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Effects of dietary betaine supplementation on back fat thickness and serum IGF-1 in late finishing pigs

Muttarin Lothong^{1A} Kittipong Tachampa¹ Pornchalit Assavacheep² Kris Angkanaporn^{1*}

Abstract

This study was performed in finishing pigs to investigate the effects of betaine HCl on carcass fat deposition and lipid metabolism in relation to growth hormone (GH) and insulin-like growth factor 1 (IGF-1) alterations. Sixty cross-bred finishing pigs (30 gilts and 30 barrows) with an average initial body weight of 74.0 ± 1.8 kg were randomly allocated into two groups, each with six replicate pens. Each group was fed either 0% or 0.125% betaine HCl supplementation for 41 days. Results showed that the betaine HCl supplementation had no effect on growth performance ($P > 0.05$). In the betaine-supplemented group, Lenden-speck-quotient index, average back fat thickness, serum triglycerides concentrations and fatty acid synthase (FAS) activities were lower than those in the untreated control group ($P < 0.05$, $P < 0.01$, $P < 0.05$ and $P < 0.05$, respectively). The betaine supplementation did not alter serum GH concentrations ($P > 0.05$), but serum IGF-1 concentrations were higher in the betaine group ($P < 0.01$). In conclusion, betaine reduced lipogenesis by reducing the FAS activity and increased serum IGF-1, leading to improvement in carcass characteristics.

Keywords: back fat thickness, betaine, finishing pigs, growth hormone, IGF-1, lipogenic enzyme

¹Department of Veterinary Physiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330

²Department of Veterinary Medicine, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330

^AGraduate student

*Correspondence: akris@chula.ac.th

Introduction

Growth performance and carcass characteristics are the main factors influencing profitability in finishing pig production. Late finishing pigs increase feed intake and feed conversion ratio while increasing lipogenesis, which can promote carcass fat deposition. For premium pork, excessive carcass fat results in a down-grade and price penalty. Some farmers are known to use β -agonists as feed additives in pigs to reduce carcass fat accretion. Although there are few reports on the residual effects of β -agonists on consumer health, adverse effects on some at-risk groups of human are inevitable (Sears, 2002). In addition, β -agonists increase shear-force and reduce meat tenderness (Vestergaard et al., 1994). These disadvantages focus interest on safer alternative lipotropic agents such as betaine.

Lipid accumulation results from the balance between lipogenesis and lipolysis (Saleh et al., 1999). In pig, lipogenesis plays a major role in lipid accumulation. Pig obtains fat from lipogenesis in adipose tissue than from dietary lipid (O'Hea and Leveille, 1969). Lipogenesis is the formation of fat by converting acetyl coA to fatty acids, and subsequently esterifying with glycerol to form triglyceride (Saleh et al., 1999). The rate-limiting step of lipogenesis is mediated via fatty acid synthase (FAS) enzyme (Huang et al., 2006). Previous studies suggested that betaine affected fat metabolism in pig (Matthew et al., 2001a; 2001b; Fernandez-Figares et al., 2002; Feng et al., 2006; Huang et al., 2006). However, the mechanism of betaine modulating fat metabolism is still unclear.

Betaine (trimethylglycine) is a methyl derivative of glycine, found in large amounts in sugar beet. Its physiological functions are defined as an osmolyte, methyl donor and carcass modifier (Eklund et al., 2005). The product "betaine" is commonly used as an animal feed additive. There are two forms of feed-grade betaine, consisting of betaine anhydrous and betaine hydrochloride (HCl). Although the stability of these two forms is not different (EFSA, 2013), the acidity of betaine HCl aids gastric digestion. In addition, betaine HCl can reduce contamination of microorganism during supplementation. The level of up to 2,000 mg/kg in complete animal feed is considered safe for consumers (EFSA, 2013). In pig production, betaine has many valuable effects,

depending on age of the animal. Dietary supplementation of betaine in finishing pigs has been reported to reduce carcass fat deposition and increase leanness (Matthew et al., 2001a; Lawrence et al., 2002). In finishing pigs, betaine induces GH and IGF-1 secretions and affects lipid metabolism via GH induction. This results in reduction in carcass fat deposition by reducing lipogenesis and increasing lipolysis (Huang et al., 2006). Nakev et al. (2009) demonstrated that 0.1% dietary betaine supplementation did not affect carcass fat and lean in finishing pigs. The effect of betaine HCl and its mechanisms on fat metabolism are still controversial. Therefore, this study was conducted to examine the effects of dietary betaine supplementation on carcass characteristics and fat metabolism in relation to serum GH and IGF-1 alterations in finishing pigs.

