Effects of dietary energy and crude protein levels on growth performance, blood profiles, and nutrient digestibility in weaning pigs

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Objective: This experiment was conducted to investigate the effect of reducing dietary metabolic energy (ME) and crude protein (CP) levels on growth performance, blood profiles, and nutrient digestibility in weaning pigs.

Methods: A total of 240 crossbred pigs (Duroc×[Landrace×Yorkshire]) with an average body weight of 8.67±1.13 kg were used for a 6-week feeding trial. Experimental pigs were allotted to a 2×3 factorial arrangement using a randomized complete block design. The first factor was two levels of dietary ME density (low ME level, 13.40 MJ/kg or high ME level, 13.82 MJ/kg) and the second factor was three dietary CP levels based on subdivision of early and late weaning phases (low CP level, 19.7%/16.9%; middle CP level, 21.7%/18.9%; or high CP level, 23.7%/20.9%).

Results: Over the entire experimental period, there were no significant difference in body weight among groups, but a decrease in diet energy level was associated with an increase in average daily feed intake (p = 0.02) and decrease in gain-feed ratio (G:F) ratio (p<0.01). Decreased CP levels in the diet were associated with a linear increase in average daily gain (p<0.05) and quadratic increase in G:F ratio (p<0.05). In the early weaning period, blood urea nitrogen concentration tended to increase when ME in the diet decreased and decrease when CP level in the diet decreased (p = 0.09, p<0.01, respectively). Total protein concentration tended to increase when CP level was reduced (p = 0.08). In the late weaning period, blood urea nitrogen concentration decreased linearly as CP level decreased (p<0.01). The CP and crude fat digestibility decreased when ME was decreased by 0.42 MJ/kg (p = 0.05, p = 0.01, respectively). The CP digestibility increased linearly as CP level decreased (p = 0.01).

Conclusions: A weaning pig diet containing high ME level (13.82 MJ/kg) and low CP level (19.7%/16.9%) can improve pig growth performance and nutrient digestibility.

Keywords: Energy; Crude Protein; Growth Performance; Blood Profiles; Nutrient Digestibility; Weaning Pigs

INTRODUCTION

Nutritional concentration of feed is very important in the swine industry as it affects both growth performance and profitability. Lower nutritional concentrations can decrease the growth rate of pigs, while higher concentrations can have a negative effect on the environment and increase production costs [1-3]. After weaning, piglets experience nutritional, physiological, environmental, and social stresses [4], which are linked to low feed intake, poor growth, and incidence of diarrhea [5]. In general, a high feed intake could increase the health of weaning pigs and improve growth performance [6,7].

Energy concentration in feed is an important factor of feed intake, as a high energy level can decrease feed intake, while a lower energy level can decrease the deposition of protein...
Deposition and intake of protein and amino acids is related to energy concentration [9]. However, the optimal energy requirements of weaning pigs are difficult to determine accurately [10], because different average daily feed intake (ADFI) and weaning stress. Metabolizable energy (ME) requirements in editions of NRC differ, with an increased in ME from 1998 to 2012 [9,11]. However, other studies were reported that ME levels in diets did not affect growth performance in weaning pigs [12,13]. In general, fat and oil are important energy sources in pig diets [14], but weaning pigs do not utilize fat and oil very efficiently [8].

In addition, recommended total N levels have decreased largely over the years in editions of NRC (1998 to 2012). However, other studies reported that the pigs fed low crude protein (CP) diet, showed better fecal consistency score, improved enteric health and growth performance [15–18]. Decreasing the protein level in feed can decrease the risk of post-weaning diarrhea, as undigested proteins can be utilized by pathogenic bacteria such as enterotoxigenic *Escherichia coli* [19]. Moreover, Jensen et al. [20] reported that from birth to 56 days, the digestive enzymes of piglets have low activity for digestion of energy and protein. That means there is much more surplus protein and energy that cannot be digested in diets for piglets than requirement. Other study reported that digestibility of CP at the terminal ileum was from 60% to 80% in weaning pigs [21]. Hence, optimizing energy and protein levels in the weaning pig diet is therefore important.

For these many reasons, this experiment was conducted to investigate the effect of reducing dietary ME and CP levels on growth performance, blood profiles, and nutrient digestibility in weaning pigs.

