Effect of the valine-to-lysine ratio on the performance of sows and piglets in a hot, humid environment

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A R T I C L E   I N F O

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A B S T R A C T

To determine the effect of the valine-to-lysine (Val: Lys) ratio on the performance of sows and piglets in a hot, humid environment, eleven Large White × Landrace sows (parity 2 or 3) were selected and randomly assigned to 3 groups. The diets contained total dietary Val: Lys ratios of 0.72, 0.87, or 1.01:1. Sows were fed from d 29 prepartum to d 21 postpartum in a hot, humid environment (temperature: 22–31 °C, relative humidity: 69–96%). The results showed that dietary valine improved the average daily feed intake (ADFI) of the sows and increased serum glucose in the sows and increased serum albumin in the piglets. Additionally, dietary valine affected metabolite and metabolic hormone concentrations. The increase in the ratio of dietary Val: Lys decreased the blood urea nitrogen and increased serum glucose in the sows and increased serum albumin in the piglets. In addition, increasing dietary Val: Lys increased the serum concentration of estradiol-17β in the sows. In conclusion, in a hot, humid environment, dietary valine could improve the performance of sows and piglets by increasing colostrum lactose and serum immunoglobulin concentration in piglets and by influencing serum glucose in sows.

1. Introduction

It is important for producers to maximize the reproductive potential of sows during their lifetime. However, the fertility and prolificacy of sows often decrease under hot, humidity environments during the late summer and early autumn months (Bertoldo et al., 2012). Temperature and humidity become the determining factors under heat stress conditions (Huynh et al., 2005). This suggests that the sows often suffer from heat stress when the ambient temperature is greater than 26 °C (Gourdine et al., 2006), and high humidity can enhance the negative effects of high temperatures (Myer, 2001). Heat stress can be mitigated using different strategies, such as physical modification of the environment, genetic development of less sensitive breeds and improved nutritional management schemes (Aggarwal and Upadhyay, 2013). Dietary formula modification is often used on farms due to its convenience and cost.

Valine is the second limiting amino acid (AA) in a corn-soybean meal diet for lactating sows based on the National Research Council (NRC)-recommended requirements. During gestation and lactation, heat-stressed sows reduce their feed intake, lose body weight, have a reduced litter size, and reduce milk production, which can negatively affect piglet growth (Nardone et al., 2006; Williams et al., 2013). Valine is a potential agent for alleviating thermal stress-induced seasonal infertility of sows. First, dietary valine can increase the average daily feed intake of sows (Xu et al., 2017). In addition, valine enters the tricarboxylic acid cycle and participates in glucose metabolism in the body (Dickinson, 2000). Glucose be converted to lactose in pig mammary tissue (Ebner, 1971). However, thermal heat causes a protein metabolism imbalance (Holmes, 2010), while the nitrogen retention and nitrogen balance increase with an increase in lysine or protein (Dourmad et al., 1998). Thus, it is possible to alleviate a protein imbalance by increasing the amino acid level (e.g., valine) at high temperatures. Furthermore, newborn piglets must acquire maternal immunoglobulins (Ig) from ingested colostrum and milk for passive immune protection. Colostrum intake is the main determinant of piglet survival through its provision of energy and immune protection and has potential long-term effects on piglet growth and immunity (Devillers et al., 2011). L-arginine supplementation enhances immunity in early weaned piglets by promoting IgG and IgM concentrations in piglet serum (Tan et al., 2009). However, little is known about the effect of dietary valine supplementation on the immune status of piglets.

Therefore, valine plays a critical role in the physiological functions of nutrition through glucose metabolism and protein synthesis, and of immunity. We hypothesized that increasing the dietary Val: Lys ratios...
may alleviate the seasonal infertility caused by heat stress and improve sow reproductive performance and piglet growth performance. In this study, we analysed the performance of sows and piglets and the colostrum composition. Some relevant parameters, such as colostrum and serum immunoglobulins, metabolites and hormones, were measured to investigate the function of valine in sows and piglets.

2. Materials and methods

The present study was approved by Nanjing Agricultural University Institutional Animal Care and Committee, China.