Materials and Methods

Animals and management: This experiment was approved by the Animal Care and Use Committee of the Faculty of Veterinary Science, Chulalongkorn University, No. 12310081. Sixty cross-bred barrows and gilts (Yorkshire x Landrace x Duroc) with an average body weight of 74.0 ± 1.8 kg were randomly allocated into 2 groups. Each group comprised six replicate pens with three pens of barrow and three pens of gilts (5 pigs per replicate pen). The pigs were reared in an open-system house with concrete floor pens, with dimensions of 4 x 3.5 m². Prior to the experiment, all pigs were fed similar commercial basal diet for 1 week (finishing pig formula, SPMTM, Thailand). The basal diet was pellets, delivered via a pig feeder. Nutrients of the basal diet were analyzed by proximate analysis (AOAC, 1990) and are shown in Table 1. In this study, each group of pig was fed the basal diet either containing 0% or 0.125% betaine HCl (HibetaTM, RCI, Australia) supplementation. The betaine HCl was calculated and mixed with rice bran before adding on top of the basal diet on a daily basis. The control group was also supplemented with the same amount of rice bran as in the betaine group. The pigs were fed water and diet *ad libitum* throughout the experiment. This study lasted until the pigs reached market slaughtering weight of approximately 110.0 ± 2.8 kg (41 days).

Table 1 Analyzed composition of the basal diet (g/kg)

Nutrients	
GE (kcal/kg)	3,920
DE (kcal/kg)*	3,100
Crude fat (g/kg)	43.3
Crude protein (g/kg)	130.7
Calcium (g/kg)	8.4
Phosphorus (g/kg)	6.7

* DE was calculated from gross energy according to King and Taverner (1975).
(DE: Digestible energy)

At day 0 and day 41 of the experiment, all overnight (12 hr) fasted pigs were weighed. Feed intake was recorded weekly and was calculated from total given feed minus leftover. Average daily gain (ADG) and feed conversion ratio (FCR) were

calculated based on the replicate weight gain and feed intake. Average values for each replicate pen were calculated for growth performance per group (n=6).

Sample collection: Fasted blood samples (10 ml) were randomly collected from 2 pigs per pen on the last day of the experiment (day 41). Each experimental group comprised 12 blood samples and 12 adipose tissue samples (pooled samples). The blood samples were centrifuged at 1,500 rpm for 5 minutes. Serum samples were collected and kept frozen at -80°C until subsequent analyses.

The adipose tissue samples were collected from back fat at the 10th rib of warm carcasses. All samples were chilled in ice during 2-hour transportation and stored at -80°C until subsequent analyses.

Carcass evaluation: At the end of the study, carcasses were evaluated at a slaughterhouse. The greatest number of carcasses that could be randomly evaluated in each group was 14. Five positions of back fat thickness were measured by digital vernier caliper (Fowler®, USA). Thicknesses of back fat at the 1st rib (BF1), at the 13th rib (BF2), at the front base of *Gluteus medius* (BF3), at the thinnest part of back fat (BF4) and on the top of *Gluteus medius* (BF5) were measured respectively. The width of distance from the front base of *gluteus* muscle up right to the spinal cord was also measured (b). Lenden-speck-quotient (LSQ) index was used to evaluate fat-lean ratio (Pfeiffer and Falkenberg, 1972). The fourteen carcasses were evaluated according to the LSQ index and average back fat thickness. Briefly, LSQ was calculated as (BF3 + BF4) / (2b), and back fat thickness was calculated as {BF1 + BF2 + [BF3 + BF4 + BF5] / 3}. Comparison of LSQ index to carcass lean percentage, carcass fat percentage and grading of pork was described in the study of Sethakul et al. (1996). Lower LSQ index refers to greater carcass lean percentage and lower carcass fat percentage.

