Effect of supplementation of lysine and methionine on growth performance, nutrients digestibility and serum biochemical indices for growing sika deer (Cervus Nippon) fed protein deficient diet

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Abstract

This study was conducted to investigate the effect of supplementation of lysine (Lys) and methionine (Met) on growth performance, nutrients digestibility and serum biochemical indices for growing sika deer fed crude protein (CP) deficient diet. Sixteen 5-month-old growing male sika deer were randomly assigned to 4 groups receiving diets (n=4): i) CP-adequate (16.63%) diet; ii) CP-deficient (13.77%) diet with 3 g/kg Lys; iii) CP-deficient with 3 g/kg Lys and 1 g/kg Met; iv) CP-deficient diet with 3 g/kg Lys and 2 g/kg Met. The digestibility of dry matter (P<0.01), organic matter (P<0.01), CP (P<0.01), serum albumin (P<0.01), and total protein (P<0.01) concentrations of groups receiving CP-adequate or Met supplementation were improved. The average daily gain (P=0.10), gain to feed ratio (P=0.07), the digestibility of acid detergent fibre (P=0.09) and neutral detergent fibre (P=0.09), and the serum globulin (P=0.08) concentrations had a tendency to increase as the Met or CP level increased. Meanwhile, blood urea nitrogen (P<0.01) and alanine aminotransferase (P<0.01) were decreased for CP-deficient, but no response to Met-added diets; aspartate aminotransferase (P=0.04) depressed for both CP-deficient and Met-added diets. Therefore, amino acids added to CP-deficient diets show high efficiency: they remain among the simplest ways for growth performance, while cutting down environmental waste and economic consumption.

Introduction

Giving current global environmental protection and economic development, it has become a growing concern to find dietary strategies that can reduce nitrogen losses more efficiently from livestock production (Kamran et al., 2010). Meanwhile, low crude protein (CP) intake has long been a prominent problem in regions with insufficient protein resources. Nevertheless, protein nutrition of the predominant livestock is dependent primarily on essential amino acids (AA) digestion and absorption. In light of this, supplementation with AA may be a potential strategy for alleviating the possible negative production effects of decreased dietary protein supply. Cows and pigs fed low CP diets supplemented with AA were able to achieve the same performance as those fed adequate CP diet (Leonardi et al., 2003; Zhang et al., 2013). Methionine (Met) and lysine (Lys) are known as top limiting AA for ruminant (Abe et al., 1998; Greenwood and Titgemeyer, 2000), but very little information exists in the importance of Met and Lys in sika deer. Yet, it remains to be unclear for the sika deer how Met and Lys perform as limiting factors, despite that the National Research Council (2007) provides estimation of metabolisable protein requirement. It is important to be aware of the estimates about possible effects of low-CP with AA on sika deer production. Therefore, the objective of this study is to evaluate the responses of adding Lys and Met to a CP-deficient (DCP) diet in sika deer.

Materials and methods

Animal and diet

The experiment was conducted under the animal care and use guidelines at the Antler-Deer Farm of the Institute of Special Animals and Plant, Chinese Academy of Agricultural Science, during the period from 7th Nov 2013 to 15th Jan 2014. Sixteen 5-month-old healthy growing sika deer [average initial body weight (BW)=29.13±2.92 kg] were randomly divided into 4 dietary treatments (n=4): i) CP-adequate (16.63% CP) diet (ACP); ii) DCP (13.77% CP) diet with 3 g/kg Lys (DLN); iii) DCP with 3 g/kg Lys and 1 g/kg Met (DLL); iv) DCP with 3 g/kg Lys and 2 g/kg Met (DLH). The concentration of diet was determined based on National Research Council (2007). Diets were offered twice daily at 08:00 and 17:00 as total mixed rations with allowances made for refusals of 10%. The chemical composition of each diet is listed in Table 1. The theoretical and actual Met and Lys concentrations in diets are described in Table 2.