**MATERIALS AND METHODS**

**Experimental animals and management**

All experimental procedures involving animals were conducted in accordance with the Animal Experimental Guidelines provided by the Seoul National University Institutional Animal Care and Use Committee (SNUIAUC; SNU-160819-9). A total of 240 crossbred pigs (*Duroc*×*Landrace*×*Yorkshire*) with an average body weight (BW) of 8.67±1.13 kg were used for a 6-week feeding trial. Pigs were reared at Seoul National University experimental farm. Weaning pigs were allotted to one of six treatments with five replications with 8 pigs per pen. Four male pigs and four female pigs were assigned to each pen of a weaning facility based on BW, and pigs in each pen were maintained until the end of the experiment. Pigs were randomly allotted to their respective treatments by an experimental animal allotment program [22]. Pigs were reared in weaning (1.54×1.96 m) facilities for 6 weeks. Feed and water were provided *ad libitum* during the entire experimental period by a 4-hole stainless feeder and two nipples installed in each pen.

The BW and feed intake were recorded at 0, 3, and 6 weeks to calculate average daily gain (ADG), ADFI, and gain-feed ratio (G:F ratio).

**Experimental design and diet**

Experimental pigs were allotted to a 2×3 factorial arrangement using a randomized complete block design. The first factor was two levels of dietary ME density (low ME level, 13.40 MJ/kg or high ME level, 13.82 MJ/kg) and the second factor was three dietary CP levels based on subdivision of weaning phases (low CP level, middle CP level, or high CP level), early weaning phase CP percentages of 23.7%, 21.7%, and 19.7% and late weaning phase percentages of 20.9%, 18.9%, and 16.9%.

Experimental diets were formulated for two phases, namely early weaning phase (0 to 3 weeks) and late weaning phase (4 to 6 weeks). Formulee and chemical compositions of the experimental diet are provided in Table 1, 2.

**Blood analysis**

Blood samples were taken from the jugular vein of six randomly selected pigs in each treatment when BW were recorded to measure blood urea nitrogen (BUN), glucose, albumin, and total protein after a 3 hours fasting. All blood samples were collected in serum tubes (SST II Advance, BD Vacutainer, Becton Dickinson, Plymouth, UK). Collected blood samples were centrifuged for 15 min at 3,000 rpm at 4°C (5810R, Eppendorf, Hamburg, Germany). Serum was carefully transferred into 1.5 mL plastic tubes and stored at –20°C until analysis. Total BUN (kinetic UV assay, Roche, Mannheim, Germany), albumin (Cobas 8000, Roche, Germany), and glucose (enzymatic kinetic assay, Roche, Germany) concentrations were analyzed using a blood analyzer. Total protein concentration was measured by a kinetic colorimetry assay using a blood analyzer (Modular Analytics, PE, Roche, Germany).

**Digestibility trial**

A digestibility trial was conducted using a completely randomized design with three replicates. A total of 18 weaning barrows (*Yorkshire*×*Landrace*×*Duroc*) with an average BW of 13.94±1.64 kg were individually allotted to an individual metabolic crate (0.4×0.8×0.9 m). A 5-day adaptation period was followed by 5 days of data collection.

The daily feed allowance required to provide 2.6 times the maintenance requirements for ME [106 kcal ME/kg (0.44 MJ/kg) of BW<sup>0.72</sup>] was calculated [9,23]. Experimental diets were provided during the late weaning phase at 07:00 and 19:00. At the first and last feeding, 1% ferric oxide and chromium oxide were added to the experimental diet. Water was provided *ad libitum*. Excreta and urine were collected daily and preserved at –20°C until later analysis. When collection was finished, the excreta were dried in an air-forced drying oven at 60°C for 96 h, and ground into 5 mm particles in a Wiley mill for
Statistical analyses
All collected data were analyzed using least squares mean comparisons and evaluated using the general linear model procedure implemented in the statistical software package SAS (SAS Institute Inc., Cary, NC, USA). Every pen was considered one unit in the feeding trial, and individual pigs were the experimental units in the digestibility trial and when assessing blood profiles. Orthogonal polynomial contrasts were used to detect linear and quadratic responses to CP levels when the significance of CP was evaluated. Differences were declared significant at p<0.05 or highly significant at p<0.01, while p≥0.05 and p<0.10 was considered to indicate a trend in the data.

