

Research Article



Generating Background Information of Laboratory Chinese Jue-ma Minipigs, a Plateau Swine Species

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Abstract | Today, minipigs have been proved to be available for biomedical research and become one appropriate non-rodent species for drug preclinical safety testing. But the species selection based on scientific information is far behind the needs of science. Our work team has been contributing to select different Chinese minipig species for different scientific purposes since the 1990s. Here, we introduce laboratory Chinese Jue-ma minipigs (*Sus scrofa*) initially grazing at high altitude of temperate zone (Northeast Tibetan plateau) to producers or researchers concerned with minipigs. Jue-ma minipigs have two main appearances of complete black and spotted colours. The litter sizes of laboratory Jue-ma minipigs at birth and weaning are significant higher than grazing ones ($P < 0.05$), presenting values of 5.70 ± 1.97 and 5.20 ± 2.31 , respectively. The growth performances of laboratory Jue-ma minipigs resemble grazing ones except those of birth, which also resemble Gottingen minipigs showing a rapid to slight increase. There are no significant differences between males and females for hematology, blood biochemistry, absolute and relative organ weights. When compared to human reference values, 8 hematological parameters and 10 blood biochemical parameters fall within human reference ranges. Our results show that laboratory Jue-ma minipigs tends to be gentle as farming pigs with small body size, slow growth rate, and high fertility rate after continuous selection. It is also concluded that laboratory Jue-ma minipigs have more higher and acceptable reproductive traits than grazing ones. Current data present that captive Jue-ma minipigs are suitable laboratory animal for scientific uses.

Keywords | Chinese Jue-ma minipigs, Reproductive performance, Growth performance; Hematology and blood biochemistry, Organ weight

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INTRODUCTION

Even though minipigs have been proved to be available for biomedical research and began to be used in biomedical research for half a century, minipigs are still not the most appropriate non-rodent species for preclinical studies (Ganderup et al., 2012; Swindle et al., 2012; Dalgaard, 2014). Besides the relative larger body sizes than monkeys or dogs, the lack of minipig species for different scientific objects is the main reason, though there are some famous minipigs in developed countries, such as Gottingen minipigs, Sinclair minipigs, Minnesota Hormel minipigs, and Hanford minipigs. Even China owns rich resources of minipigs, but the laboratory minipig work is far behind the

developed countries. To enrich the laboratory minipig species, a series of studies into the selection and de-selection of minipig species have been carried out by our research team since the 1990s. Conventional and specific pathogen free (SPF) Chinese Wuzhishan (WZS) minipigs (Min et al., 2014; Pan et al., 2015), Jue-ma Minipigs (*Sus scrofa*), and Lantang minipigs (*Sus domestica*) are preserved in our breeding farm and continuously cultivated for different scientific demands. For example, the outbred WZS minipig cultivated by us is most often used in biomedical research in China now, which was initially grazed in subtropical zone (Hainan Island) and suit for tropical medicine. Lantang minipig is a species of Guangdong region with high reproductive performance and can be used for

reproductive research. Different from the other two minipigs, Jue-ma minipig is a species living at high altitude of temperate zone (Northeast Tibetan plateau), which can be used for research of plateau medicine.

In this study, we generated the scientific knowledge and background information about the Jue-ma minipigs. Grazing Jue-ma minipigs were firstly collected from different herdsmen to keep genetic diversity at 2009. Strict pedigrees were recorded for the selection of minipigs. A large outbred colony formed by the fourth generations has been established for biomedical researches. Now, a great deal of background data about laboratory Jue-ma minipigs is available for scientific usage, such as productive performances, growth performances, hematology and blood biochemistry, absolute and relative organ weights.

MATERIALS AND METHODS

LABORATORY MINIPIGS

An outbred Jue-ma minipig colony formed by 34 sows and 15 males was set as the objective. All information was obtained from them and their offspring in 2011-2014 periods. The laboratory animal administrative license number is SCXK (Yue) 2015-0036.

