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ผลของการให้อาหารที่มีวิตามินอีและซีลีเนียมต่ำต่อพยาธิสภาพของห่านและเป็ดเทศ

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บทคัดย่อ: ห่าน 100 ตัวและเป็ดเทศ 100 ตัว ที่เริ่มเลี้ยงในฟาร์มของโครงการแบบเกษตรอินทรีย์โดยให้กินอาหารที่มาจากธรรมชาติ คือ หยวกกล้วยและรำข้าว พบว่าเมื่ออายุ 3 สัปดาห์ ห่านเริ่มแสดงอาการเดินเซ กล้ามเนื้อกระตุก และทยอยตายตั้งแต่อายุ 3-8 สัปดาห์รวมจำนวน 50 ตัว ในขณะที่เป็ดเทศเริ่มแสดงอาการเบื่ออาหารและขาอ่อนแรงในช่วงเวลาเดียวกันรวมจำนวน 30 ตัว ทำการเจาะเลือดสัตว์ป่วยเมื่ออายุ 2 เดือนชนิดละ 10 ตัวอย่าง เก็บตัวอย่างอาหารสัตว์และน้ำส่งตรวจทางห้องปฏิบัติการ นอกจากนี้ได้เจาะเลือดห่านและเป็ดเทศที่มีสุขภาพดีจากฟาร์มอื่นชนิดละ 5 ตัวอย่างเพื่อใช้เป็นตัวอย่างควบคุม ผลการผ่าซากพบสมองห่านมีลักษณะบวมและอ่อนเหลว ผลการตรวจทางจุลพยาธิวิทยาพบรอยโรค encephalomalacia และ satellitosis ในสมองส่วน cerebrum และ cerebellum ของห่าน และพบรอยโรค satellitosis แต่ไม่พบรอยโรค encephalomalacia ในสมองส่วนดังกล่าวของเป็ดเทศ นอกจากนั้นยังพบการเสื่อมของกล้ามเนื้อเรียบที่กระเพาะบดและกล้ามเนื้อหัวใจ และพบการตายของเซลล์เนื้อเยื่อตับอ่อนในห่านและเป็ดเทศ ตรวจไม่พบเชื้อไวรัสและแบคทีเรีย ผลการตรวจทางห้องปฏิบัติการชีวเคมีและพิษวิทยาพบว่าระดับวิตามินอีในซีรัมของห่านและเป็ดเทศมีค่าเฉลี่ย0.15±0.02SE µg/mL และ1.56±0.13SE µg/mL ตามลำดับ และระดับซีลีเนียมในซีรัมของห่านและเป็ดเทศมีค่า 2.01±0.10SE µg/dL และ 1.93±0.12SE µg/dL ตามลำดับ ซึ่งมีค่าต่ำและแตกต่างอย่างมีนัยสำคัญเมื่อเทียบกับค่าในตัวอย่างควบคุม (P<0.05) ในการศึกษาติดตามหลังการรักษาโดยใช้รำข้าวที่สดใหม่หรือรำข้าวที่ผ่านการนึ่งหรืออบ (รำข้าวเสถียร) ร่วมกับเสริมวิตามินอีขนาด 100 IU และซีลีเนียม0.25 ppm ต่ออาหาร 1 กิโลกรัม เป็นเวลา 2 เดือน ไม่พบสัตว์ป่วยเพิ่มและพบว่าระดับวิตามินอีและซีลีเนียมในซีรัมของห่านและเป็ดเทศมีค่าเพิ่มขึ้นอย่างมีนัยสำคัญ (P<0.05) และในการศึกษาติดตามครั้งที่ 2 หลังการรักษาเป็นเวลา 7 เดือน พบว่าสัตว์ทุกตัวไม่แสดงอาการป่วยและระดับวิตามินอีและซีลีเนียมในซีรัมของห่านและเป็ดเทศมีค่าไม่แตกต่างจากตัวอย่างควบคุม (P<0.05) การศึกษาครั้งนี้พบว่าการให้รำข้าวและหยวกกล้วยซึ่งเป็นอาหารที่มีวิตามินอีและซีลีเนียมต่ำแก่ห่านและเป็ดเทศส่งผลให้เกิดรอยโรคที่สมองและกล้ามเนื้อ โดยห่านเป็นสัตว์ปีกที่มีความไวรับต่อภาวะของการขาดวิตามินอีและซีลีเนียมมากกว่าเป็ดเทศ

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Effects of Vitamin E and Selenium Deficient Diets on the Pathology of Geese and Muscovy Ducks

