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Optimizing the ratios of standardized ileal digestible (SID) methionine plus SID cystine and SID threonine to SID lysine in low-protein diets for working boars

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KEYWORDS

Amino acids pattern

Boar

Semen quality

SID Met plus Cys to Lys

SID Thr to Lys

ABSTRACT

This study aimed to optimize the ratios of standardized ileal digestible (SID) methionine (Met) plus cystine (Cys), and threonine (Thr) to SID lysine (Lys) in low-protein diets for working boars. Forty-eight working Duroc boars were randomly allocated to one of 12 dietary treatments in a 3x4 factorial experimental design in which factor 1 was the ratios of SID Met plus Cys to SID Lys (50, 60, 70%), factor 2 was the ratios of SID Thr to SID Lys (40, 50, 60, 70%). Semen was collected at a 4 days interval for 6 weeks for 10 ejaculates. Semen volume (V), percentage of sperm with progressive motility (A), sperm concentration (C), and the total number of motile sperm per ejaculate (VAC) were measured. The results of the study revealed that the ratios of SID Met plus Cys to SID Lys in the diets affected the C and VAC. Values of C and VAC were highest at the ratios of SID Met plus Cys to SID Lys of 70% and lowest at 50% ($P<0.05$). Similarly, the ratios of SID Thr to SID Lys affected the C and VAC. Further, the values of C and VAC were highest at the ratio of SID Thr to SID Lys of 60% and lowest at 40% ($P<0.05$). There was no interaction effect between the two factors. In conclusion, the ratios of SID Met plus Cys to SID Lys of 70% and SID Thr to SID Lys of 60% in a 13.5% CP diet are optimal for working boars.

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1 Introduction

The semen quality of boars plays an important role in pig breeding, and it depends on various factors. In terms of nutrition, the contents of dietary essential amino acids highly influence boar semen quality. In general, for pigs, lysine (Lys) is normally the first limiting amino acid in practical diets (Ho et al. 2019). According to Louis et al. (1994), the dietary Lys requirement for working boars should be 0.60% or 12.0g of total Lys/day. Further, as per the NRC (1998), the nutrient requirements for boars are 3265 kcal ME/kg, 13% Crude Protein (CP), and 0.6% Lys at 2.0 kg/day feed intake. Rupanova (2006) reported that boars fed a diet containing 1.03% Lys have better semen quality than those fed a 0.86% Lys diet with no changes in ejaculate volume, while Golushko et al. (2010) determined that the total Lys requirement for boars is about 0.92% (0.76% digestible Lys). In addition, several recent studies showed that a Lys:Met:Thr:Trp ratio of 100:60:65:19 in boar diets improved the reproductive performances (Kiefer et al. 2012), and boars fed a low CP diet (13%) with a ratio of Lys:Thr:Trp: Argas 100:76:38:120 resulted in similar or better reproductive performance than a 17% CP diet (Ren et al. 2015). On the other hand, Ho et al. (2019) suggested that it is necessary to estimate Met plus Cys requirement when formulating diets for pigs because the amount of Met needed in the diet depends on the amount of Cys present. From the above-mentioned results, it is believed that the essential amino acid ratios in low-protein diets are very important for the semen quality of boars. To our knowledge, there are few studies regarding the effect of different amino acid ratios of SID Met plus Cys and Thr to SID Lys in low-protein diets on boar semen quality. Therefore, the objective of this study was to optimize the ratios of SID Met plus Cys and Thr to SID Lys in 13.5% CP diets for working boars.

2 Materials & Methods

2.1 Experimental design

Forty-eight 11 months old working Duroc boars, weighing approximately 150 kg each, were randomly allocated to one of 12 dietary treatments in a 3X4 factorial experimental design in which factor 1 (M) was the three ratios of 50, 60, and 70% SID Met plus Cys to 100% SID Lys, and factor 2 (T) was the four ratios of 40, 50, 60 and 70% SID Thr to 100% SID Lys. The treatments are denoted in Table 1.

The boars were trained to mount a dummy sow for one month before experimenting to provide uniform semen production, and they were individually housed. The experiment was conducted at the Binh Thang Pig Research and Development Center, Institute of Animal Sciences for Southern Vietnam.

