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Basic nutritional investigation

Leucine improves growth performance of intrauterine growth retardation piglets by modifying gene and protein expression related to protein synthesis

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ABSTRACT

Objective: Leucine has been reported to alter the gene expression of proteins, the activation of signaling components, and the fractional rates of protein synthesis in multiple organs of piglets. The aim of this study was to investigate the effects of leucine on molecular mechanisms regulating protein synthesis and degradation in skeletal muscle and determine how these adaptations affect body weight gain in intrauterine growth retardation (IUGR) pigs.

Methods: Thirty-two weaned piglets were randomly assigned to the following four experimental groups (n = 8 per group): normal birth weight, normal birth weight supplemented with leucine, IUGR, and IUGR supplemented with leucine. Piglets were fed from 14 to 35 d of age. Growth performance, major serum biochemical parameters, and enzyme activities were evaluated. The messenger RNA expression of muscle mammalian target of rapamycin (mTOR), muscle atrophy F-box (MAFbx), and muscle-specific ring finger-1 were investigated. Additionally, total and phosphorylated levels of mTOR and ribosomal protein S6 kinase 1 were measured in longissimus muscle.

Results: Average daily weight gain and average daily feed intake were increased by leucine in IUGR pigs. At the end of the experiment, IUGR pigs had lower liver and kidney weight compared with the normal piglets. However, IUGR supplemented with leucine decreased serum concentration of urea. Leucine supplementation affected the concentrations of isoleucine, valine, lysine, and phenylalanine in serum. There was no significant difference in the expression of mTOR and muscle-specific ring finger-1 in IUGR piglets, whereas muscle atrophy F-box was reduced only by IUGR dependent of leucine. Compared with the IC group, dietary supplementation with 0.35% L-leucine increased the phosphorylated levels of mTOR and ribosomal S6 kinase 1 in IUGR piglets.

Conclusions: The present study identified a major role for leucine in the activation of the mTOR signaling pathway and reducing muscle atrophy in IUGR piglets, which contributed significantly to differences in body weight gain.

Introduction

The growth performance of pigs is generally related to birth weight [1–3]. Pigs with lower birth weight exhibit a slower rate of growth after weaning and require a greater number of days to reach the common slaughter weight [4]. When the birth weight is <10th percentile, the piglet is regarded as intrauterine growth restriction/retardation (IUGR) [5–7], which is considered a major health problem and an important cause of neonatal morbidity and mortality. To maximize the growth of these IUGR pigs, renewed focus on the earlier stages of growth is needed.

Skeletal muscle is a critical organ in maintaining normal physiological metabolism for animals, which represents 40% to 50% of the body composition of newborn piglets [5]. During the neonatal period, the gain in protein mass of skeletal muscle is

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more rapid than other tissues of the body [6]. The efficiency of protein synthesis in skeletal muscle of the newborn is mediated partly via the mammalian target of rapamycin (mTOR) signaling pathway integrating several different upstream and downstream signals that regulate mRNA translation [7]. The ubiquitin-proteasome pathway (UPP) is the primary cytosolic protein degradation pathway [8] that is activated during sepsis [9]. The UPP involves three types of ubiquitin enzymes: ubiquitin-activating (E1), ubiquitin-conjugating (E2), and ubiquitin-ligase (E3) enzymes. In skeletal muscle cells, the E3 enzyme is coordinated by two muscle-specific enzymes, muscle-specific ring finger-1 (MuRF-1) and muscle atrophy F-box (MAFbx/atrogin-1) [10].

Evidence has shown that amino acids play an important role in regulating protein synthesis and animal growth [11]. Among amino acids, leucine plays a major role in stimulating protein synthesis and in inhibiting protein degradation in skeletal muscle [12]. Leucine also has been validated to enhance the availability of specific eukaryotic initiation factors, as well as to augment the activity of proteins involved in messenger RNA (mRNA) translation [13].

Many researchers have demonstrated that parenteral infusion of a balanced amino acid mixture or leucine alone stimulates muscle protein synthesis in neonates by enhancing mTOR activation and its downstream effectors [14–16]. However, it is still not known whether leucine supplementation would have beneficial effects on the development of skeletal muscle in IUGR neonatal piglets. Therefore, the aim of this study was to evaluate the growth performance, organ development, and expression of these muscle growth-related genes in response to dietary leucine status, which also could provide fundamental information for the understanding of the growth in low birth-weight infants.

Materials and methods

Animals and groups

All experimental procedures were conducted in conformity with the Guidelines on Ethical Treatment of Experimental Animals (2006) No. 398 set by the Ministry of Science and Technology in China and the Regulation regarding the Management and Treatment of Experimental Animals (2008) No. 45 set by the Jiangsu Provincial People's Government. The experimental protocol was specifically approved by the Animal Ethics Committee of Nanjing Agricultural University. At the time of parturition (day 114 [SD 1] of gestation), 16 pairs of IUGR and normal birth-weight piglets (Duroc × [Landrace × Yorkshire]) from 16 sows were selected. In each litter, one normal piglet and one IUGR littermate were chosen according to a previous study [17]. The average birth weights of normal birth-weight and IUGR piglets used in the present study were 1.52 (SD 0.06) and 0.87 (SD 0.04) kg, respectively.