Sample collection and treatment

Growth and feed consumption were measured at the last day of the study. On 35th and 70th days of the study, blood samples were collected with vacuum tubes (Vacutainer; Becton Dickinson and Co., Rutherford, NJ, USA) containing sodium heparin or ethylene diamine tetraacetic acid by puncture of the jugular vein, and centrifuged for 10 min at 4000×g to obtain plasma. Samples were stored at -20°C for later analysis of glucose (GLU), urea, total proteins (TP), albumin (ALB), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Selectra E Chemistry Analyzer; Selectra Technology, Sumatra, Indonesia) using enzymatic (glucose-oxidase and urase), colorimetric (biuret and bromocresol green) methods and velocity (lactate dehydrogenase). Feed provided and refusals were weighed and recorded daily. Feed offered and faecal output were sampled daily for eight days from 18th to 26th December 2013. Samples of each feed and faeces were dried at 65°C in a forced air oven for 48 h, ground to pass through a 1-mm mesh. Feed and feces were analysed for dry matter (DM), organic matter (OM), CP (Kjeldahl-N, 6.25), ash, Ca, P and ash insoluble in hydrochloric acid according to

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Key words: Sika deer, Crude protein-deficient, Methionine, Growth performance, Serum biochemical indices.

Acknowledgments: this work was financially supported by Science and Technology Pillar Program from Jilin Province (20140203018NY), Scientific Research Funds from Chang Chun city (13Nk07).

Received for publication: 1 September 2014. Accepted for publication: 8 December 2014.

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Italian Journal of Animal Science 2015; 14:3640
doi:10.4081/ijas.2015.3640
AOAC (2003), and for neutral detergent fibre (NDF), acid detergent fibre (ADF) content (Ankom 2000 fibre analyser; Ankom Technology, Fairport, NY, USA). For AA analysis, samples were quantified by ion-exchange chromatography (AOAC, 2003) (Hitachi L8900 amino acid analyser; Hitachi Technology, Tokyo, Japan). The digestibility of DM, OM, CP, NDF, ADF, Ca, and P was calculated using the method of 2N ash insoluble in hydrochloric acid. The apparent digestibility of DM, OM, CP, Ca, P, NDF, and ADF was determined according to the following equation:

$$D = 100\left(1 - \frac{A}{A_1} - \frac{B}{B_1}\right)$$

where D represents the nutrients apparent digestibility; A is the determination of hydrochloric acid insoluble ash in diet; A₁ is the determination of hydrochloric acid insoluble ash in faeces; B is the determination of nutrients in diet; B₁ is the determination of nutrients in faeces.

### Statistical analyses

Data were analysed by ANOVA as a randomised complete block design using the GLM procedure of SAS (version 9.2, SAS Institute Inc., Cary, NC, USA). DUNCAN test was used to compare the results from each of the experimental. P<0.01 was considered as greatly significant, while P<0.05 was considered as significant.

### Results

#### Growth performance

Body weight showed no difference after dietary treatment in this study (Table 3). The average daily feed intake (ADFI) was depressed by CP-deficient or Met-added, sika deer fed with DLHN decreased ADFI (P<0.05), compared with that of ACP group. The average daily gain (ADG, P=0.10) and gain to feed ratio (G/F, P=0.07) had a tendency to improve as the Met or CP level increased. Average daily gain in ACP group was higher (P<0.05) than that of DLN group. The G/F was increased (P<0.05) when supplemented with Met, and a similar result was observed in the ACP diet and other diets.

#### Nutrients apparent digestibility

The digestibility of DM, OM, and CP improved (P<0.01) as the Met or CP level increased (Table 4), and sika deer fed DLHN diet was lower (P<0.01) than that of sika deer fed ACP and DLHN diets, while no difference was observed between ACP group and DLHN group. There was no response of NDF, ADF, P digestibility for sika deer containing the CP levels reduced, while the digestibility of NDF, ADF were increased (P<0.05), and P digestibility was decreased (P<0.05) for Met-supplemented diet.

#### Serum biochemical indices

The concentrations of TP and ALB decreased (P<0.01) in response to the decrease of CP level, and increased (P<0.01) when Met was added. However, TP and ALB concentrations showed no difference among treatments with various Met or CP levels added (Table 5). The concentration of GLOB was increased by the DLL diet, compared with the DLN diet (P<0.05). The AST and BUN concentration was lower (P<0.05 or P<0.01) in the DCP groups compared to ACP group, and AST concentration was decreased for DLL compared with DLN (P<0.05), but BUN was unaffected by dietary treatment throughout the DCP diets. There was no difference in ALT concentration for sika deer fed with diets containing different concentrations of Met with a low-CP content, while the feed conversion of sika deer receiving the DLL was poorer (P<0.05) than that of sika deer fed with ACP.