2.1. Animals, experimental design and diets

The study was conducted at Jiangsu Zhirun Animal Husbandry Co., LTD, where located at 120.18 degrees north latitude and 31.97 degrees east longitude (from 26th of August to 14th of October 2017). A total of 11 Large White × Landrace sows (parties 2 or 3) were selected from a sow pool system. The sows were randomly assigned to 3 treatments with 3 dietary ratios of total Val:Lys, 0.72:1, 0.87:1, 1.01:1. Each group had 3 or 4 replicates with one sow per replicate. The animals were studied from d 29 prepartum to d 21 postpartum (7 weeks), and the piglets were weaned on d 21 postpartum.

The basal diet was based on corn and soybean meal, and its lysine concentration was 0.96% and Val: Lys ratio was 0.72:1 (Table 1). All diets were supplemented with 0.04% lysine to meet the NRC-recommended requirements of nutrients for lactating sows. Crystalline l-valine was added to the 3 diets, and they made isonitrogenous by the addition of appropriate amounts of crystalline l-alanine: (1) 0.22% l-alanine + 0.00% l-valine (Val: Lys=0.72:1); (2) 0.11% l-alanine + 0.15% l-valine (Val: Lys=0.87:1) and (3) 0% l-alanine + 0.29% l-valine (Val: Lys=1.01:1) (Li et al., 2010). During gestation, the sows were fed twice daily (0700 and 1600 h) with 1.5 kg of the experimental diets. During lactation, feed was provided twice daily as well. Starting from the day after farrowing, the amount of the ration fed was gradually increased by 1 kg/d until day 5 postpartum, and then the sows were allowed ad libitum to access to experimental diets and water. The unconsumed feed was weighed daily, and the average daily feed intake (ADFI) was evaluated.

2.2. Housing and management

During gestation, the sows were housed in individual stalls (2.15 m × 0.65 m) in an enclosed building. On d 107 of gestation, the sows were moved into farrowing crates (2.15 m × 1.75 m), which contained a warm area for the newborn pigs on each side.

The environment was under a day/night cycle, and the sows were exposed to ambient temperature. The temperature and relative humidity in the pigsty were recorded daily by a temperature and relative humidity recorder (RC-4HC, Jingchuang Electric Co., Ltd., Jiangsu, China) at intervals of one hour. The recorder was placed at 1.0 m from the floor and distributed in front, middle and rear of pigsty. The temperature-humidity index (THI) was calculated as follows: (1.8 °AT + 32)-(0.55 °RH/100)+(1.8 °AT+32)-58, ambient temperature (AT, °C), relative humidity (RH, %) (Wegner et al., 2016). Negative pressure ventilation and wet curtain air-conditioning systems were used to control the indoor environment. On d 1 post birth, the piglets received 200 mg of iron (iron dextran solution), their ears were notched, their teeth were cut and the tails and navel cords were docked. The male pigs were castrated on d 3.

2.3. Records and sampling

Sow backfat thickness (P2, 6 cm from the mid line at the top of the last rib) was measured at d 1 of postpartum and at weaning with an ultrasonic device using 5 MHz frequency (Renco B-07, USA). Within 24 h postpartum, the number of the total born, live born, still born, weak (weight less than 0.8 kg) and dead piglets were recorded. The piglets were cross-fostered within 3 days after birth. The piglets were weighed at d 1, d 3, d 7, d 14, and d 21 (weaning) of postpartum to calculate average daily gain (ADG).

At d 85 and d 100 of gestation and d 7 and d 21 of lactation, blood samples were collected from all sows via jugular venipuncture 2 h after the morning feeding. Piglet blood samples were collected from 8 randomly selected piglets per group at d 21 of lactation by puncture of the vena jugularis. In each litter, 2 or 3 piglets with similar weight and in similar body condition were selected. The collected blood samples were centrifuged at 3500 r/min for 10 min at 4 °C. The isolated serum samples were stored at −20 °C until analysis. The colostrum of the sows was collected at farrowing, and part of it was centrifuged at 10,000 r/min for 10 min at 4 °C to obtain the skim milk. The colostrum and skim milk samples were transferred into polypropylene tubes and frozen at −20 °C until analysis. The total milk yield was calculated as [litter weight (day 21) – litter weight (day 1)] × 4.2 (Van der Peet-Schweruing et al., 1998). On the day of weaning, the sows were moved to an environmentally regulated breeding facility for observation of estrus. The detection of the wean-to-estrus interval (WEI) was conducted by using boar stimuli twice every day.