2.2 Diets

The diet compositions and calculated nutritive values of the twelve experimental diets are shown in Table 2 and Table 3, respectively. The level of SID Lys was kept constant at 0.8% of all diets. All SID Met plus Cys and SID Thr levels in the diets were adjusted by using synthetic Met and Thr. The energy level was set at 3,000 kcal ME/kg, and the CP level was set at 13.5% for all diets. The ratio of SID tryptophan to SID Lys was fixed at 0.22% for all diets. Other amino acids, vitamin, macro, and micro-mineral element concentrations were formulated to equivalency in all diets. The animals were offered 3.0 kg of feed/day and were provided with *ad libitum* access to water. All boars were fed their experimental diets for one week before collecting semen to evaluate semen quality.

Table 1 Experimental design and denoted treatments

Factor 1 (M)	Factor 2 (T)	Treatments
50 M	40T	50M.40T
	50T	50M.50T
	60T	50M.60T
	70T	50M.70T
60M	40T	60M.40T
	50T	60M.50T
	60T	60M.60T
	70T	60M.70T
70M	40T	70M.40T
	50T	70M.50T
	60T	70M.60T
	70T	70M.70T

Table 2 Developed Diet ingredients and different combination

Ratio of SID Met plus Cys to Lys (%)	50				60				70			
Ratio of SID Thr to Lys (%)	40	50	60	70	40	50	60	70	40	50	60	70
Treatment	50M X 40T	50M X 50T	50M X 60T	50M X 70T	60M X 40T	60M X 50T	60M X 60T	60M X 70T	70M X 40T	70M X 50T	70M X 60T	70M X 70T
Corn	428	429	430	431	429	430	431	432	430	431	432	433
Rice bran	250	250	250	250	250	250	250	250	250	250	250	250
Wheat	100	100	100	100	100	100	100	100	100	100	100	100
Wheat bran	86	83	81	78	83	81	78	76	81	78	76	73
Soybean meal	82	83	84	84	83	84	84	85	84	84	85	85
Fish meal 60%	20	20	20	20	20	20	20	20	20	20	20	20
DCP	9	9	9	9	9	9	9	9	9	9	9	9
Limestone	12	12	12	12	12	12	12	12	12	12	12	12
Salt	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7
Vit-min. premix	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Phytase	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Kenzyme V	1	1	1	1	1	1	1	1	1	1	1	1
Lys 98%	2.97	2.96	2.96	2.95	2.96	2.96	2.95	2.94	2.96	2.95	2.94	2.94
DL-Met	0.09	0.09	0.09	0.09	0.91	0.91	0.91	0.91	1.72	1.72	1.72	1.72
L-Thr 98%	0.22	1.04	1.85	2.66	0.22	1.04	1.85	2.66	0.22	1.03	1.85	2.66
L-Try 98%	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000

Table 3 Calculated nutritive values of imposed diets in various combinations

Ratio of SID Met plusCys to Lys (%)	50			60			70					
Ratio of SID Thr to Lys (%)	40	50	60	40	50	60	40	50	60	40	50	60
Treatment	50M X 40T	50M X 50T	50M X 60T	50M X 40T	50M X 50T	50M X 60T	50M X 40T	50M X 50T	50M X 60T	50M X 40T	50M X 50T	50M X 60T
DM (%)	88.41	88.42	88.43	88.44	88.42	88.43	88.44	88.44	88.43	88.44	88.44	88.45
ME (kcal/kg)	3,000	3,000	3,000	3,000	3,000	3,000	3,000	3,000	3,000	3,000	3,000	3,000
CP (%)	13.50	13.50	13.50	13.50	13.50	13.50	13.50	13.50	13.50	13.50	13.50	13.50
EE (%)	5.90	5.90	5.89	5.89	5.90	5.89	5.89	5.89	5.89	5.89	5.89	5.88
CF (%)	4.56	4.54	4.51	4.49	4.54	4.51	4.49	4.46	4.51	4.49	4.46	4.44
Ca (%)	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Total P (%)	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83
Available P (%)	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Salt (%)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
SID Lys (%)	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
SID Met (%)	0.22	0.22	0.22	0.22	0.30	0.30	0.30	0.30	0.38	0.38	0.38	0.38
SIDMet plus Cys (%)	0.40	0.40	0.40	0.40	0.48	0.48	0.48	0.48	0.56	0.56	0.56	0.56
SID Thr (%)	0.40	0.48	0.56	0.64	0.40	0.48	0.56	0.64	0.40	0.48	0.56	0.64
SID Trp (%)	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
SID Arg (%)	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77
SID Val (%)	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55
SID Iso (%)	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42
SID Leu (%)	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95
SID His (%)	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
SID Phe (%)	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53

2.3 Semen collection and evaluation

Semen collection from the boars was carried out at an interval of 4 days during 6 weeks for a total of 10 ejaculates by the well-trained technician using a dummy sow with an artificial vagina. Samples collected from the boars for one ejaculate throughout the experiments were subjected to semen quality evaluation.