### Discussion

This study can be viewed as a pioneer step in investigating the feeding of sika deer with Met and Lys in a low CP diet. One of the notable effects in this study was the trend of depressed ADFI with the DCP diet. A recent meta-analysis reported increased feed intake with increasing dietary protein intake (Lee et al., 2012a, 2012b). Yang et al. (2014) observed similar trends with low CP diet. Together with the apparent reduction in nutrients digestibility, particularly fibre, it was suggested that rumen function was modified due to RDP and ammonia deficiency (Weigel et al., 1997; Allen, 2000). Therefore, our data suggested that nutrients digestibility (except Ca and P) was decreased as a consequence of decreased CP. Tomkins and McMeniman (2006) reported the

### Table 1. Composition and nutrient levels of basal diets.

| Ingredients, g/100 g | ACP | DCP | Proximate composition | ACP | DCP |
|----------------------|-----|-----|-----------------------|-----|-----|
| Corn                 | 10.00 | 19.00 | DM, % | 93.37 | 93.30 |
| Soybean meal         | 16.00 | 8.00  | OM, % | 80.95 | 82.22 |
| Corn fibre           | 4.50  | 7.00  | CP, % | 16.07 | 13.77 |
| Distiller dried grain soluble | 5.00  | 4.00 | ME, MJ/kg | 9.33 | 3.81 |
| Corn germ            | 10.00 | 7.50  | EE, % | 1.85  | 1.99  |
| Alfalfa meal         | 50.00 | 50.00 | NDF, % | 52.53 | 54.94 |
| Corn                 | 10.00 | 7.50  | NDF, % | 52.53 | 54.94 |
| Syrup                | 3.00  | 3.00  | ADF, % | 29.56 | 28.42 |
| NaCl                 | 0.50  | 0.50  | Ca, % | 1.00  | 0.96  |
| Premix°              | 1.00  | 1.00  | Total P, % | 0.45  | 0.42  |
| Total                | 100.00 | 100.00 | Total P, % | 0.45  | 0.42  |

ACP, crude protein-adequate; DCP, crude protein-deficient; DM, dry matter; OM, organic matter; CP, crude protein; ME, metabolic energy; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre. ME was a calculated value, while the others were measured values. “One kg of premix contained the following: MgO, 0.076 g; ZnSO₄·H₂O, 0.036 g; MnSO₄·H₂O, 0.843 g; FeSO₄·H₂O, 0.053 g; Na₂SO₃·H₂O, 0.031 g; vitamin A, 2484 U; vitamin D, 3498.8 U; vitamin E, 0.828 U; vitamin K, 0.00023 g; vitamin B₁₂, 0.000025 g; vitamin B₉, 0.000093 g; vitamin B₆, 0.00138 mg; folic acid, 0.023 mg; niacin acid, 0.00162 g; calcium pantothenate, 0.0015 g; CaHPO₄, 5.17 g; CaCO₃, 4.57 g.”
apparent digestibility of the DM, OM and CP decreased when the CP content of the diet decreased, which presumably depressed the availability of AA and energy. Smith et al. (1975) and Yang et al. (2014) reported a similar response to protein digestibility when deer were fed with pelleted diets varying in CP content. Analogous results were found that CP and ADF digestibility of deer were depressed by CP deficient (Holter et al., 1979). With the depressing nutrients digestibility, the rate of body protein synthesis and catabolism, and amino acid transformation were also depressed. The decreased protein metabolism correlated in serum with decreased levels of CP in the diet was observed in our study. The concentrations of BUN aligned with the results obtained by Tomkins and McMeniman (2006), Yang et al. (2014). Similar with the experiment done by Bartlett et al. (2006) showing that TP and BUN concentrations increased linearly as CP increased for calves fed at 1.75% of BW daily. Based on those, the ADG and G/F decreased for CP reduced. Similar results were observed in Bartlett et al. (2006), Blome et al. (2003) and Wang et al. (2011).