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Abstract: This study 100 geese and 100 Muscovy ducks were fed diets of banana stalk and rice bran. At 3 weeks of age, the geese showed ataxia, myoclonus and finally died. The total numbers of geese dying from 3 to 8 weeks of age are 50. Meanwhile, 30 Muscovy ducks showed anorexia, ataxia and no mortality was found. Three geese and three Muscovy affected carcasses were necropsied. Blood samples from affected birds together with feed and water were collected. Besides, blood samples from 5 healthy geese and Muscovy ducks from another farm were collected as control samples. Macroscopically, the most prominent lesions were soften and swollen brains in geese. Microscopically, encephalomalacia and diffuse satellitosis were found in cerebrum and cerebellum of affected geese while diffuse satellitosis without encephalomalacia was found in cerebrum and cerebellum of Muscovy ducks. In addition, degeneration of the gizzard smooth-muscle cells and cardiac-muscle cells, necrosis of pancreatic acinar cells were found in both affected geese and Muscovy ducks. No bacteria and virus were found from all specimens. Importantly, the average concentration of vitamin E in geese and Muscovy ducks was 0.15±0.02SE µg/mL and 1.56±0.13SE µg/mL, respectively which were significantly (P<0.05) lower than concentrations in unaffected birds. Furthermore, the average concentration of Selenium (Se) in geese and Muscovy ducks were 2.01±0.10SE µg/dL and 1.93±0.12SE µg/dL, respectively, which were significantly (P<0.05) lower than concentrations in unaffected birds. In the follow-up study, 2 months after supplementation with vitamin E 100 IU and Se 0.2 mg/kg of diet, the conditions had improved and the serum concentrations of vitamin E and Se were significantly higher than when birds were on the banana stalk and rice bran diet (P<0.05) and at 7 months on the second follow-up study, all birds were healthy and the serum concentrations of vitamin E and Se were the same as control birds. It was found that the feeding of banana stalk and rice bran which is considered to be vitamin E and Se deficient could affect brain lesions and muscular dystrophy in geese and Muscovy ducks. The data also suggests that geese are more sensitive to vitamin E and Se than Muscovy ducks.

Keywords: Vitamin E, Selenium, Pathology, Geese, Muscovy ducks

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Introduction

Vitamins and minerals are required only in small amount but are essential for growth and production. The deficiency of vitamin E is one of nutritional diseases that affect poultry including chickens, ducks and turkeys. Diets deficient in vitamin E can produce encephalomalacia, exudative diathesis, and muscular dystrophy in chicks; enlarged hocks and dystrophy of the gizzard smooth muscle in turkeys; and muscular dystrophy in ducks. Encephalomalacia is a nervous syndrome characterized by ataxia, opisthotonus, myoclonus and prostration. The signs usually begin between 15-56 days of age. In exudative diathesis, the lesions include vasculitis which blood vessel walls become abnormally permeable. In muscular dystrophy, the fibers of skeletal, smooth and cardiac muscles can degenerate (Klasing and Austic, 2003). Importantly, vitamin E deficiency mostly occurs in poultry that are fed rations high in polyunsaturated fats which support vitamin E becoming rancid and the vitamin is no longer bio-available (Mezes *et al*., 1997).

Selenium (Se) is an essential mineral in poultry. The deficiency of Se in chickens, especially in combination with low vitamin E supply is responsible for the development of a range of diseases including exudative diathesis (Nouguchi *et al*., 1973), encephalomalacia (Combs and Hady, 1991) and pancreatic atrophy (Cantor *et al*., 1975). The relationship between vitamin E and Se is not fully understood, however, Se appears to effectively prevent myopathies of gizzard and heart in young poults (Surai, 2002).

Disease in poultry is usually thought of as being caused only by infectious agents. However, poultry can be affected by a wide range of diseases caused by non-infectious agents such as nutritional diseases. The objective of this study was, therefore, to investigate the pathology of geese and Muscovy ducks fed vitamin E and Se deficient diet, as well as perform follow up studies after treatment.

Materials and Methods

Case history

One hundred geese and 100 Muscovy ducks, belonging to a farmer in Thailand, were reared from about one week after hatching in a large floor pen under initiative organic farming project. At 3 weeks of age, the geese showed ataxia, myoclonus and finally died (Fig 1). The total numbers of geese dying from this problem at 3 to 8 weeks of age are 50. Meanwhile 30 Muscovy ducks showed anorexia, ataxia (Fig 2) and no mortality was found. All were fed with diets from natural sources of rice bran and banana stalk. Three geese and 3 affected Muscovy ducks

carcasses were necropsied and tissue samples were collected for histopathology and further laboratory examination. Then 10 blood samples from affected geese and Muscovy ducks, together with feed and water samples were submitted for analysis. In addition, blood samples from 5 healthy geese and Muscovy ducks from another farm were collected as control samples.