2.4. Chemical analyses

The analysis of the crude protein (CP) content of the basal diets
(Table 1) was conducted according to the Association of Official Analytical Chemists Method (AOAC 2003). Feed samples of the basal diet were submitted to the State Key Laboratory of Crop Genetics & Germplasm Enhancement (Nanjing, Jiangsu, China) for amino acid analysis using high-performance liquid chromatography (L-8800 AA Analyzer; Hitachi, Tokyo, Japan).

The colostrum composition analysis was conducted using MilkoScan TM FT2 (Combifoss FT, FOSS Electric, Hillerød, Denmark). The blood metabolites in the serum such as triglyceride (TG); glucose (GLU); albumin (ALB); total protein (TP), and blood urea nitrogen (BUN) were also analysed. The assay was conducted with the commercial kits purchased from Nanjing Kehua Biotechnology Co., Ltd. The measurements for IgA, IgG and IgM concentrations were conducted using ELISA kits following the manufacturer’s instructions (Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China).

To determine the estradiol level in the serum, estradiol was extracted as follows. The serum samples (200 μl) were extracted by 2 ml of diethyl ether with by vortexing the tubes for 3 min. These samples were placed in a mixture of dry ice and 90% ethanol, and the ether phase was poured into another tube after the aqueous phase was frozen. The ether phase was dried down on a dry heater, rinsed with 500 μl of diethyl ether and again dried down. The dried samples were dissolved in 300 μl of PBS-based 1% BSA by vortexing for 3 min. These solutions were used for samples to measure estradiol-17β. The serum content of estradiol-17β was determined with a double-antibody RIA system with 125I-labeled radio ligands. This antiserum to estradiol-17β (GDN244) was kindly provided by Dr G.D. Niswender (Fort Collins, Co, USA). The intra- and inter-assay CVs were 10.7 and 11.3 for estradiol-17β.

2.5. Statistical analyses

Sow and litter were considered as the experimental units. Before analysis, the data was tested for normality and homoscedasticity. The statistical analyses were performed by using the SPSS 20.0 package and consisted of a one-way ANOVA performed on the 3 treatment groups and when the effect was significant, a post-hoc Tukey test. Results were expressed as means ± standard error of the mean (SEM). *P < 0.05 was considered significant.

3. Results

3.1. Temperature and humidity in the pig facility

During the experimental period, the temperature, relative humidity and maximum (Max) THI in the pig house are shown in the Fig. 1. According to the Fig. 1c, the Max THI during the experiment was close to or more than the threshold Max THI which is 74.

3.2. Feed intake and performance

As shown in Fig. 2, the ADFI in week 3 and average daily valine intake significantly increased in sows during lactation with increasing Val: Lys ratios (P < 0.05).

As shown in Table 2, the sow back fat thickness was not affected by dietary treatment. The mean-to-estrus interval was affected by the diet (P < 0.05), litter size and milk yield were not affected by the dietary treatments. According to Fig. 3, increasing the dietary Val: Lys ratios increased piglets ADG during d 7–14 postpartum (P < 0.05). The litter weight, daily litter gain, and mean piglet weight of the piglets did not differ among the groups.

3.3. Colostrum composition, immunoglobulin in serum and colostrum

As shown in Fig. 4, the fat, protein, total solid and urea nitrogen levels in the colostrum were similar among the three groups. However, the lactose level in the colostrum increased with increasing Val: Lys ratios (P < 0.05). The immunoglobulin levels in the colostrum and serum were shown in Fig. 5. There was no effect on the IgA, IgG and IgM concentrations in the colostrum after increasing the Val: Lys ratios. However, the IgM concentrations in the serum increased in the piglets (P < 0.05), and there was an increasing tendency towards a dietary effect on IgA (P = 0.09) and IgG (P = 0.06).

