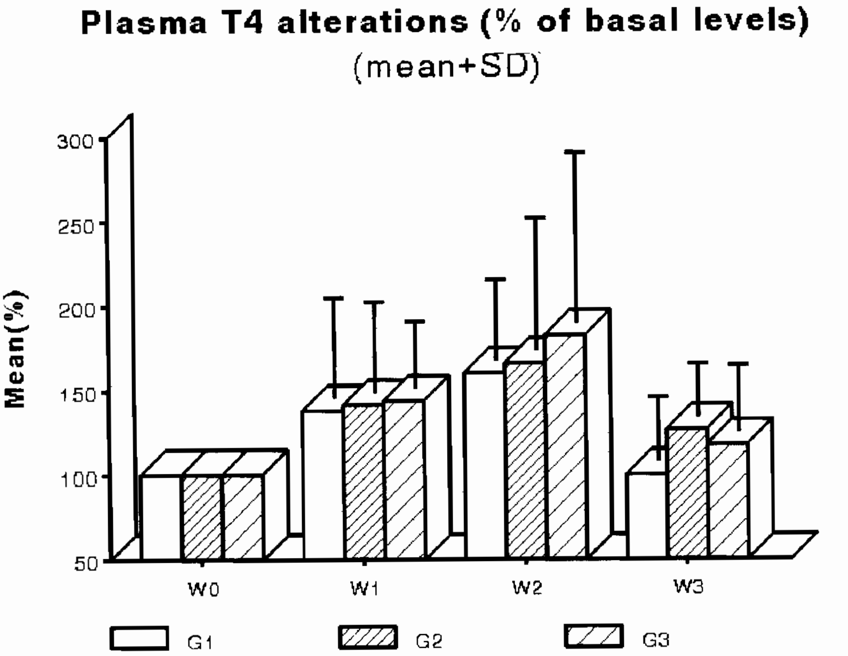


Fig. 7: Mean percental thyroxine changes (% T4) in plasma of broilers. Hormone T4 was increased and highest at W2 and decreased at W3, hormone T4 was higher in Mg-treated group than control group.

rate (FCR) and to decrease cholesterol [12]. In the present experiments increasing amounts of Mg as the MAH were added to the drinking water of broilers to investigate the relations of thyroid hormone to plasma levels of Mg, of Ca and to the growth of broilers in order to answer why FCR is improved. In view of the relationship between the metabolic effects of thyroid hormones and the role of Mg^{2+} ions in energy metabolism, some researchers have attempted to prove an association between thyroid function and Mg status [13, 17]. In the present experiments, it was found that Mg supplementation via drinking water can increase T3 and T4, but the level of T3 is more increased than T4 . These results are harmonious to the experiments of *Mahoney* in 1992 [8] showing that very young chick fed a Mg-deficient diet had lowered serum triiodothyronine levels (T3) whereas serum thyroxine (T4) remained generally unaffected. Mg is a cofactor for thyroidal adenylate cyclase [10] which converts adenosine monophosphate (AMP) to cyclic adenosine monophosphate (cAMP). Cyclic AMP is required for thyroid stimulating hormone (TSH) to stimulate the synthesis and secretion of thyroid hormone [16]. Thyroid hormones are the principle hormones responsible for the development of normal growth in the domestic fowl [14]. Especially T3 was more effective than T4 [4, 9, 14]. However it was found that supplementation with T3 or T4 at l ppm in the diet of broiler chicken resulted in respective serum T3 and T4 concentrations of approximately three times the control levels. Supplementation with T3 decreased weight gain and feed efficiency, while T4 did not adversely affect performance [9]. In our experiments, level of T3 increased significantly after Mg supplements, but less than 3 fold the controls, while T4 slightly increased. The moderately increased T3 could be the factor inducing better feed efficiency and growth of broilers. Chicken do not have a specific tyroid-binding globulin. Serum albumin, produced in the liver, is the primary thyroid binding protein. Therefore alterations of hepatic synthesis of albumin could affect total circulating levels of thyroid hormones [2]. Hepatic protein synthesis is a process that requires Mg at many steps [11]. Hence, theoretically, high synthesis of hepatic albumin in Mg-supplemented chicken could lead to increased thyroid binding protein and consequently to decreased free thyroid hormone levels. Total thyroid hormone exeretion by glomerular filtration could be reduced. For the higher T3 to T4 ratio, liver also plays an important role for T3 production in chicken by hepatic enzyme 5'-deiodinase [7]. For further studies it seems worthy to measure this enzyme in order to answer why T3 had increased more than T4. Studies on broilers rendered hypothyroid with anti-thyroid drugs like methimazole [4] or on obese chicken strain with spontaneous hereditary thyroiditis could also be suitable to show interactions between Mg supplements and altered levels of thyroid hormones. Such data could be clinically relevant, for example to improve the efficacy of thyroid hormone treatment with Mg in premature infants or hypothyroid patients.