Histopathology

Tissue samples from the brain, lung, heart, pancreas, liver, kidney, spleen, proventriculus, gizzard and intestine were fixed in 10% neutral buffered formalin, routinely processed, and stained with hematoxylin and eosin (HE) for histological examination.

Virology and bacteriology

Tissue samples from the same organs were prepared to 10% suspension in PBS with antibiotics. For virus isolation, serial ten fold dilution of the suspensions was inoculated in 0.1 mL to primary chicken kidney cell in microplate, four wells per dilution, and observed daily for cytopathic effects for five days. The homogenates were also inoculated into the allantoic cavity of 10-day-old embryonated eggs. Inoculated eggs were incubated for five days at 37^0C, and the allantoic fluids were tested for hemagglutination activity. For bacteriological examination, the specimens were cultured on blood agar and MacConkey agar and identified according to Quinn *et al*. (1998).

Biochemistry and toxicology

Serum samples were precipitated of 0.2 mL serum with 0.5 mL n-hexane and centrifuged. Evaporation of supernatant to the dryness and then the residue was dissolved in iso-propanol. Finally, detection of serum vitamin E was measured by using High Performance Liquid Chromatography (HPLC) and fluorescent detector. For Se detection, serum samples were digested with acid mixture (nitric acid : perchloric acid 3:1). Then they were detected with hydride generator techniques by inductively couple plasma optical emission spectrometry (ICP-OES). Additionally, rice bran samples were extracted and aflatoxin was detected by using fluorometry method. Rice bran, water, liver, kidney and gizzard content were extracted and detection of pesticides such as organophosphate and carbamate were carried out using Thin Layer Chromatoghraphy (TLC) and Gas Chromatography – Mass Spectrometry (GC-MS) techniques.

Treatment

Two months after using fresh or stabilized rice bran and supplementation

vitamin 100 IU and Se 0.2 mg/kg of diet for 2 months, no clinical signs had been observed. Blood collection from 10 geese and Muscovy ducks were taken.

Seven months after treatment in a second follow-up study, blood samples from 10 geese and Muscovy ducks were collected to investigate for vitamin E and Selenium concentrations.

Statistical analysis

Analysis was calculated on data using parametric (ANOVA). The difference in means was considered statistically significant when $P<0.05$.

Results

Pathological findings

The most prominent gross lesion was soften and swollen brains (Fig 3) in all geese. Microscopic findings of some organs from geese and Muscovy ducks were presented in Table1. In brains, diffuse satellitosis and encephalomalacia with presence of severe vasculitis (Fig 4) were found in cerebrum and cerebellum of geese while only diffuse satellitosis (Fig 5) was found in cerebrum and cerebellum of Muscovy ducks.

Virological and bacteriological findings

No bacteria and virus were found in the submitted specimens.

Biochemistry and toxicological findings

The average concentrations of vitamin E and Se in serum samples of geese and Muscovy ducks were shown in Table 2. In addition, aflatoxin was not detected in rice bran and pesticides such as organophosphate and carbamate were not detected in rice bran, water, liver, kidney and gizzard contents.

Table 1 Histopathological changes in geese and Muscovy ducks fed diets formulated predominantly from rice bran and banana stalk

Tissues	Lesions	Geese No.			Muscovy No.		
		1	2	3	1	2	3
Brain	Encephalomalacia	+	+	+	-	-	-
	Diffuse satellitosis	+	+	+	+	+	+
Heart	Degeneration of cardiac myocytes	+	+	+	+	+	+
Gizzard	Degeneration of gizzard smooth muscle cells	+	+	+	+	+	+
Pancreas	Necrosis of acinar cells	+	+	+	+	+	+

- lesion absent; + lesion present

Table 2 The average concentrations of vitamin E and Selenium in serum of geese and Muscovy ducks

Group	Geese (Mean±SE) (n=10)		Muscovy ducks (Mean±SE) (n=10)	
	Vitamins E µg/mL	Selenium µg/dL	Vitamins E µg/mL	Selenium µg/dL
Control (n=5)	3.65^{b}±0.12	3.95^{b}±0.11	3.86^{b}±0.11	3.87^{b}±0.09
Before treatment	0.15^{a}±0.02	2.01^{a}±0.10	1.56^{a}±0.13	1.93^{a}±0.12
Two months after treatment	2.60^{a}±0.11	4.37^{a}±0.13	3.72^{b}±0.17	4.03^{b}±0.16
Seven months after treatment	4.90^{b}±0.19	3.88^{b}±0.16	4.82^{b}±0.22	4.35^{b}±0.04

Values in brackets are the mean concentration for the control birds within columns values with uncommon superscripts are significantly different ($P < 0.05$)

Statistical analysis from data presented in Table 2 showed that mean values of vitamin E level in serum of geese and Muscovy ducks before treatment were 0.15 µg/mL and 1.56 µg/mL, respectively which was significantly (P<0.05) lower than control group. In addition, the level of Se in geese and Muscovy ducks were 2.01 µg/dl and 1.93 µg/dl, respectively that they were significantly (P <0.05) lower than control group.