The semen volume (V) was determined immediately after the collection by filtering the ejaculate through gauze to remove the gelatinous fraction, and the gelatinous-free fraction was weighed using an electronic scale and converted to volume as described by Lovercamp et al. (2013). Sperm concentration (C) was determined by hemacytometer counts as described by Melrose (1966) and Almquist (1973).

Percentage of sperm with progressive motility (A) was determined by light microscopy under ×200 magnification on a warm stage immediately after straining the ejaculate based on the scoring method described by Swierstra (1973).

Total number of motile sperm per ejaculate (VAC) was calculated by multiplying the semen volume (V), sperm concentration (C), and percentage of sperm with progressive motility (A) (Louis et al. 1994).

2.4 Statistical analysis

Collected data were analyzed using an ANOVA by Minitab version 16.2.0 (2010). The Tukey-Test was used to compare average values with a 95% confidence interval. Mean values were considered significantly different when P values were less than 0.05. For statistical analysis, the used statistical Model is as follows

$$Y_{ijk} = \mu + M_i + T_j + MxT_{ij} + e_{ijk}$$

Y_{ijk} = the value of the dependent variable of observation k in factor A level i and factor B level j (i = 1,2,3; j = 1, .., 4; k = 4); In which, μ: general mean; M_i: effects of methionine plus cystine; T_j: effects of threonine; MxT_{ij}: effects of interaction M and T; and e_{ijk}: random error.

3 Results

3.1 Effect of different ratios of SID methionine plus cystine to SID lysine on boar semen quality

Results given in Table 4 revealed that except for the semen volume of boars, the other semen characteristics were affected by the ratios of SID Met plus Cys to SID Lys in the diet (P<0.05). The percentage of sperm with progressive motility from boars fed a diet with a ratio of SID Met plus Cys to SID Lys of 50% was higher than those in the diet with the ratios of SID Met plus Cys to SID Lys of 60 and 70% (P<0.05). However, the sperm concentration and VAC were highest in boars fed the diet with a ratio of SID Met plus Cys to SID Lys of 70% and lowest in boars fed the diet with a ratio of SID Met plus Cys to SID Lys of 50% (P<0.05). It can be seen that the ratio of SID Met plus Cys to SID Lys of 70% in the diet improved the semen quality of boars.

3.2 Effect of different ratios of SID threonine to SID lysine on boar semen quality

Results presented in Table 5 show that the ratios of SID Thr to SID Lys in boar diets did not affect semen volume and the percentage of sperm with progressive motility (P>0.05), but they did affect sperm concentration and VAC (P<0.05). The sperm concentration in boars fed the diet with the ratio of SID Thr to SID Lys of 40% was the lowest and was highest in boars fed the diet with the ratio of SID Thr to SID Lys of 60%. However, there were no significant differences in sperm concentration between in boars fed the diets with the ratios of SID Thr to SID Lys of 60 and 70%(P>0.05), as well as in boars fed the diets with the ratio of SID Thr to SID Lys of 40 and 50%. The VAC was lowest in boars fed the diet with a ratio of SID Thr to SID Lys of 40%, followed by 50%, and highest in boars fed the diet with a ratio of SID Thr to SID Lys of 60%. Therefore, it can be concluded that the ratio of SID threonine to SID lysine of 60% in the diet would be optimal for working boars.