3.4. Serum metabolites

The metabolites in the serum of the sows were shown in Fig. 6, and there was no effect of the dietary Val: Lys ratios on the triglyceride, albumin, and total protein in the serum of the sows. However, a linear increase was observed in glucose at d 21 of lactation (P < 0.05), while a linear decrease was observed in blood urea nitrogen at d 100 of gestation (P < 0.05). As shown in Fig. 7, with the increasing Val: Lys ratios, the albumin level in the serum had a linear response in the piglets (P < 0.05).

3.5. Hormone level

According to Fig. 8, the levels of estradiol-17β were significantly increased with increasing dietary Val: Lys ratios at d 100 of gestation (P < 0.05).

4. Discussion

The objective of this study was to investigate the effect of different total Val: Lys ratios on sows and piglet performance in a hot, humid environment. Overall, the results showed trends that could be helpful in improving the performance of sows and piglets through regulating...
nutrition-related physiological functions and immune functions.

Heat stress impacts the reproductive performance of sows and during summer months. Valine may alleviate heat damage in sows by improving feed intake. High temperatures decreased sow feed intake and farrowing rate during lactation, increased the sows lactation length and prolonged the weaning-to-first-mating interval (Koketsu et al., 2017). Inadequate feed intake will lead to increased losses of back fat and body weight, and extra energy for body weight gain is needed (Eissen et al., 2000). During the experiment, the maximum THI was close to or more than the threshold THI, which was 74 (Brown-Brandl et al., 2016). High THI reduced the litter size, the number of liveborn piglets and weaned piglets, also increased stillborns (Wegner et al., 2016). It was possible to conclude that the experimental sows suffered from thermal stress. In the present study, increasing the dietary Val: Lys ratio enhanced the ADFI of the sows in late lactation, while the valine intake increased from 33.77 g/kg to 53.05 g/kg. When feed intake is insufficient amino acid supply often results in a reduction in N retention (Dourmad and Etienne, 2002). However, supplementing branched-chain amino acids (BCAAs) to reduced protein diets increases feed intake and contributes to better growth performance in piglets (Li et al., 2016). The reason why and how feed intake is affected by a valine supply is not fully understood. A possible explanation is that the hypothalamic general AA control (GAAC) or mammalian target of rapamycin (mTOR) pathway play important roles in feed intake regulation after dietary BCAA supplementation (Li et al., 2016). Research with growing pigs (Liu et al., 2015) and sows (Xu et al., 2017) also showed that the ADFI increased when the dietary valine: lysine ratio increased. In addition, it is reported that the higher feed intake, the lower the backfat loss was in sows (King and Dunkin, 1986). Sows synthesize milk protein by mobilizing their body proteins to provide free amino acids, which will result in an increase in body tissue loss (Eissen et al., 2003).

In our study, no effect of dietary treatment on sow backfat loss was detected. The result shows that increasing valine can effectively prevent the body tissue loss caused by heat stress. In contrast, in a previous study, sows had a reduced backfat loss after being fed diets with increasing valine levels, which could be explained by the fact that valine can promote protein synthesis under normal environmental temperature and humidity conditions (Xu et al., 2017). The WEI is one of the main evaluations of non-productive days and can influence reproductive performance. In our study, the WEI of sows was prolonged. The WEI may be influenced by lactation length, parity order, litter size, season, nutrition, boar exposure after weaning, genetics, diseases and management (Koketsu et al., 1997). Therefore, it is necessary to increase the number of experimental sows in our next study. In the earlier studies, Richert et al. (1996) found that increasing the valine content of a relatively low protein basal diet had no effect on sow live weight or fat loss. The same results were found by Dunabeitia et al. (2005) when sows were fed diets containing higher levels of BCAA. Taken together,