Discussion

In this study, all birds were fed banana stalk and rice bran. Although banana stalk contains crude protein, crude fibers but there is no fat and vitamin E or Se in nutritive value (Poyyamozhi and Kadirvel, 1986; Viswanathan *et al*., 1989). Rice bran has been used as a feed ingredient in poultry but it contains a large amount of oil and lipase which are susceptible to oxidation and destroy nutritive value (Linfield *et al*., 1985). It was likely that the diets used in this study would be considered as being deficient in vitamin E especially when it was found that rice bran had along storage time and became rancid.

At first (before treatment), geese showed severe nervous signs, soften and swollen brains. Microscopic examination revealed encephalomalacia with presence of severe vasculitis in both cerebellum and cerebrum related to the concentration of vitamin E in affected geese was 0.15 µg/mL which was significantly (P<0.05)lower than in the control samples taken from unaffected birds(Table2). Moreover, the lesion was typically consistent with that of vitamin E deficiency in chicks described by Klasing and Austic (2003), Van Vleet and Ferrans (1976) and Ikumo (1980). These reports described that encephalomalacia can occur in cerebellum of chickens. To our knowledge,

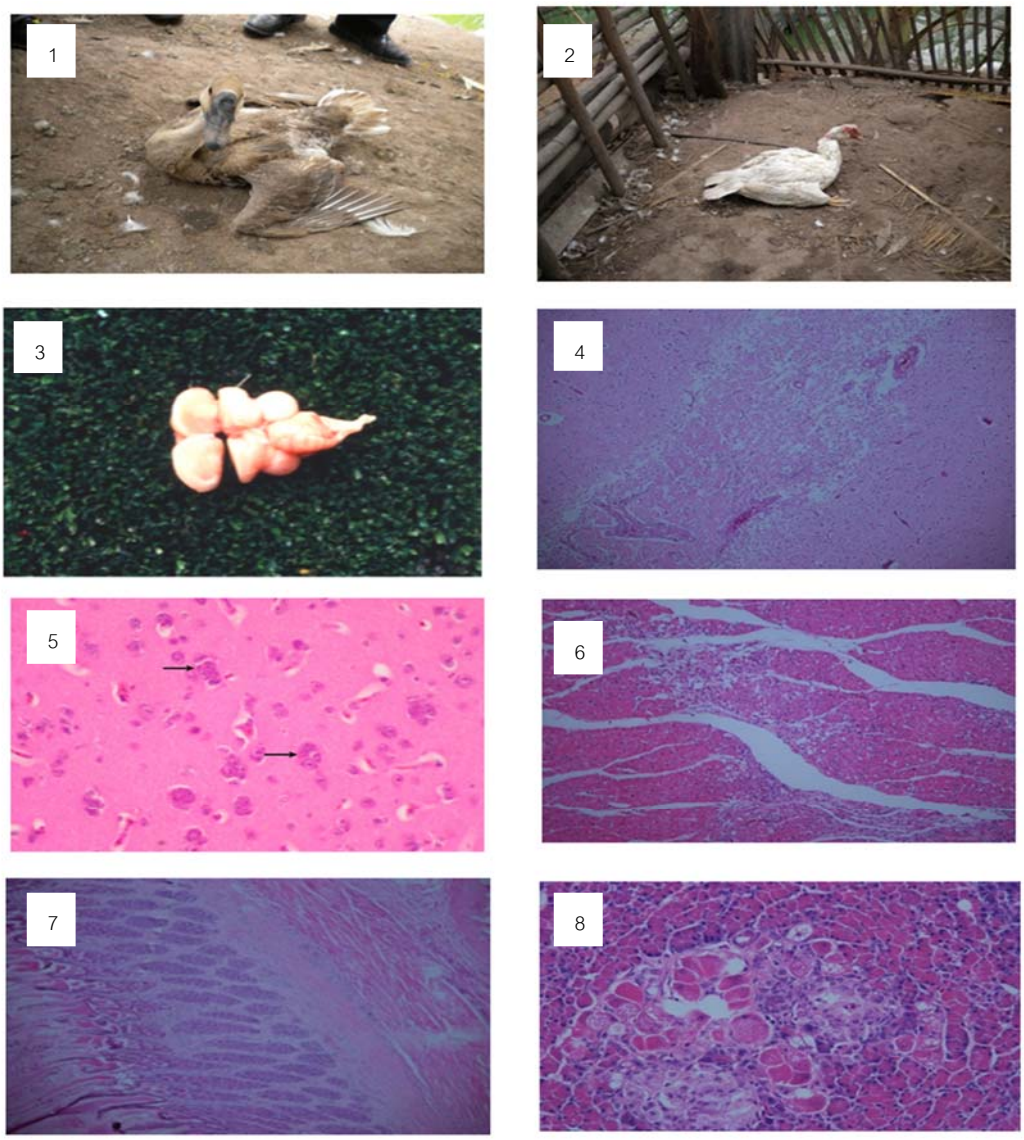

Fig.1-8. *1-*, Clinical signs of affected goose showed ataxia, myoclonus and prostration. *2-*, A Muscovy duck showed difficulty walking. *3-*, Macroscopic finding: cerebrum and cerebellum were soft and swollen. 4-,Microscopic finding: the cerebrum of goose showed foci of encephalomalacia with presence of severe vasculitis HE.x 100. *5-*,Microscopic finding in cerebellum of Muscovy ducks was limited to diffuse satellitosis HE.x400. *6-7-*, Cardiac myocytes and gizzard smooth muscle in a Muscovy duck revealed degenerative change HE.x200. *8-,* Degeneration and necrosis of some pancreatic acinar cells with lymphoid cells infiltration observed in a Muscovy duck HE.x400.

this is the first report which confirms that encephalomalacia can occur in geese. Moreover, Garland and Pritchard (2008) reported that cerebellum was the target organ of vitamin E deficiency while the cerebrum is not affected which was not in