Table 4 Effect of different ratios of SID methionine plus cystine to SID lysine on boar semen quality

Item	Ratios of SID Met plusCys to SID Lys (%)			SEM	P-value
	50M	60M	70M		
V, mL	298.0 ± 1.49	297.9 ± 1.25	298.2 ± 5.37	0.92	0.967
A, %	84.5^{a} ± 0.28	83.9^{b} ± 0.43	83.9^{b} ± 0.87	0.12	0.012
C, $\times 10^{6}$ mL	331.6^{c} ± 2.05	338.3^{b} ± 2.69	343.9^{a} ± 4.43	1.17	<0.001
VAC,$\times 10^{9}$/ejaculate	83.10^{c} ± 0.19	84.71^{b} ± 0.61	86.31^{a} ± 1.87	0.295	<0.001

Values given in table are mean of selected replicates; mean value followed by the different letter in same row are significantly different at P<0.05

Table 5 Effect of different ratios of SID threonine to SID lysine on boar semen quality

Parameters	Ratios of SID Thr to SID Lys (%)				SEM	P-value
	40T	50T	60T	70T		
V, mL	296.3	297.2	299.5	299.1	1.06	0.122
A, %	84.2	84.1	84.2	84.1	0.16	0.946
C, $\times 10^6$/mL	331.2^{c}	336.3^{bc}	343.3^{a}	340.9^{ab}	1.35	<0.001
VAC,$\times 10^9$/ejaculate	82.66^{c}	84.19^{b}	86.15^{a}	85.84^{a}	0.34	<0.001

Values given in table are mean of selected replicates; mean value followed by the different letter in same row are significantly different at P<0.05

Table 6 Interaction effect between ratios of SID methionine plus cysteine to SID lysine and SID threonine to SID lysine on boar semen quality

Factor 1	50M				60M				70M				SEM	p-value
Factor 2	40T	50T	60T	70T	40T	50T	60T	70T	40T	50T	60T	70T		
V, mL	294.9	297.9	299.1	300.0	298.6	296.0	299.0	298.0	295.4	297.8	300.3	299.4	1.838	0.709
A, %	84.4	84.5	84.6	84.4	84.2	84.0	84.0	83.7	83.9	83.9	83.9	84.2	3.112	0.879
C, $\times 10^6$/mL	424.9^{f}	328.4^{ef}	338.9^{abcde}	334.1^{cdef}	330.7^{def}	337.3^{bcde}	342.0^{abcd}	343.1^{abcd}	337.9^{abcde}	343.1^{abc}	349.2^{a}	345.6^{ab}	2.332	0.816
VAC, $\times 10^9$/ ejaculate	80.9^{e}	82.6^{de}	84.3^{cd}	84.6^{bcd}	83.1^{cde}	84.0^{cd}	86.0^{abc}	85.7^{abcd}	83.9^{cd}	85.9^{abc}	88.1^{a}	87.2^{ab}	0.589	0.872

Values given in table are mean of selected replicates; mean value followed by the different letter in same row are significantly different at P<0.05

3.3 Interaction effect of ratios of SID methionine plus cystine and threonine to SID lysine

Results presented in Table 6 show that there were no statistically significant differences (P>0.05) in semen volume, the percentage of sperm with progressive motility, sperm concentration, or VAC among dietary treatments. Nor was there an interaction effect between the two factors (ratios of SID Met plus Cys to SID Lys and ratios of SID Thr to SID Lys) in this study.

4 Discussion

The main aim of this study was to optimize the amino acids ratios of SID Met plus Cys and Thr to SID Lys in low-protein diets for working boars by evaluating the effect of different ratios of SID Met plus Cys and Thr to SID Lys in 13.5% CP diets on boar semen quality. The present study found that the semen volume of boars ranged from 294.9 mL to 300.3 mL per ejaculate, and there were no significant differences in semen volume per ejaculate among the twelve treatments. These data suggest that the semen volume of boars was not affected by the different ratios of SID Met plus Cys to SID Lys and/or SID Thr to SID Lys in the diets. These results are consistent with previous reports, that there was no effect on sperm production when boars were fed either 6.8 g Lys/kg or 12 g Lys/kg diets (Kemp and Hartog 1989), and that no significant differences in semen volume between the two dietary treatments composed of total Lys levels of 0.64% and 0.96% with the Lys:Met:Thr:Trp ratio of 100:27:73:69 were found (Dong et al. 2016). Rozeboom (2000) also reported that the semen volume of different boar breeds fluctuated from 100 mL to 500 mL in the ejaculate, so the recorded results of boar semen volume in the current study are quite high.