Serum analyses of GH, IGF-1, triglycerides and free fatty acids: Serum concentrations of GH were determined using porcine GH sandwich ELISA kit (Cloud-Clone®, USA) following the manufacturer's instructions. The samples and standards were analyzed in triplicate. Optical density (O.D.) was measured at a wavelength of 450 nm immediately using a microplate reader (Model Synergy HT, Biotek®, Vermont, USA). The concentration of GH in the samples was determined by comparing the O.D. of the sample to the standard curve.

Serum concentrations of IGF-1 were measured by chemiluminescence assay (Faculty of Medicine, Chulalongkorn University). Serum triglyceride concentrations were determined by glycerol enzymatic assay (Faculty of Veterinary Science, Chulalongkorn University).

Serum free fatty acids (FFA) were determined by enzyme-based colorimetric method using FFA quantification kit (Biovision®, California, USA) following the manufacturer's guidelines. The samples and standards were evaluated in triplicate. O.D. measurement was performed at a wavelength of 570 nm in a microplate reader (Model Synergy HT, Biotek®, Vermont, USA) using Gen 5.1.11 program. FFA concentrations of the samples were evaluated by comparing the samples' O.D. to the standard curve.

Fatty acid synthase (FAS) activities: Measurement of FAS activity was performed using the protocol of Moibi et al. (2000) modified from Nepokroeff et al. (1975). Two grams of adipose tissue was homogenized with 6 ml of phosphate bicarbonate buffer (70 mM KHCO₃, 85 mM K₂HPO₄, 9 mM KH₂PO₄, 1 mM DTT; pH 8). The homogenate was centrifuged at 8,330 g for 10 minutes to collect the supernatant (Refrigerated centrifuge model U16R, Hettich company, Germany). The supernatant fluid was further centrifuged at 100,500 g for 60 minutes at 4°C (Beckman XL-20, Beckman Coulter Inc., USA). The collected supernatant was saturated with 35% of saturated ammonium sulfate solution (containing 3 mM EDTA and 1 mM β-mercaptoethanol; pH 7) at 4°C. The solution was centrifuged at 100,000 g for 60 minutes at 4°C to collect protein precipitate. The precipitate was dissolved in phosphate bicarbonate buffer and protein concentrations were determined. The retained solution was reserved at -20°C for FAS activity determination.

The determination of protein content of the solution was performed by Bradford (1976) assay using Bradford reagent (Bio-Rad Laboratories Inc., California, USA). O.D. was measured at a wavelength of 595 nm. The protein concentration in the samples was determined by comparing the O.D. of the sample to the standard curve.

The FAS activity was determined by measuring the oxidation of NADPH within the reaction mixture of malonyl coA, acetyl coA and fatty acid synthase. Briefly, the supernatant of 100 μl sample (containing 50-100 ng of protein) was mixed with 100 μl of buffer (containing potassium phosphate buffer 2 M, EDTA 2 mM, β-mercaptoethanol 2 mM, acetyl coA 12 mM and NADPH 60 mM; pH 7.4). O.D. of the reaction mixture was measured and recorded at a wavelength of 340 nm using a microplate reader (Model Synergy HT, Biotek®, Vermont, USA). Subsequently, 5 ml of malonyl coA (4.1 mM) was added and incubated for 15 minutes prior to measuring absorbance again at 340 nm. Change in concentrations of NADPH during oxidation was calculated using the following equation: $\Delta C = \Delta A / E$, where ΔC = Δ change in concentration of NADPH during oxidation, ΔA = change in absorbance and E = extinction coefficient of NADPH (E_{340 nm} = 6.22 mM⁻¹·cm⁻¹). The FAS activity was reported as picomole NADPH oxidized·min⁻¹·mg protein⁻¹.

Statistical analysis: All data were presented as means ± SEM. Student's t-test was calculated using SigmaStat 3.5 (Systat Software Inc., California, USA). Differences between means were considered significant at P<0.05. If data did not pass the normality test, the Mann-Whitney rank sum test was used.

Results

Effect of betaine HCl supplementation on growth performance: The effect of betaine HCl supplementation on growth performance of finishing pigs is shown in Table 2. After 41 days of treatment, there were no significant differences in the initial or final body weight, ADG, ADFI and FCR between the

control group and the 0.125% betaine-supplemented group.