### Table 2

<table>
<thead>
<tr>
<th>Item</th>
<th>Val: Lys ratio</th>
<th>SEM^1</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.72</td>
<td>0.87</td>
<td>1.01</td>
</tr>
<tr>
<td>No. of sows</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Litter size (head)</td>
<td>2.67</td>
<td>2.25</td>
<td>2.00</td>
</tr>
<tr>
<td>Total born</td>
<td>10.00</td>
<td>11.00</td>
<td>12.75</td>
</tr>
<tr>
<td>Live born</td>
<td>9.00</td>
<td>10.75</td>
<td>12.25</td>
</tr>
<tr>
<td>Still born</td>
<td>0.33</td>
<td>0.00</td>
<td>0.25</td>
</tr>
<tr>
<td>Weak</td>
<td>0.66</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>After cross-foster</td>
<td>9.33</td>
<td>9.75</td>
<td>9.25</td>
</tr>
<tr>
<td>Weaning</td>
<td>9.00</td>
<td>9.25</td>
<td>8.75</td>
</tr>
<tr>
<td>Mortality (Foster-wean)</td>
<td>0.04</td>
<td>0.06</td>
<td>0.05</td>
</tr>
<tr>
<td>Sow back fat thickness (mm)^1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>19.02</td>
<td>20.66</td>
<td>21.33</td>
</tr>
<tr>
<td>Day 21</td>
<td>17.59</td>
<td>20.23</td>
<td>20.27</td>
</tr>
<tr>
<td>Loss Day 1–21</td>
<td>1.43</td>
<td>0.44</td>
<td>1.07</td>
</tr>
<tr>
<td>Wean-to-estrus interval (d)</td>
<td>3.60B</td>
<td>4.00B</td>
<td>4.50B</td>
</tr>
<tr>
<td>Total milk yield (kg)</td>
<td>152.39</td>
<td>175.27</td>
<td>173.35</td>
</tr>
</tbody>
</table>

Different letters indicate statistically significant differences (P < 0.05).

^1SEM, standard error of means.

^2Sow back fat thickness was measured at day 1 and day 21 of postpartum.
increasing the Val: Lys ratio improves the ADFI of sows in a hot, humid environment but has no effect on the reproductive performance of sows.

High temperatures also reduced the birth weight of the piglets or their preweaning growth rate and increased the number of stillborn piglets (Koketsu et al., 2017). Before weaning, piglets receive most of their nutrients from colostrum and milk. Sow nutrient intake, milk yield, and subsequently piglet weight can be increased through nutrient during lactation (Craig et al., 2016). In addition, milk production and protein production increase with increasing levels of valine (Rousselow and Speer, 1980), and both the weaned piglet weight and daily piglet weight gain are increased indirectly due to the increase in milk and protein production (Xu et al., 2017). In this study, milk yield had an increasing tendency, although there was no significant difference among the treatment groups. Interestingly, the concentration of lactose in the colostrum was affected by the diet and showed a linear increase. It is suggested that the colostrum of cows exposed to high ambient temperatures had lower mean percentages of total lactose (Nardone et al., 1997). The increasing concentration of lactose in the colostrum indicated that valine can alleviate the shortage of lactose caused by high ambient temperatures. During milk synthesis in mammary epithelial cells, lactose is responsible for controlling the milk volume, as lactose synthesis drives water into the mammary secretory vesicles from the mammary epithelial cell cytoplasm and then becomes a major part of milk. (Rigout et al., 2002; Liu et al., 2013). Therefore, in the present study, the improvement in the lactose concentration in the higher valine groups was a crucial factor in indirectly influencing milk yield and enhancing piglet ADG. In fact, the piglet ADG increased during d 7–14 postpartum in our study. Pierce et al. (2005) also found that the pigs receiving 295 g/kg lactose had a higher overall ADG than pigs receiving 175 g/kg lactose. Similarly, Moser et al. (2000) reported that there was an improvement in litter growth rate as the Val: Lys ratio increased from 0.89 to 1.33:1 for sows that were nursing large litters (≥ 10 pigs/litter). Strath et al. (2016) reported that there was no improvement in litter growth and the contents of milk fat, protein, and lactose. The differences between previous reports and our study might be due to differences in valine intake, pig breeds and litter sizes. In conclusion, these results suggest that increasing the Val: Lys ratio might improve the growth performance of piglets by increasing the concentration of lactose in colostrum.