RESULTS AND DISCUSSION

Growth performance
The effect of dietary energy and CP levels on growth performance are presented in Table 3. In the early weaning period, ADG (linear, p<0.01) and ADFI (linear, p<0.05) increased when diet CP level decreased, and ADFI increased when dietary ME decreased (p = 0.04). In the late weaning period, ADFI (p = 0.03) increased and the G:F ratio (p<0.01) decreased when ME level was decreased by 0.42 MJ/kg. There were no significant differences in BW over the experimental period, but decreased ME levels were associated with an increase in ADFI (p = 0.02) and a decrease in G:F ratio (p<0.01). A decrease in CP level resulted in a linear increase in ADG (p<0.05) and a
quadratic increase in G:F ratio (p<0.05). Therefore, decreasing the ME level by 0.42 MJ/kg did not affect growth performance, and reducing the CP level by 4% improved ADG and the G:F ratio.

The effect of dietary energy density on weaning pig performance is a topic of debate among researchers. Tokach et al [24] and Hastad et al [25] reported that decreasing dietary energy concentration had no effect on growth. Ribeiro et al [12] reported that reducing dietary ME from 3,700 kcal/kg to 3,250 kcal/kg (15.49 MJ/kg to 13.61 MJ/kg) had no influence on growth performance. Beaulieu et al [10] reported that decreasing the digestible energy concentration from 3,650 kcal/kg to 3,350 kcal/kg (15.28 MJ/kg to 14.03 MJ/kg) in the weaning pig diet increased BW, ADG, and ADFI linearly, while the G:F ratio decreased linearly.

In this study, reducing the soybean meal (SBM) level to decrease CP level improved growth performance over the whole experiment period. A similar result for ADG and G:F ratio was reported by Li et al [26,27]. Le Bellego and Noblet [28] described a reduction in N excretion and an increase in feed intake when CP level was reduced from 22.4% to 20.4%. In other studies, a lower CP level had no negative effects on growth performance [29,30]. Hermes et al [29] reported that decreasing the CP level from 20% to 16% in the weaning pig (9 to 18 kg) diet had no effect on growth performance after weaning for 3 weeks. Nyachoti et al [30] reported a significant reduction in ADG and ADFI for diets containing a CP level of 19% or less.

Table 2. Formulae and chemical compositions of diets in late weaning pigs

| Ingredient (%) | Low energy \(^1\) | Middle CP \(^2\) | High CP \(^3\) | Low energy \(^1\) | Middle CP \(^2\) | High CP \(^3\) |
|----------------|-----------------|----------------|----------------|-----------------|----------------|----------------|
| Corn           | 43.00           | 38.28          | 33.49          | 40.91           | 36.07          | 31.19          |
| Soybean meal   | 21.09           | 25.48          | 29.86          | 21.66           | 25.94          | 30.22          |
| Barley         | 25.65           | 25.50          | 25.44          | 25.04           | 25.10          | 25.17          |
| Whey powder    | 3.00            | 3.00           | 3.00           | 3.00            | 3.00           | 3.00           |
| Lactose        | 3.00            | 3.00           | 3.00           | 3.00            | 3.00           | 3.00           |
| Soypeptide     | 0.81            | 1.81           | 2.81           | 0.75            | 1.80           | 2.83           |
| Soy-oil        | 0.26            | 0.13           | 0.00           | 2.45            | 2.30           | 2.17           |
| Mono-dicalcium phosphate | 1.22 | 1.12 | 1.02          | 1.25            | 1.13           | 1.03           |
| Limestone      | 0.82            | 0.84           | 0.85           | 0.81            | 0.83           | 0.85           |
| L-lysine-HCl, 78% | 0.38  | 0.19           | 0.00           | 0.37            | 0.18           | 0.00           |
| DL-methionine, 80% | 0.07  | 0.04           | 0.00           | 0.06            | 0.04           | 0.01           |
| L-threonine, 99% | 0.17  | 0.08           | 0.00           | 0.17            | 0.08           | 0.00           |
| Vit. Mix \(^3\) | 0.10           | 0.10           | 0.10           | 0.10            | 0.10           | 0.10           |
| Min. Mix \(^4\) | 0.10           | 0.10           | 0.10           | 0.10            | 0.10           | 0.10           |
| Salt           | 0.30            | 0.30           | 0.30           | 0.30            | 0.30           | 0.30           |
| ZnO            | 0.03            | 0.03           | 0.03           | 0.03            | 0.03           | 0.03           |