The experiment was designed as a 2 × 2 factorial trial. Treatments were litter composition (I = IUGR pigs and N = normal birth-weight pigs) and provision of L-leucine supplement (98%; Sigma-Aldrich Co., St. Louis, MO, USA) from day 14 to 35 (yes or no). All piglets were weaned at 14 d of age and randomly allocated to one of two dietary treatments with eight replicates of one piglet (four males and four females) per treatment:

1. Normal birth weight (NC) group (n = 8): normal piglets receiving the control diet (1.45% L-leucine);
2. Normal birth weight supplemented with leucine (NL) group (n = 8): normal piglets receiving the control diet (1.45% L-leucine) and supplementation with 0.35% L-leucine;
3. IUGR (IC) group (n = 8): IUGR piglets receiving the control diet (1.45% L-leucine); and
4. IUGR supplemented with leucine (IL) group (n = 8): IUGR piglets receiving the control diet (1.45% L-leucine) and supplementation with 0.35% L-leucine.

The compositions of the diets are presented in Table 1.

Piglets were housed individually in plastic floored pens (1 m × 0.6 m) at an ambient temperature of 28°C in an environmentally controlled room and had free access to water. At 35 d of age, piglets were weighed after feed deprivation for 12 h to calculate average daily weight gain (ADG), and feed consumption was recorded by replicate to calculate average daily feed intake (ADFI) and feed conversion ratio.

Table 1
Composition of basal diets (as-fed basis)

Ingredients (g/100 g)	NC/IC	NL/IL
Corn	40	40
Rice, broken	15	15
Soybean meal, fermented	10	10
Soybean meal, dehulled	6	6
Spray-dried animal plasma	5	5
Whey powder	7	7
Fish meal	4	4
Sugar	4.5	4.5
Glucose	3	3
Soybean oil	1.5	1.5
L-leucine	–	0.35
L-lysine-HCl (98%)	0.3	0.3
L-methionine	0.15	0.15
L-threonine	0.2	0.2
L-tryptophan	0.05	0.05
L-isoleucine	0.05	0.05
L-valine	0.05	0.05
Salt	0.3	0.3
Limestone	1.1	1.1
$CaHPO_4$	0.8	0.8
Vitamin mixture*	0.2	0.2
Mineral mixture†	0.8	0.8
Total	100	100
Nutrient composition (%)		
Crude protein	20.2	20.2
Digestible energy (Mcal/kg)	3.4	3.4
Total calcium	0.85	0.85
Total phosphorus	0.7	0.7
Digestible lysine	1.45	1.45
Digestible methionine + cystine	0.79	0.79
Digestible threonine	0.81	0.81
Digestible tryptophan	0.23	0.23
Digestible isoleucine	0.74	0.74
Digestible leucine	1.45	1.8
Digestible valine	0.89	0.89

IC, IUGR piglets; IL, IUGR piglets supplemented with L-leucine; NC, normal weight piglets; IUGR, intrauterine growth retardation piglets; NL, normal weight piglets supplemented with L-leucine

* The vitamin mixture supplied the following per kg complete diet: vitamin A 15 000 IU; vitamin D_3, 3000 IU; vitamin E, 150 mg; vitamin K_3, 3 mg; vitamin B_1, 3 mg; vitamin B_2, 6 mg; vitamin B_6, 5 mg; vitamin B_{12}, 0.03 mg; niacin, 45 mg; vitamin C, 250 mg; calcium pantothenate, 9 mg; folic acid, 1 mg; biotin, 0.3 mg; choline chloride, 500 mg.

† The mineral mixture supplied the following per kg complete diet: iron, 170 mg; copper, 150 mg; iodine, 0.90 mg; selenium,0.2 mg; zinc, 150 mg; magnesium, 68 mg; manganese, 80 mg; and cobalt, 0.3 mg.

Sample collection

At 35 d of age, the piglets were sacrificed by intramuscular injection of sodium pentobarbital (50 mg/kg body weight) at 12 h after the last meal. Heart, liver, spleen, kidney, and pancreas were separated and weighed to calculate the relative organ weight. After the feeding trial, blood samples were collected for the determination of serum parameters. The sample from longissimus doris muscle was excised within 5 min postmortem and snap-frozen in liquid nitrogen and thereafter stored at −80°C for the determination of total protein, RNA, and gene expression. Homogenates of the muscles were analyzed for activities of lactate dehydrogenase (LDH) and creatine kinase (CK) according to the previously described methods [18,19].

Serum profile

The concentrations of total protein and urine nitrogen in serum were measured according to the manufacturer's instructions (Nanjing JianCheng Bioengineering Institute, Jiangsu, China). The concentrations of individual amino acids were analyzed using a high-performance liquid chromatography method (Venusil-AA HPLC column; Agela Technologies, Newark, DE, USA).