Minipigs were maintained in closed shelters, which were equipped with cooling water curtains, infrared thermal lights, and air supply fans. Environmental temperatures normally ranged from 18 to 29°C and air exchanges were kept at about 10 times per hour.

CLINICAL OBSERVATION

Animals were observed by animal care technicians who recorded all clinical signs daily. If necessary, the veterinarian would provide on-site inspection. All records were verified by veterinarian periodically.

REPRODUCTIVE PERFORMANCE

According to Chinese national standard- *Records for Swine Breeds* (SAC, 1982), the reproductive performances of female Jue-ma minipigs were recorded continuously for at

least 3 birth frequencies. The estrus and reproduction behaviours were observed by experienced animal care technicians. Litter performances were recorded by animal care technicians on duty, which include the litter sizes, litter weights, and individual weights at birth and weaning (45d).

GROWTH PERFORMANCE

Body weight (BW) was taken by weighbridge monthly up to 6 months and at 2 months interval up to 12 months. After weighing body weights each time, animals were handled or fixed to determine 14 indices of body measurements using the tailor's tape measure as previous reported (Min et al., 2014). They were height-at-withers (HAW), body length (BL), heart girth (HG), abdomen girth (AG), heart depth (HD), heart breadth (HB), abdomen girth (AG), shank girth (SG), buttock-knee length (BKL), buttocks breadth (BB), head length (HL), forehead breadth (FB), jaw width (JW), caudal length (CL), caudal girth (CG).

HEMATOLOGY AND BLOOD BIOCHEMISTRY

Reference values for 19 hematological indices and 12 blood biochemistry indices were determined by automatic hematology analyser (Sysmex XT-2000iv) and automatic biochemical analyzer (Hitachi 7020) respectively. Samples were collected from minipigs of more than 4 months old.

ABSOLUTE AND RELATIVE ORGAN WEIGHT

The absolute and relative organ weights were determined as described in rats (Gur and Waner, 1993). Nine castrated males and 12 females were slaughtered according to standard procedures. The main internal organs were picked out quickly and weighted accurate to 0.01g. After that, the relative (% of body weight) organ weights that were also called organ coefficients were calculated.

DATA ANALYSIS

Data are expressed as means \pm SD. Student's *t*-tests were used to analyse between-group differences. Variances of different time points in the same group were analysed by one-way ANOVA. A value of *P* less than 0.05 was considered statistically significant. All analyses were done by using SAS software, version 8.01 (SAS Institute, Cary, North Carolina).



Figure 1: Typical appearances of Jue-ma minipigs: (A) the complete black minipigs; (B) the spotted minipig

Table 1: Litter performances of Jue-ma minipigs at birth and weaning per birth order

| Birth order | Litter size | | | Litter weight (kg) | | Individual weight (kg) | |
|--------------------|-------------|-----------|------------|--------------------|------------|------------------------|------------|
| | Total | At birth | At weaning | At birth | At weaning | At birth | At weaning |
| Firstborn (n=32) | 5.84±1.63 | 5.59±1.62 | 4.94±2.54 | 4.24±1.62 | 15.22±3.71 | 0.76±0.19 | 3.04±1.13 |
| Second-born (n=19) | 5.95±2.07 | 5.68±1.73 | 5.35±1.99 | 4.46±2.16 | 15.96±3.58 | 0.79±0.22 | 3.00±1.09 |
| Multi-born (n=15) | 6.27±2.76 | 5.93±2.87 | 5.57±2.16 | 4.63±2.58 | 17.95±3.59 | 0.77±0.08 | 3.22±1.63 |
| ANOVA, P | 0.8049 | 0.8623 | 0.7685 | 0.8134 | 0.6253 | 0.9835 | 0.9543 |