Effect of betaine HCl supplementation on average back fat thickness and LSQ index: As shown in Table 3, adding 0.125% betaine HCl in the diet improved carcass qualities. The average back fat thickness and LSQ index of the betaine-supplemented group was significantly lower than the control group ($P<0.01$ and $P<0.05$, respectively).

Effect of betaine on serum GH, IGF-1, TG, FFA concentrations and FAS activities in adipose tissue:

The effects of betaine HCl supplementation on serum GH, IGF-1, TG and FFA concentrations in finishing pigs are demonstrated in the Table 4. There were no differences in serum GH and FFA between the groups. Compared to the control pigs, in the betaine-treated pigs the serum IGF-1 concentrations were greater ($P<0.01$), whereas the serum TG concentrations were lower ($P<0.05$).

The activities of FAS in adipose tissue affected by betaine HCl are also shown in Table 4. The betaine-fed group had significantly lower FAS activity compared to the untreated control group ($P<0.05$).

Table 2 Effects of dietary betaine HCl supplementation on growth performance

Parameter	Betaine HCl		P-value
	0% (n=6)	0.125% (n=6)	
Initial weight (kg)	74.5±1.8	73.2±2.8	0.69
Final weight (kg)	111.4±2.7	108.9±2.7	0.52
ADG (kg/pig)	0.90±0.04	0.85±0.03	0.35
ADFI (kg/pig)	5.27±0.02	5.19±0.19	0.58
FCR	5.91±0.28	6.31±0.26	0.32

Data are presented as mean±SD (ADG: average daily gain, ADFI: average daily feed intake, FCR: feed conversion ratio).

Table 3 Effects of betaine HCl supplementation on carcass characteristics

Parameter	Betaine HCl		P-value
	0% (n=14)	0.125% (n=14)	
Average back fat thickness (mm) ^a	29.5±1.4	24.0±0.6	<0.01**
LSQ index ^b	0.32±0.03 (grade 3)	0.24±0.01 (grade 2)	0.04*

Data are presented as mean±SD (LSQ: Lenden-speck-quotient).

^a Average back fat thickness was determined by averaging 5 positions of back fat thickness at the 1st rib, the 13th-14th rib, the base of *Gluteus Medius*, the middle of *Gluteus Medius* and the top of *Gluteus Medius*.

^b LSQ was determined by averaging back fat thickness at the base and the middle of *Gluteus Medius*, divided by the width of *Longissimus* muscle.

* Significance: $P<0.05$

** Significance: $P<0.01$

Table 4 Effects of betaine HCl on serum GH, IGF-1, TG, FFA concentrations and FAS activities in adipose tissues

Parameter	Betaine HCl		P-value
	0% (n=12)	0.125% (n=12)	
GH (ng/ml)	2.59±0.25	2.31±0.72	0.72
IGF-1 (ng/ml)	172.19±12.09	231.79±16.49	0.01*
Free fatty acid (µM)	39.71±1.28	37.53±2.24	0.41
TG (mg/dl)	44.33±2.70	34.50±3.10	0.03*
FAS activities (pmole NADPH oxidized·min ⁻¹ ·mg protein ⁻¹)	47.10±5.51	12.20±2.88	0.04*

Data are presented as mean±SD (GH: growth hormone, IGF-1: insulin like growth factor-1, TG: triglyceride, FAS: fatty acid synthase).

* Significance: $P<0.05$

Discussion

This study was performed in late-finishing pigs with the average weight of 74±1.8 kg, which showed a tendency to develop more adipose tissue than lean tissue (Dunshea and D'Souza, 2015). The current study showed that the growth performance was inferior (FCR 5.9-6.3, ADG 0.85-0.90 kg/pig and ADFI 5.19-5.21 kg/pig) to normal values in conventional finishing pigs. In this study, the high FCR may be relevant to excessive feed intake during the *ad*

libitum feeding. In addition, this study had fewer pigs per pen, meaning more pen space per pigs. This led to increased energy expenditure, which resulted in the increased feed intake and FCR. The betaine HCl supplementation had no effect on growth performance in the current study. These data are in agreement with numerous other studies (Feng et al., 2006; Kitt et al., 2000; Lawrence et al., 2002; Matthews et al., 2001b; Nakev et al., 2009). However, betaine HCl improves ADG, FCR and feed intake in growing pigs (Wray-Cahen et al., 2004; Yu et al., 2004). Production stage in

which betaine was fed, length of time fed, sex and genotype of pig may cause the differences (Henman, 1995; Lawrence et al., 2002). Since there were no differences between the sexes of pigs in this study, data are not shown.