agreement with this study. However, Klasing and Austic (2003) reported that lesions in cerebrum may occur but not common. Microscopic examination of affected Muscovy ducks revealed diffuse satellitosis in brain (Table1).The average level of vitamin E in Muscovy ducks was 1.56 µg/mL which was significantly (P<0.05) lower than in the control samples (Table2). It is possible that the lesions found were caused by vitamin E deficiency which allows accumulation of excessive lipid hydroperoxides, which resulted in brain tissue damage (Garland and Pritchard, 2008). In this study, geese showed obvious brain lesions at death while Muscovy ducks showed mild degree of brain lesions and were able to recover from the disease after treatment.

Both geese and Muscovy ducks revealed degeneration of cardiac myocytes (Fig 6) and gizzard smooth muscle cells (Fig 7). In this study, the concentration of Se in affected geese and Muscovy ducks were 2.01 µg/dL and 1.93 µg/dL, respectively and were significantly (P<0.05) lower than concentrations seen in the control samples (Table2). These findings are supported by those of Klasing and Austic, (2003); Dhillon and Winterfield (1983) and Van Vleet and Ferrans (1976) who have reported on the characteristics of vitamin E and Se deficiency in ducklings and chicks. In addition, the concentration of Se were significantly low (P<0.05) and necrosis of acinar cells was found in pancreas (Fig 8).These findings supported the reports by Thompson and Scott (1969) and Cantor *et al*. (1975) who found that vitamin E and Se deficiency in chicken is responsible for nutritional pancreatic degeneration. Additionally, the data also suggested that geese were more sensitive to vitamin E and Se deficiency than Muscovy ducks.

However, neurologic signs and brain lesions resulting from vitamin E deficiency must be differentiated from other viral diseases such as flavivirus infection, avian influenza and avian encephalomyelitis. In this report, no virus was found from virus isolation. Hence, history taking, blood collection and histopathology should be taken into account for investigation before making a final diagnosis. In this study, after using fresh or stabilized rice bran and taking a daily vitamin E 100 IU and Selenium 0.2 mg/kg of diet for 2 months (recommended dose by the manufacturer), it was found that the conditions had improved.

As presented in Table2, on the first follow-up study in 2 months after treatment, the condition was improved and the concentrations of vitamin E and Se significantly (P<0.05) increased. In the meantime, the new flock of geese and

Muscovy ducks, about 1 week of age, were brought and reared in this farm and fed high quality rice bran with vitamin E and Se supplement. On the second follow-up study in 7 months after treatment, all birds were healthy and the concentrations of vitamin E and Se of the old flock were normal.

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