Table 2: Values of body weights, daily gain, and relative growth rate of Jue-ma minipigs

| Age class | Sex | N | Body weight, kg | Daily gain, g | Relative growth rate ¹ |
|-----------|--------|-----|-----------------|---------------|-----------------------------------|
| Birth | Total | 104 | 0.75±0.17 | — | — |
| | Male | 55 | 0.79±0.16* | — | — |
| | Female | 49 | 0.67±0.19 | — | — |
| 1 week | Total | 99 | 1.02±0.26 | 38.23 | 0.0435 |
| | Male | 53 | 1.07±0.27* | 40.48 | 0.0439 |
| | Female | 46 | 0.96±0.23 | 41.90 | 0.0521 |
| 2 weeks | Total | 97 | 1.52±0.36 | 71.43 | 0.0570 |
| | Male | 51 | 1.54±0.32 | 67.14 | 0.0520 |
| | Female | 46 | 1.50±0.41 | 77.14 | 0.0638 |
| 1 month | Total | 93 | 2.55±1.01 | 73.21 | 0.0368 |
| | Male | 49 | 2.50±0.84 | 68.47 | 0.0346 |
| | Female | 44 | 2.61±1.23 | 79.61 | 0.0397 |
| 2 months | Total | 80 | 4.67±1.72 | 70.83 | 0.0202 |
| | Male | 41 | 4.55±1.77 | 68.38 | 0.0200 |
| | Female | 39 | 4.81±1.68 | 73.18 | 0.0203 |
| 3 months | Total | 74 | 7.32±2.08 | 90.41 | 0.0153 |
| | Male | 38 | 7.08±2.48 | 84.33 | 0.0147 |
| | Female | 36 | 7.58±1.94 | 92.17 | 0.0151 |
| 4 months | Total | 65 | 10.12±1.25 | 91.28 | 0.0105 |
| | Male | 31 | 9.91±1.67 | 94.48 | 0.0112 |
| | Female | 34 | 10.37±0.50 | 93.06 | 0.0105 |
| 5 months | Total | 57 | 13.07±2.13 | 98.28 | 0.0085 |
| | Male | 27 | 12.60±2.97 | 102.13 | 0.0080 |
| | Female | 30 | 13.43±1.67 | 89.69 | 0.0086 |
| 6 months | Total | 52 | 17.19±3.83 | 137.33 | 0.0091 |
| | Male | 25 | 16.93±3.95 | 144.44 | 0.0098 |
| | Female | 27 | 17.47±3.88 | 134.88 | 0.0088 |
| 8 months | Total | 46 | 19.07±2.58 | 31.32 | 0.0017 |
| | Male | 21 | 18.81±3.16 | 31.33 | 0.0018 |
| | Female | 25 | 19.29±2.14 | 30.35 | 0.0017 |
| 10 months | Total | 38 | 20.46±2.34 | 23.26 | 0.0012 |
| | Male | 15 | 20.54±1.92 | 28.76 | 0.0015 |
| | Female | 23 | 20.33±1.97 | 17.43 | 0.0009 |
| 12 months | Total | 23 | 22.55±2.64 | 34.79 | 0.0016 |
| | Male | 11 | 21.98±2.47 | 24.00 | 0.0011 |
| | Female | 12 | 22.93±2.59 | 43.28 | 0.0020 |

Note: ¹Relative growth rate = (lnW2-lnW1)/(D2-D1); W1 and W2 were body weights at D1 and D2, respectively; Results of t- test, comparison between males and females; * p<0.05.

RESULTS

BASIC PHYSICAL TRAITS

Jue-ma minipigs have two main appearances. One typical appearance is a complete black body with thick and bushy coats (Figure 1A), and the other appearance is a black body with white abdomen, back and legs (Figure 1B). Its head is long and narrow with a long snout. Ears are relative smaller and of prickly ear shape. Trotters (feet) are small but strong and solid. The hindquarters are slightly higher than the forequarters in a normal upright manner.

Grazing Jue-ma minipigs are normally active with sensitive response, especially for postpartum. While after selection and controlled cultivation, the captive Jue-ma minipigs' temperament tends to be gentle as farming pigs.