Our hypothesis is that, in this study, AA supplementation with CP-deficient diet improved nutrients digestibility, as well as AA metabolism and ability, which in turn increased ADG and G/F, and achieve normal CP level diet. However, addition of 1 g/kg diet Met tended to decrease ADFI, and the supplementation of 2 g/kg Met further decreased it to the extent of being significantly lower than the ADFI of the CP-adequate diet. Wekes et al. (2006) reported feed intake depression with AA-rich diet. In contrast, more evidences to previous conclusions that feed intake depression with AA-deficient diets (Allen, 2000; Lee et al., 2012a, 2012b). Maybe there is a potential physiological effect of AA on feed intake. Gratifyingly, nutrients digestibility (except P), AA metabolism ability and balance were all improved by adding Met. Data from the current study confirmed that Met was an important limiting AA in sika deer under dietary conditions, which is a well-known phenomenon in ruminant and rumen microorganisms (Abe et al., 1998; Greenwood and Titgemeyer, 2000).

Clark and Peterson (1988) and Gordon and Ashes (1984) observed that rumen microorganisms populations were stimulated by Met supplementation. In previous studies, increased post ruminal supply of Met tended to gradually increase degradation of cellulose, hemicellulose (Li et al., 2007), DM, OM and CP (Hall et al., 1990; Huisman et al., 1988), but some reports suggested unaffected nutrients digestibility in cows or lambs as Met supplementation.

### Table 2. Content of lysine and methionine in diets.

| Item (level), % | ACP | DLN | DLL | DLH | SEM | P   |
|---------------|-----|-----|-----|-----|-----|-----|
| Lys in basal diet | 0.89 | 0.63 | 0.63 | 0.63 |     |     |
| Met in basal diet | 0.25 | 0.18 | 0.18 | 0.18 |     |     |
| Addition of Lys | -   | 0.27 | 0.27 | 0.27 |     |     |
| Addition of Met | -   |     | 0.10 | 0.20 |     |     |
| Lys in test diet | 0.89 | 0.90 | 0.90 | 0.90 |     |     |
| Met in test diet | 0.25 | 0.18 | 0.28 | 0.38 |     |     |

ACP, crude protein-adequate diet; DLN, crude protein-deficient (DCP) diet with 3 g/kg lysine; DLL, DCP with 3 g/kg lysine and 1 g/kg methionine; DLH, DCP with 3 g/kg lysine and 2 g/kg methionine; Lys, lysine; Met, methionine.

### Table 3. Effects of supplementation of lysine and methionine on growth performance of deer.

| Item | ACP | DLN | DLL | DLH | SEM | P   |
|------|-----|-----|-----|-----|-----|-----|
| Initial BW, kg | 28.93 | 29.80 | 29.68 | 28.85 | 3.23 | 0.96 |
| Final BW, kg | 38.25 | 37.58 | 38.75 | 38.15 | 3.28 | 0.97 |
| ADG, g/d | 133.21a | 111.07b | 129.64ab | 131.43ab | 12.90 | 0.10 |
| ADFI, kg/d | 1.65a | 1.60ab | 1.56ab | 1.53ab | 0.07 | 0.11 |
| G/F | 0.079ab | 0.068b | 0.081b | 0.084b | 0.008 | 0.07 |

ACP, crude protein-adequate diet; DLN, crude protein-deficient (DCP) diet with 3 g/kg lysine; DLL, DCP with 3 g/kg lysine and 1 g/kg methionine; DLH, DCP with 3 g/kg lysine and 2 g/kg methionine; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G/F, gain to feed ratio. Least square means in the same row with different superscript letters are significantly different (P<0.05).

### Table 4. Effects of supplementation of lysine and methionine on nutrients digestibility of deer.

| Apparent digestibility, % | ACP | DLN | DLL | DLH | SEM | P   |
|---------------------------|-----|-----|-----|-----|-----|-----|
| DM                        | 62.33abc | 56.32abc | 58.31abbc | 60.49abbc | 1.91 | <0.01 |
| OM                        | 64.05abc | 58.14abc | 59.86abc | 62.41abc | 2.22 | 0.01 |
| CP                        | 74.72abc | 67.99abc | 69.90abc | 72.32abc | 1.91 | <0.01 |
| NDF                       | 65.51abc | 63.40abc | 64.38abc | 68.35abc | 2.60 | 0.09 |
| ADF                       | 53.79abc | 50.82abc | 52.71abc | 55.95abc | 2.43 | 0.07 |
| Ca                        | 28.70 | 27.31 | 27.30 | 31.23 | 2.96 | 0.39 |
| Total P                   | 68.87abc | 71.88abc | 71.89abc | 68.14abc | 1.66 | 0.02 |

ACP, crude protein-adequate diet; DLN, crude protein-deficient (DCP) diet with 3 g/kg lysine; DLL, DCP with 3 g/kg lysine and 1 g/kg methionine; DLH, DCP with 3 g/kg lysine and 2 g/kg methionine; DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre. Least square means in the same row with different superscript letters are significantly different (P<0.05).