Chemical composition (calculated value)

| Metabolic energy (MJ/kg) | 13.40 | 13.40 | 13.40 | 13.82 | 13.82 | 13.82 |
| Crude protein (%)        | 16.90 | 18.90 | 20.90 | 16.90 | 18.90 | 20.90 |
| Total lysine (%)         | 1.15  | 1.15  | 1.15  | 1.15  | 1.15  | 1.15  |
| Total methionine (%)     | 0.31  | 0.31  | 0.31  | 0.30  | 0.31  | 0.31  |
| Total threonine (%)      | 0.80  | 0.80  | 0.80  | 0.80  | 0.80  | 0.80  |
| Calcium (%)              | 0.70  | 0.70  | 0.70  | 0.70  | 0.70  | 0.70  |
| Total phosphorus (%)     | 0.60  | 0.60  | 0.60  | 0.60  | 0.60  | 0.60  |

Chemical composition (analyzed value)

| Crude protein (%)        | 18.64 | 19.65 | 22.63 | 18.26 | 18.61 | 20.47 |
| Crude fat (%)            | 1.95  | 1.56  | 1.63  | 4.26  | 5.34  | 4.04  |
| Crude ash (%)            | 4.91  | 5.23  | 5.67  | 5.01  | 5.86  | 5.84  |

CP; crude protein; ME, metabolic energy.

1) Low energy level, ME (13.40 MJ/kg); high energy level, ME (13.82 MJ/kg).
2) Low CP level: early weaning phase CP percentages of 19.7% and late weaning phase percentages of 16.9%; middle CP level: early weaning phase CP percentages of 21.7%, and late weaning phase percentages of 18.9%; high CP level: early weaning phase CP percentages of 23.7%, and late weaning phase percentages of 20.9%.
3) Provided per kg of diet. Vitamins per kg of complete diets: vitamin A, 8,000 IU; vitamin D\(_3\), 1,800 IU; vitamin E, 60 IU; vitamin K\(_3\), 2 mg; thiamine, 2.00 mg; riboflavin, 7.0 mg; pantothenic acid, 25 mg; niacin, 27 mg; pyridoxine, 3 mg; d-biotin, 0.2 mg; folic acid, 1 mg; vitamin B\(_12\), 0.03 mg.
4) Provided per kg of diet. Minerals per kg of complete diet: Se, 0.3 mg; I, 1 mg; Mn, 51.6 mg; Cu, 105 mg; Fe, 150 mg; Zn, 72 mg; Co, 0.5 mg.
Table 3. Effects of dietary energy and crude protein levels on growth performance in weaning pigs