REPRODUCTIVE PERFORMANCE REPRODUCTIVE BEHAVIOUR

The reproduction behaviours of Jue-ma minipigs are similar with the other minipigs, such as WZS minipigs. Female oestrus is characterized by swollen and red vulvar lips with a thin, mucous discharge. Depressed appetite, restlessness, pacing, grunting, and chomping of the jaws are always observed during the estrus. Estrus cycle is a little shorter than other minipigs, showing a value of about 18 d. And the behavioural estrus sustains 3-4 d. The pregnancy is 114.61±4.35 d (n=46). Maternal behaviours of postpartum sows do not differ from domestic pigs, but the frequencies of changing postures from lying to standing and back are higher than domestic ones, demonstrating the greater ability of protecting and nursing the piglets.

LITTER PERFORMANCE

The overall litter performances of the present study are given in Table 1, including litter sizes, litter weights, and individual weights at birth and weaning. As birth orders increasing, the total litter sizes and litter sizes at birth and weaning are increasing slightly, showing a small positive correlation with birth orders (ANOVA, P>0.05); the litter weights at birth and weaning resemble those of litter sizes. Different from litter sizes and litter weights, the individual weights at birth and weaning have no correlations with birth orders (ANOVA, P>0.05).

Table 3: Values of body measurements of Jue-ma minipigs

| Age Class | Sex | N | HAW (cm) | BL (cm) | HG (cm) | HD (cm) | HB (cm) | AG (cm) | BKL (cm) | BB (cm) | SG (cm) | HL (cm) | FB (cm) | JW (cm) | CL (cm) | CG (cm) |
|-----------|-----|-----|------------|------------|------------|-----------|------------|------------|-----------|------------|-----------|------------|----------|-----------|------------|-----------|
| Birth | T | 104 | 14.54±1.55 | 19.57±1.13 | 19.19±1.62 | 6.26±0.62 | 4.88±0.62 | 20.65±1.88 | 6.53±0.61 | 4.17±0.50 | 4.33±0.39 | 8.50±0.87 | 4.36±0.2 | 5.03±0.30 | 6.61±0.50 | 2.16±0.21 |
| | M | 55 | 14.67±1.80 | 20.0±1.36* | 19.47±1.53 | 6.35±0.67 | 4.96±0.59 | 20.99±1.58 | 6.63±0.64 | 4.25±0.42 | 4.40±0.40 | 8.64±0.85 | 4.5±0.3* | 5.07±0.29 | 6.53±0.48 | 2.15±0.17 |
| 1 month | F | 49 | 14.39±1.21 | 19.08±0.80 | 18.87±1.72 | 6.15±0.55 | 4.78±0.66 | 20.33±2.16 | 6.42±0.58 | 4.08±0.57 | 4.26±0.37 | 8.35±0.89 | 4.17±0.1 | 4.98±0.31 | 6.71±0.52 | 2.17±0.25 |