Sows undergo large changes in metabolite concentrations during gestation, farrowing, and lactation, and these processes are all affected by heat stress (Lucy and Safranski, 2017). Losses in BUN increase and retention nitrogen is reduced at the high temperature, leading to a protein metabolism imbalance (Holmes, 2010). Amino acid requirements often have been based on serum or plasma BUN concentrations. When the amino acid requirement is adequate, BUN will decrease to its lowest observed level and then increase as the amino acid requirement is exceeded (Richert et al., 1997). In this study, as the Val: Lys ratio increased from 0.72:1–1.01:1, BUN tended to decrease, indicating that, based on BUN, the requirements for the dietary Val: Lys ratio of sows might be more than 1.01:1 in a hot, humid environment. In addition, O’Brien et al. (2010) and Shehab-El-Deen et al. (2010) found that heat stress can decrease the concentration of glucose in the circulating blood and significantly decrease the glucose levels in serum. In this study, glucose increased in sow serum after valine treatment in a hot, humid environment. In lactating sow mammary tissue, glucose can be converted to lactose by a biosynthesis pathway (Ebner, 1971). Therefore, the increasing level of glucose in our study indicated that metabolites of valine entered glucose metabolism and further increased the concentration of lactose in the colostrum. These results imply that increasing the Val: Lys ratio could affect serum BUN and glucose levels in sows and alter metabolite concentrations in sow serum in the hot, humid environment.

Most components of the immune system of piglets are present at birth but are functionally undeveloped, and several weeks of life are necessary before the immune system becomes fully developed (Rooke and Bland, 2002). Therefore, newborn piglets need immediate immune protection via large amounts of immunoglobins from porcine colostrum.

![Image](https://example.com/image.png)
Four protein fractions (total protein, globulin, IgG and albumin) were analysed in piglets serum, and the concentration of globulin decreased in the serum of piglets from heat-stressed sows (Machadoneto et al., 1987). The present study showed that dietary valine increased serum albumin concentration in the piglets, probably because valine promotes the absorption and metabolism of protein in piglets in a hot, humid environment. We found no effect of valine on colostrum IgA, IgG, and IgM concentrations. However, the serum IgM of piglets was higher when the Val: Lys ratio increased at weaning. A newborn piglet is reliant on IgG absorbed from colostrum for humoral immune protection until its own immune system has sufficiently matured to respond (to produce antibodies against) to foreign antigens. IgM, rather than IgA, was the dominant type of immunoglobulin in the gut of the piglets after 10 d of age (Butler et al., 1981). These results show that valine did not change colostrum immunoglobulin level but did affect the serum albumin and IgM levels. Dietary valine may affect colostrum intake and have potential long-term effects on piglet immunity.

Fig. 4. Effect of dietary valine-to-lysine ratio (Val:Lys) on colostrum composition (n = 3 or 4, mean ± SEM). Sows were fed three diets with different Val:Lys ratios of 0.72, 0.87, or 1.01:1. Different letters indicate statistically significant differences (P < 0.05).
All estrogens are involved in the growth and development of the mammary glands before parturition and synergism with prolactin to promote lactation (Kensinger et al., 1986). Estrogen is essential for the initiation and maintenance of pregnancy in mammals (Matsuura et al., 2004). In this study, the content of estradiol-17β increased in the valine treatment groups at d 100 of gestation. While it is known that elevated levels of estrogen are correlated with the increase in the rate of fetal-placental development, as well as fetal growth and litter size (Edgerton et al., 1971). Foisnet et al. (2008) suggested that colostrum production in primiparous sows was positively correlated with prolactin concentrations. Therefore, we deduced that valine improved the estradiol-17β concentration in sow serum before parturition, resulting in
improved colostrum yield after parturition.

5. Conclusion

Dietary valine could enhance the ADPI of sows in hot, humid environments. Increasing the Val:Lys ratio improves the growth performance of piglets by altering serum metabolites in sows, the lactose concentration in colostrum and serum immunoglobins in piglets. These findings suggest that in hot, humid environments, it may be useful to supplement the appropriate amount of valine to alleviate heat stress-induced seasonal infertility in sows.

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References