In the present study, the effect of betaine HCl on carcass characteristics was characterized using the average back fat thickness and LSQ index. Betaine reduced the average back fat thickness, consistent with other studies (Fernandez-Figares et al., 2002; Feng et al., 2006; Huang et al., 2006; Matthews et al., 2001b; Sales, 2011). By comparing the width of back fat thickness to the width of Longissimus muscle, LSQ is an accurate index for evaluating meat and fat percentage in carcass (Pfeiffer and Falkenberg, 1972). Due to its simplicity, this index is widely used in Thai slaughterhouses to evaluate and grade carcass qualities. Lower LSQ index means lower carcass fat percentage, greater carcass lean percentage and superior pork grade (Sethakul et al., 1996). Our result demonstrated that the betaine-supplemented group had significantly lower LSQ index, which indicates that betaine decreased carcass fat/lean ratio. Fernandez-Figares et al. (2002) revealed that betaine altered nutrient partitioning including carcass protein deposition and fat reduction. In this study, the *ad libitum* feeding may favor the substantial effect of betaine on carcass fat reduction. However, some studies reported that betaine did not affect carcass fat deposition (Lawrence et al., 2002; Nakev et al., 2009). In pigs, betaine influences carcass fat and lean depending on energy level (Haydon et al., 1995; Matthews et al., 1998). High dietary protein reduces carcass fat percentage and increases carcass lean percentage (Zhao et al., 2010). In this study, the crude protein was slightly lower than normal values, which may lead to promote carcass fat accretion because of higher energy to protein ratio. Betaine HCl supplementation could be used as feed additive to reduce carcass fat and helps to save cost compared to inclusion of higher percentage of crude protein in diet.

GH regulates growth and metabolism directly and/or indirectly via stimulating IGF-1 to target organs. In this study, the betaine supplementation had no effect on the serum GH concentrations but significantly increased the serum IGF-1 concentration in the finishing pigs. GH secretion can be stimulated by betaine (Etherton, 2000; Huang et al., 2006; 2007). GH is secreted in a pulsatile fashion in nature (Klindt and Stone, 1984). Kraetzl et al. (1994) reported that pigs had an episode of GH secretory profiles, with a constant basal course and clearly distinct amplitudes. Inter-peak interval was shorter at night, while GH amplitude was higher at night than at day. The blood collection in this study was performed at noon and measured only a single point of GH secretion. Therefore, the effect of betaine on single point GH secretion might not be obvious. Thus, the effects of betaine on GH secretion during the day should be further investigated. The betaine supplementation increased serum IGF-1 in the current study, consistent with the findings of other studies (Huang et al., 2006; Yan, 2001). The IGF-1 concentrations might be increased by betaine throughout the GH action, but no differences in the

serum GH level were observed. On the other hand, the increment of serum IGF-1 concentration might be influenced directly by betaine. Recent studies have shown that IGF-1 production can also be stimulated directly by amino acids (Brameld et al., 1999; Stubbs et al., 2002). Betaine is a derivative of glycine amino acid and, as a result, may stimulate IGF-1 production irrespective of GH level. The reduction in LSQ can partly account for the increment of carcass lean in the betaine group, which might be a consequence of the increment of serum IGF-1.

In this study, the betaine HCl-fed pigs had decreased FAS activities. Therefore, it is possible that betaine reduced FA synthesis in adipose tissue, resulting in the reduction in fat accumulation and lower serum TG concentrations. Betaine causes alteration in blood lipoprotein profiles (Barak et al., 1994; Zeisel, 2006), which may contribute to the redistribution of fat metabolites. In this study, the lower FFA concentrations may be due to the test limit to detection of only FFA above C₈. The serum FFA concentrations were not influenced by betaine HCl, which is consistent with previous studies (Matthews et al., 1998; Overland et al., 1999). Hence, it may be deduced that the reduction in carcass fat deposition in the betaine group resulted from decreased lipogenesis. However, circulating plasma FFA concentration may not produce sufficient evidence for lipolysis in adipose tissue deposition in pigs. Thus, the effect of betaine on lipolysis cannot be excluded.