| | T | 93 | 22.57±3.33 | 32.00±4.05 | 31.89±4.59 | 11.2±1.64 | 8.33±1.19 | 36.41±3.94 | 12.27±2.5 | 7.95±1.16 | 6.35±0.60 | 12.61±1.10 | 6.5±0.67 | 6.69±0.81 | 10.70±1.97 | 3.42±0.41 |
| 2 months | M | 49 | 22.06±2.40 | 31.43±4.14 | 31.44±4.40 | 10.9±1.56 | 8.19±1.10 | 35.94±3.56 | 11.8±1.64 | 7.73±1.19 | 6.26±0.49 | 12.50±0.92 | 6.46±0.5 | 6.78±0.81 | 10.54±2.04 | 3.34±0.35 |
| | F | 44 | 23.13±4.13 | 32.63±3.94 | 32.40±4.79 | 11.5±1.72 | 8.48±1.28 | 36.93±4.32 | 12.7±3.28 | 8.20±1.12 | 6.45±0.70 | 12.73±1.27 | 6.7±0.79 | 6.58±0.80 | 10.88±1.89 | 3.50±0.47 |
| 3 months | T | 80 | 20.91±2.70 | 33.29±4.77 | 31.06±5.16 | 10.9±1.91 | 7.62±1.47 | 34.7±5.67 | 10.9±2.02 | 6.96±1.47 | 6.14±1.05 | 12.55±1.23 | 6.28±0.4 | 6.79±0.85 | 10.58±1.39 | 3.55±0.66 |
| | M | 41 | 20.86±2.55 | 33.68±5.10 | 30.98±5.19 | 11.08±2.3 | 7.80±1.77 | 34.93±5.75 | 11.05±2.4 | 7.09±1.67 | 6.23±0.81 | 12.60±1.07 | 6.38±0.4 | 6.87±1.07 | 10.25±1.63 | 3.42±0.59 |
| 4 months | F | 39 | 20.96±2.85 | 32.89±4.39 | 31.14±5.13 | 10.8±1.33 | 7.44±1.06 | 34.54±5.59 | 10.8±1.47 | 6.82±1.23 | 6.04±1.25 | 12.50±1.38 | 6.2±0.39 | 6.70±0.54 | 10.92±1.08 | 3.68±0.73 |
| | T | 74 | 27.87±1.84 | 47.24±4.82 | 46.90±2.59 | 17.0±0.65 | 12.57±0.49 | 53.53±3.04 | 16.16±1.6 | 11.33±1.61 | 7.97±0.70 | 15.23±1.57 | 7.16±0.3 | 8.33±0.89 | 14.91±1.31 | 5.01±0.71 |
| 5 months | M | 38 | 28.22±1.54 | 47.00±2.65 | 46.33±2.89 | 17.6±0.5* | 12.67±0.58 | 53.33±2.89 | 16.50±1.5 | 11.00±1.53 | 7.90±0.75 | 14.93±1.76 | 6.8±0.3* | 8.17±1.04 | 14.83±1.61 | 4.87±0.42 |
| | F | 36 | 27.50±2.12 | 47.50±6.36 | 47.50±2.22 | 16.5±0.71 | 12.46±0.37 | 53.75±3.18 | 15.8±1.71 | 11.67±1.69 | 8.05±0.64 | 15.55±1.35 | 7.5±0.32 | 8.50±0.71 | 15.00±0.88 | 5.15±0.92 |
| 6 months | T | 65 | 30.97±1.26 | 55.90±3.03 | 48.11±2.47 | 17.19±1.3 | 11.22±1.23 | 50.35±0.72 | 19.1±0.78 | 10.30±0.45 | 8.78±0.62 | 16.55±1.01 | 7.57±0.7 | 8.23±0.67 | 15.71±1.31 | 5.16±0.38 |
| | M | 31 | 31.33±1.15 | 55.33±3.06 | 45.6±2.29* | 16.87±1.5 | 10.97±1.06 | 47.8±0.58* | 19.0±1.00 | 9.57±0.59* | 8.77±0.75 | 16.37±0.76 | 7.40±0.9 | 8.11±0.87 | 15.67±1.61 | 5.08±0.46 |
| 7 months | F | 34 | 30.58±1.35 | 56.50±3.00 | 50.75±2.63 | 17.5±1.16 | 11.48±1.36 | 53.00±0.82 | 19.25±0.5 | 11.08±0.25 | 8.80±0.48 | 16.75±1.19 | 7.75±0.5 | 8.36±0.42 | 15.75±0.96 | 5.25±0.30 |