| Items                        | Low energy<sup>1</sup> | High energy<sup>1</sup> | SEM | p-value |
|------------------------------|-------------------------|-------------------------|-----|---------|
|                              | Low CP (%)<sup>2</sup>  | Middle CP (%)<sup>2</sup> | High CP (%)<sup>2</sup> | Low CP (%)<sup>2</sup>  | Middle CP (%)<sup>2</sup> | High CP (%)<sup>2</sup> | ME | CP | M×C |
| Body weight (kg)             |                         |                         |     |         |
| Initial                      | 8.66                    | 8.66                    | 8.67 | 8.67    | 8.67 | 0.206 | 0.99 | 1.00 | 1.00 |
| 3 weeks                      | 13.59                   | 12.23                   | 12.88 | 13.33   | 12.23 | 12.34 | 0.280 | 0.65  | 0.22  | 0.93 |
| 6 weeks                      | 24.28                   | 22.24                   | 23.45 | 24.52   | 22.54 | 22.94 | 0.406 | 0.99  | 0.16  | 0.90 |
| ADG (g)                      |                         |                         |     |         |
| 0-3 weeks<sup>3</sup>        | 234<sup>a</sup>         | 170<sup>c</sup>         | 201<sup>b</sup> | 222<sup>a</sup> | 170<sup>c</sup> | 175<sup>c</sup> | 7.6 | <0.01 | 0.72 |
| 4-6 weeks                    | 509                     | 477                     | 505  | 533     | 491   | 505   | 7.8  | 0.41  | 0.17  | 0.84 |
| 0-6 weeks<sup>3</sup>        | 372                     | 323                     | 352  | 377     | 330   | 340   | 6.5  | 0.99  | 0.01  | 0.76 |
| ADFI (g)                     |                         |                         |     |         |
| 0-3 weeks<sup>3</sup>        | 433<sup>a</sup>         | 362<sup>b</sup>         | 388<sup>b</sup> | 391<sup>a</sup> | 335<sup>b</sup> | 325<sup>b</sup> | 10.6 | 0.04  | 0.01  | 0.84 |
| 4-6 weeks                    | 986                     | 970                     | 1,030 | 924     | 925   | 915   | 16.7 | 0.03  | 0.82  | 0.66 |
| 0-6 weeks                    | 709                     | 666                     | 704  | 658     | 630   | 620   | 11.9 | 0.02  | 0.44  | 0.67 |
| G:F ratio                    |                         |                         |     |         |
| 0-3 weeks                    | 0.542                   | 0.462                   | 0.528 | 0.569   | 0.508 | 0.548 | 0.0148 | 0.31  | 0.15  | 0.93 |
| 4-6 weeks                    | 0.518<sup>bc</sup>      | 0.498<sup>b</sup>       | 0.490<sup>b</sup> | 0.576<sup>b</sup> | 0.532<sup>ab</sup> | 0.554<sup>b</sup> | <0.008 | <0.01 | 0.17  | 0.65 |
| 0-6 weeks<sup>3</sup>        | 0.524<sup>bc</sup>      | 0.488<sup>b</sup>       | 0.502<sup>b</sup> | 0.575<sup>b</sup> | 0.526<sup>b</sup> | 0.549<sup>bc</sup> | 0.0080 | <0.01 | 0.04  | 0.91 |
| CP, crude protein; SEM, standard error of the mean; ME, metabolic energy; ADFI, average daily gain; G:F, gain-feed ratio.
| Low energy level, ME (13.40 MJ/kg); high energy level, ME (13.82 MJ/kg).
| Low CP level: early weaning phase CP percentages of 19.7% and late weaning phase percentages of 16.9%; middle CP level: early weaning phase CP percentages of 21.7%, and late weaning phase percentages of 18.9%; high CP level: early weaning phase CP percentages of 23.7%, and late weaning phase percentages of 20.9%.
| Linear response (p < 0.05) to dietary CP levels when a significant CP effect was detected.
| Quadratic response (p < 0.05) to dietary CP level when a significant CP effect was detected.
| Means with different superscripts in the same row differ significantly (p < 0.05).

Blood profiles

Blood profiles during the feeding trial are presented in Table 4. The BUN concentration during the early weaning period tended to increase when ME decreased and decrease when CP levels decreased (p = 0.09, p < 0.01, respectively). Total protein concentration tended to increase when CP levels decreased.

Table 4. Effects of dietary energy and crude protein levels on blood profiles in weaning pigs