Conclusion

From the overall results, it is concluded that the 0.125% dietary betaine HCl supplementation could reduce carcass fat without alteration in growth performance of finishing pigs. The decrease in carcass fat deposition resulted from the effect of betaine HCl on lipogenesis. Betaine HCl increased the serum IGF-1 concentrations, resulting in decreased fat/lean ratio. Therefore, dietary betaine supplementation becomes an alternative choice for carcass fat reduction in finishing pigs.

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บทคัดย่อ

ผลของการเสริมบีเทนในอาหารต่อความหนาไขมันสันหลังและระดับอินซูลินไลค์โกรทแฟคเตอร์วันในซีรัมของสุกรขุนระยะท้าย

มัธยฎน โล่ทอง^{1A} กิตติพงษ์ ทาจำปา¹ พรชลิต อัครชีพ² กฤษ อังคนาพร^{1*}

การศึกษานี้เพื่อทดสอบผลของการเสริมบีเทนไฮโดรคอลลอยด์ในอาหารต่อการสะสมไขมันในซากและเมตาบอลิซึมของไขมันที่เกี่ยวข้องกับการเปลี่ยนแปลงของโกรทฮอร์โมน (GH) และอินซูลินไลค์โกรทแฟคเตอร์วัน (IGF-1) ของสุกรขุนระยะท้าย แบ่งสุกรขุนลูกผสมระยะท้ายเพศผู้ตอน 30 ตัวและเพศเมีย 30 ตัว น้ำหนักตัวเฉลี่ย 74 ± 1.8 กิโลกรัม ออกเป็นสองกลุ่ม ๆ ละ 6 กลุ่มย่อย กลุ่มแรกเลี้ยงด้วยอาหารพื้นฐานของสุกรขุน ส่วนอีกกลุ่มทำการเสริมบีเทนไฮโดรคอลลอยด์ลงในอาหาร 0.125% เป็นระยะเวลา 41 วัน การทดลองพบว่าการเสริมบีเทนไฮโดรคอลลอยด์ลงในอาหารไม่มีผลต่อน้ำหนักตัวสุดท้าย อัตราการเจริญเติบโต และอัตราแลกเนื้อของสุกร ($P > 0.05$) แต่ทำให้ดัชนี Lenden-speck-quotient ความหนาเฉลี่ยไขมันสันหลัง ระดับไตรกลีเซอไรด์ในซีรัม และการทำงานของเอนไซม์ fatty acid synthase (FAS) ลดลงเมื่อเทียบกับสุกรกลุ่มควบคุม ($P < 0.05$, $P < 0.01$, $P < 0.05$ และ $P < 0.05$ ตามลำดับ) การเสริมบีเทนไฮโดรคอลลอยด์ลงในอาหารสุกรไม่ทำให้ระดับ GH ในซีรัมแตกต่างกัน แต่ทำให้ระดับ IGF-1 ในซีรัมเพิ่มขึ้นเมื่อเทียบกับสุกรกลุ่มควบคุม ($P < 0.01$) จากการทดลองสรุปได้ว่าการเสริมบีเทนไฮโดรคอลลอยด์สามารถลดการสะสมของไขมันในสุกรขุนระยะท้ายได้โดยลดการทำงานของเอนไซม์ FAS และเพิ่มระดับ IGF-1 ในซีรัม ซึ่งนำไปสู่การพัฒนาคุณภาพซากของสุกร

คำสำคัญ: ความหนาไขมันสันหลัง บีเทน สุกรขุนระยะท้าย โกรทฮอร์โมน อินซูลินไลค์โกรทแฟคเตอร์วัน เอนไซม์ที่สร้างไขมัน

¹ภาควิชาสัตววิทยา คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพมหานคร 10300

²ภาควิชาอายุรศาสตร์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพมหานคร 10300

^Aนิสิตบัณฑิตศึกษา สาขาสัตววิทยาการสัตว ภาควิชาสัตววิทยา คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย 10330

*ผู้รับผิดชอบบทความ E-mail: akris@chula.ac.th