Acknowledgements

We wish to thank for research grant supported by Srinakharinwirot University and to Dr. *A. Seidl*, Verla Pharm, Tutzing, Germany, for the sample of MAH.

References

[1] *Atteh, J. O.; Leson, S.*: Influence of increasing the calcium and magnesium content of the drinking water on performance and bone and plasma minerals of broiler chickens. Poult. Sci. 62 (1983) 869-874.

[2] *Butler, E. J.*: Plasma proteins. In: Bell, D. J.; Freeman, B. M. (eds): Physiology and biochemistry of the domestic fowl. Academic Press, New York, NY 1971, pp. 936-939.

[3] *Classen, H. G.; Speich, M.; Schimatschek, H. F.; Rattanatayarom, W.*: Functional role of magnesium in *vivo*. In: Golf, S.; Dralle, D.; Vecchiet, L. (eds): Magnesium 1993. J. Libbey 1994, pp. 13-30.

[4] *Decuypere, E.; Buyse, J.; Scanes, C. G.; Huybrechts, L.; Kuhn, E. R.*: Effects of hyper- or hypothyroid status on growth, adiposity and levels of growth hormone, somatomedin C and thyroid metabolism in broiler chickens. Reprod. Nutr. Dev. 27 (1987) 555-565.

[5] *Dietrich, H. M.*: Housing, breeding and selecting chicken of the obese strain (OS) with spontaneous autoimmune thyroiditis. Lab. Anim. 23 (1989) 345-352.

[6] *Grashorn, M.; Schimatschek, H. F.; Hickl, R.; Scholtyssek, S.; Classen H. G.*: Der Einfluß von Monomagnesium-L-Aspartat-Hydrochlorid auf den „Plötzlichen Herztod" und die Elektrolytverteilung bei Masthühnern. Mg.-Bull. 10 (1988) 9-14.

[7] *Lam, S. K.; Harvey, S.*: In -vitro coversion of thyroxine to tri-iodothyronine by chicken hepatic 5'-deiodinase: kinetic studies. J. Endocrininol. 110 (1986) 441-446.

[8] *Mahoney, C. P.; Alster, F. A.; Carew, L. B. Jr.*: Growth, thyroid function and serum macromineral levels in magnesium-deficient chicks. Poult. Sci. 71 (1992) 1669-1679.

[9] *May, J. D.*: Effect of dietary thyroid hormones on growth and feed efficiency of broilers. Poult. Sci. 59 (1980) 888-892.

[10] *Pastan, I., Katzen, R.*: Activation of adenyl cyclase in thyroid homogenates by thyroid stimulating hormone. Biochem. Biophys. Res. Commun. 29 (1967) 792-798.

[11] *Pike, R. L.; Brown, M. L.* (1984). Cited in Mahoney, *C. P.; Alster, F. A.; Carew, L. B. Jr.*: Growth, thyroid function and serum macromineral levels in magnesium-deficient chicks. Poult, Sci. 71 (1992) 1669-1679.

[12] *Rattanatayarom, W.; Angkanaporn, K.; Rattanatayarom, K.; Neungjamnong, J.; Classen, H. G.*: The effect of magnesium supplements on electrolyte distribution and plasma lipid of broilers. Mg-Bull. 18 (1996) 57-62.

[13] *Rude, R. K.*: Magnesium metabolism and deficiency. Endocrinol. Metabol. Cli. N. Am. 22 (1993) 377-395.

[14] *Scanes, C. G.; Dener, R. J.; Bowen, S. J.*: Effect of thyroid hormones on growth hormone secretion in broiler chickens. Poult. Sci. 65 (1986) 384-390.

[15] *Scott, T. R.; Glick, B.; Pharr, G. T.*: Avian thyroid isthmus. Poult. Sci. 66 (1987) 1885-1886.

[16] *Shell-Frederick, E.; Dumont J. E.*: Mechanism of action of thyrotrophin. In: Litwack, G. (ed): Biochemical actions of hormones. Vol. I. Academic press, New York, NY 1970, pp. 416-463.

[17] *Simsek, G.; Andican, G.; Uenal, E.; Hatemi, H.; Yigit, G.; Candan, G.*: Calcium, magnesium, zinc status in experimental hyperthyroidism. Biol. Trace. Elem. Res. 57 (1997) 131-137.

[18] *Sundick, R. S.; Herdegen, D.; Brown, T. R.; Dhar, A.; Bagchi, N.*: Thyroidal iodine metabolism in obese-strain chickens before immune-mediated damage. J. Endocrinol. 128 (1991) 239-244.

Correspondence to:
Dr. *W. Rattanatayarom*
Department of Pharmacology,
Faculty of Medicine, Srinakharinwirot University, Sukhumvit 23,
Bangkok 10110 (Thailand)