The percentage of sperm with progressive motility in experimental boars ranged from 83.70% to 84.60%, and they were in the normal range of good-quality semen for working boars. The percentage of sperm with progressive motility in boars that consumed diets with different ratios of SID Thr to SID Lys in this study were consistent at approximately 84%, while this parameter for boars fed diets containing a SID Met plus Cys to SID Lys ratio of 50% was the highest, and higher than those in boars fed two other SID Met plus Cys to SID Lys ratios of 60 and 70%. No difference in the percentage of sperm with progressive motility between the SID Met plus Cys to SID Lys ratios of 60 and 70% was found in this study. Rozeboom (2000) reported that the percentage of sperm with progressive motility in boars fluctuated from 70 to 95%, and Vuong (2010) reported that the percentage of sperm with progressive motility in 24 months old boars was 76%. In this study, the percentage of sperm with progressive motility of working boars was attained at approximately 84%, which is considered very well.

In the present study, the sperm concentration per mL of semen was significantly affected by the ratios of SID Met plus Cys to SID

Lys. Sperm concentration increased from 331.6×10^6 sperms/mL of semen in boars fed a diet with a SID Met plus Cys to SID Lys ratio of 50% to 343.9×10^6 sperms/mL in boars fed the diet with a SID Met plus Cys to SID Lys ratio of 70%. On the other hand, sperm concentration increased gradually in boars fed diets with ratios of SID Thr to SID Lys of 40 to 60%, and kept constant at a ratio of SID Thr to SID Lys of 70%. The highest sperm concentration was attained at a ratio of SID Met plus Cys to SID Lys of 70% (343.9×10^6/mL) and a ratio of SID Thr to SID Lys of 60% (343.3×10^6/mL). These values were higher than those in previous studies (Vuong, 2010; Kommisrud et al. 2002). These results are consistent with previous reports that the semen quality of boars is improved by fed a diet with a Lys:Met:Thr:Trp ratio of 100:60:65:19 (Kiefer et al. 2012), and by feeding a 13% CP diet with a Lys:Thr:Trp:Arg ratio of 100:76:38:120 (Ren et al. 2015). These findings suggest that an optimal essential amino acid ratio in diet can be established for the boar to increase semen quality. Presently, nearly all pig farms have been using artificial insemination to breed, thus the higher sperm concentration and percentage of sperm with progressive motility would be favorable to improve the economic efficiency of production.

The VAC of boars differed among treatments in this study. An increase in the ratio of SID Met plus Cys to SID Lys from 50 to 60 and 70% led to a significant increase in the VAC from 83.10×10^9 to 84.71×10^9 and 86.31×10^9. On the other hand, Increasing ratios of SID Thr to SID Lys from 40 to 60% positively affected the VAC, but not at 70%. The VAC values of boars in this study ranged from 80.9–88.1×10^9 sperms. The highest VAC value of 88.1×10^9 was observed in boars fed the diet with a ratio of SID Met plus Cys to SID Lys of 70% and a ratio of SID Thr to SID Lys of 60%. According to Rozeboom (2000), the VAC of boar fluctuated from 10-100×10^9. Knecht et al. (2014) reported that this value for Poland Duroc boars was 76.4×10^9 sperms. Thus, our recorded results of the VAC of working boars in this study can be considered very well. The results from this study show that the ratio of SID Met plus Cys to SID Lys of 70% and the ratio of SID Thr to SID Lys of 60% in 13.5% CP diets of boars improved semen quality, particularly sperm concentration and VAC. However, no interaction effect between the two factors of ratios of SID Met plus Cys to SID Lys, and ratios of SID Thr to SID Lys was found in this study. The results from the current study suggest that the ratio of SID Met plus Cys to SID Lys of 70% and the ratio of SID Thr to SID Lys of 60% in a 13.5% CP diet are optimal for working boars.

Conclusion

In conclusion, the different ratios of SID methionine plus cystine to SID lysine and SID threonine to SID lysine in 13.5% of CP diets of working boars affected semen quality. The ratios of SID methionine plus cystine to SID lysine of 70% and SID threonine to SID lysine of 60% improved sperm concentration and VAC and were found to be optimal for working boars. However, there was no interaction effect between ratios of SID methionine plus cystine to SID lysine and SID threonine to SID lysine on boar semen quality.

Conflict of Interest

The authors declare that there is no conflict of interest associated with this study.

Authors' contributions

La Van Kinh, La Thi Thanh Huyen, and Nguyen Vu Thuy Hong Loan were responsible for the design and performance of the experiments, and for writing the manuscript. Le DucNgoan and PhungThang Long interpreted the data and edited the manuscript.

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