| Items                        | Low energy<sup>1</sup> | High energy<sup>1</sup> | SEM | p-value |
|------------------------------|-------------------------|-------------------------|-----|---------|
|                              | Low CP (%)<sup>2</sup>  | Middle CP (%)<sup>2</sup> | High CP (%)<sup>2</sup> | Low CP (%)<sup>2</sup>  | Middle CP (%)<sup>2</sup> | High CP (%)<sup>2</sup> | ME | CP | M×C |
| Albumin (g/L)                |                         |                         |     |         |
| Initial                      | 39.83                   | -                       | -   | -       | -     | -     | -   | -   | -   |
| 3 weeks                      | 30.40                   | 31.80                   | 30.00 | 30.20   | 31.00 | 30.20 | 0.543 | 0.82  | 0.63  | 0.94 |
| 6 weeks                      | 31.40<sup>b</sup>       | 30.00<sup>b</sup>       | 34.20<sup>b</sup> | 30.40<sup>b</sup> | 32.60<sup>ab</sup> | 25.80<sup>b</sup> | 0.796 | 0.12  | 0.94  | 0.01 |
| BUN (mmol/L)                 |                         |                         |     |         |
| Initial                      | 3.14                    | -                       | -   | -       | -     | -     | -   | -   | -   |
| 3 weeks<sup>3</sup>          | 3.93<sup>c</sup>        | 4.92<sup>a</sup>        | 5.51<sup>a</sup> | 2.37<sup>c</sup> | 4.89<sup>b</sup> | 4.85<sup>c</sup> | 0.271 | 0.09  | <0.01 | 0.35 |
| 6 weeks<sup>3</sup>          | 2.75<sup>c</sup>        | 3.81<sup>a</sup>        | 5.47<sup>a</sup> | 2.11<sup>c</sup> | 3.46<sup>b</sup> | 4.82<sup>bc</sup> | 0.266 | 0.13  | <0.01 | 0.92 |
| Glucose (mmol/L)             |                         |                         |     |         |
| Initial                      | 6.67                    | -                       | -   | -       | -     | -     | -   | -   | -   |
| 3 weeks                      | 5.11                    | 5.42                    | 4.83  | 5.29    | 5.11  | 5.18  | 0.112 | 0.74  | 0.65  | 0.49 |
| 6 weeks                      | 5.51                    | 5.24                    | 5.59  | 5.39    | 5.79  | 5.25  | 0.094 | 0.86  | 0.92  | 0.16 |
| Total protein (g/L)          |                         |                         |     |         |
| Initial                      | 53.10                   | -                       | -   | -       | -     | -     | -   | -   | -   |
| 3 weeks                      | 51.84                   | 51.61                   | 49.02 | 50.81   | 51.78 | 46.21 | 0.758 | 0.21  | 0.08  | 0.60 |
| 6 weeks                      | 56.38                   | 54.63                   | 59.39 | 54.61   | 55.24 | 54.63 | 0.649 | 0.12  | 0.38  | 0.23 |
| CP, crude protein; SEM, standard error of the mean; ME, metabolic energy; BUN, blood urea nitrogen.
| Low energy level, ME (13.40 MJ/kg); high energy level, ME (13.82 MJ/kg).
| Low CP level: early weaning phase CP percentages of 19.7% and late weaning phase percentages of 16.9%; middle CP level: early weaning phase CP percentages of 21.7%, and late weaning phase percentages of 18.9%; high CP level: early weaning phase CP percentages of 23.7%, and late weaning phase percentages of 20.9%.
| Linear response (p < 0.05) to dietary CP levels when a significant CP effect was detected.
| Means with different superscripts in the same row differ significantly (p < 0.05).
Fat digestibility in the diet. The ability to digest fat is limited in pigs for the first 35 feed intakes because of variations in the energy density of the diet [10]. Nam and Aherne [36] and the NRC [9] report that total energy intake could be similar despite different in the diet [10]. Energy digestibility are affected by fat digestibility decreased by 3% to 4% when ME in the diet was reduced to 0.42 MJ/kg. Energy digestibility are affected by the type and proportion of ingredients as well as energy source in the diet [10]. This experiment decreased dietary ME concentration by 0.42 MJ/kg (13.82 MJ/kg to 13.40 MJ/kg) had no effects on growth performance, but decreased fat and protein digestibility. In addition, reducing CP level by 4% improved growth performance and the digestibility of proteins, and decreased the BUN concentration [40] that decreasing energy density could reduce protein and lipid gain.

The main determinants of protein digestibility are the level and balance of essential amino acids and the animals requirement [41]. In this study, lysine %, methionine %, and threonine % were the same for different CP treatments. In other words, although CP levels in the diet were reduced, but the limiting amino acid content was not. In this study, CP digestibility increased when dietary CP levels were decreased. Reducing the CP level without changing the levels of limiting amino acids can result in a more optimal amino acid composition than a high CP level.

**CONCLUSION**

Decreasing the energy content of the weaning pig diet by 0.42 MJ/kg (13.82 MJ/kg to 13.40 MJ/kg) had no effects on growth performance, but decreased fat and protein digestibility. In addition, reducing CP level by 4% improved growth performance and the digestibility of proteins, and decreased the BUN level.

A weaning pig diet containing high ME level (13.82 MJ/kg) and low CP level (19.7%/16.9%) can improve pig growth performance and nutrient digestibility.

**CONFLICT OF INTEREST**

We certify that there is no conflict of interest with any financial this experiment results obtained for the late weaning period are in agreement with those of Tokach et al [24] and Beaulieu et al [10].
organization regarding the material discussed in the manuscript.

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