### Table 5. Effects of supplementation of lysine and methionine on serum biochemical indices of deer.

| Items | ACP | DLN | DLL | DLH | SEM | P   |
|-------|-----|-----|-----|-----|-----|-----|
| ALB, g/L | 25.24abc | 23.60abc | 25.78abc | 26.09abc | 1.03 | <0.01 |
| GLOB, g/L | 33.75abc | 32.43abc | 34.90abc | 34.21abc | 1.87 | 0.08 |
| TP, g/L | 58.99abc | 56.03abc | 60.68abc | 60.38abc | 1.50 | <0.01 |
| GLU, mmol/L | 12.07 | 11.48 | 13.81 | 13.55 | 2.25 | <0.01 |
| BUN, mmol/L | 12.18abc | 11.29abc | 10.93abc | 11.35abc | 0.69 | <0.01 |
| ALT, U/L | 79.97abc | 73.26abc | 65.96abc | 69.66abc | 6.30 | <0.01 |
| AST, U/L | 79.97abc | 78.74abc | 68.84abc | 74.95abc | 8.08 | 0.04 |

ACP, crude protein-adequate diet; DLN, crude protein-deficient (DCP) diet with 3 g/kg lysine; DLL, DCP with 3 g/kg lysine and 1 g/kg methionine; DLH, DCP with 3 g/kg lysine and 2 g/kg methionine; ALB, albumin; GLOB, globuline; TP, total protein; GLU, glucose; BUN, blood urea nitrogen; ALT, alanine aminotransferase; AST, aspartate aminotransferase. Least square means in the same row with different superscript letters are significantly different (P<0.05).
ment (Broderick et al., 2008, 2009). The reason causing the different results was due to reduced ADFI in our study. With greater CP digestibility, the evidence pointed to improved protein absorption, a possible explanation for Met added, particularly the greater TP and ALB concentration. Also, there were sufficient evidences showing depressed blood plasma concentrations of BUN that could be better for protein synthesis (Awawdeh et al., 2006; Schroeder et al., 2006). While an ideal AA pattern was able to satisfy body utilisation, excessive AA level may bring extra the burden to liver and decrease nitrogen efficiency, because the catabolism of AA leads a sharp rise in BUN concentrations (Rosell and Zimmerman, 1985). Data from the current study confirmed that addition of 1 g/kg Met decreased the concentration of BUN, ALT and AST, and the supplementation of 2 g/kg Met increased. Considering those factors, increased supply of Met initially tended to increase the ADG and G/F, and such increasing trend slowed down as more Met was added. Yet a significant increase of ADG was not observed in our study. Analogous results were reported by Paula et al. (2012) and Gajera et al. (2013), who observed ADG with red deer and cows increased with Met level increased.

In both analysis, Met and Lys supplementation with CP-deficient diet improved nutrients digestibility and AA metabolism ability and balance, which compensates the lack of CP. Average daily gain and G/F were not affected for sika deer fed CP-adequate diet compared to sika deer fed CP-deficient with Lys and Met diets. Calves fed with a 12.02% CP diet containing supplemental Met showed growth performance similar to calves fed with a 14.67% protein diet (Yun et al., 2011). A similar experiment done by Yen and Veum (1982) showed that ADG of growing pigs fed with a 13% CP diet supplemented with Lys and Trp was similar to pigs fed with a 16% CP diet.

**Conclusions**

Our results indicate that the growth and metabolism of growing sika deer were improved by CP-adequate or AA supplementation. Similar nutrients digestibility, growth performance, serum protein concentration, and decreased metabolite and metabolic enzymes concentration for sika deer fed CP-deficient with balance AA diets compared to sika deer fed CP-adequate diet. Amino acids added with CP-deficient show high efficiency; they remain among the simplest ways for growth performance, while cutting down environmental waste and economic consumption.

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