| | T | 57 | 33.24±1.70 | 58.24±4.02 | 53.56±5.91 | 18.4±1.93 | 12.89±1.21 | 59.39±4.99 | 20.2±1.29 | 12.60±1.24 | 9.28±0.76 | 18.68±0.86 | 8.33±0.4 | 9.00±0.72 | 16.50±1.34 | 6.05±0.80 |
| 8 months | M | 27 | 33.50±1.29 | 59.25±3.86 | 54.38±3.30 | 17.95±1.6 | 12.88±1.43 | 60.38±5.23 | 20.2±0.96 | 12.78±0.33 | 9.30±0.88 | 18.88±0.85 | 8.4±0.55 | 9.18±0.75 | 16.50±1.87 | 6.03±0.75 |
| | F | 30 | 33.00±2.00 | 57.33±4.16 | 52.83±7.52 | 18.8±2.17 | 12.90±0.96 | 58.50±4.77 | 20.3±1.53 | 12.43±1.68 | 9.27±0.64 | 18.50±0.87 | 8.27±0.2 | 8.83±0.70 | 16.50±0.50 | 6.07±0.85 |
| 9 months | T | 52 | 35.71±1.80 | 58.71±4.49 | 55.87±4.96 | 18.28±1.4 | 13.39±1.35 | 58.97±3.29 | 22.3±1.38 | 12.51±2.08 | 9.62±0.49 | 20.79±0.86 | 8.39±0.7 | 10.1±1.51 | 18.89±1.18 | 6.13±0.45 |
| | M | 25 | 35.80±1.92 | 58.20±5.76 | 56.00±5.15 | 17.88±1.3 | 13.74±1.46 | 59.60±3.36 | 22.69±1.6 | 12.44±1.02 | 9.54±0.55 | 21.5±0.79* | 8.7±0.78 | 10.3±1.98 | 18.70±1.04 | 6.15±0.53 |
| 10 months | F | 27 | 35.63±1.69 | 59.19±2.85 | 55.75±4.77 | 18.6±1.56 | 13.06±1.23 | 58.38±3.23 | 21.9±1.15 | 12.58±2.71 | 9.70±0.43 | 20.13±0.92 | 8.1±0.66 | 9.91±0.86 | 19.06±1.29 | 6.11±0.36 |
| | T | 46 | 39.11±4.47 | 65.39±4.12 | 60.71±4.17 | 20.37±1.6 | 14.91±1.26 | 67.15±4.05 | 23.2±2.53 | 13.75±1.40 | 9.83±0.44 | 22.03±0.80 | 9.17±0.9 | 10.38±1.4 | 20.56±2.96 | 6.43±0.65 |
| 11 months | M | 21 | 38.71±3.82 | 65.79±4.69 | 62.00±5.01 | 20.76±2.0 | 15.13±1.11 | 66.14±5.04 | 23.57±3.1 | 13.83±1.68 | 10.1±0.6* | 22.20±1.04 | 9.8±0.9* | 10.00±1.2 | 21.36±1.86 | 6.53±0.68 |
| | F | 25 | 39.44±4.94 | 65.06±3.57 | 59.63±3.32 | 20.04±1.2 | 14.73±1.38 | 68.00±2.99 | 22.9±1.94 | 13.68±1.12 | 9.55±0.24 | 21.88±0.53 | 8.59±0.8 | 10.7±1.54 | 19.88±3.63 | 6.35±0.63 |
| 12 months | T | 38 | 39.84±1.38 | 67.23±3.31 | 60.80±2.33 | 20.1±1.27 | 15.11±1.28 | 67.22±4.86 | 23.4±1.87 | 13.77±1.37 | 9.73±0.36 | 22.66±2.44 | 9.48±0.5 | 10.9±0.78 | 20.54±2.09 | 6.08±0.52 |
| | M | 15 | 39.40±1.00 | 66.30±4.40 | 61.00±3.01 | 21.0±0.8* | 15.48±0.64 | 67.50±2.25 | 23.5±0.75 | 13.84±0.43 | 9.36±0.1* | 23.30±2.46 | 9.4±0.55 | 11.1±0.58 | 20.60±3.30 | 5.90±0.71 |
| 12 months | F | 23 | 40.13±1.58 | 67.83±2.36 | 60.67±1.76 | 19.5±1.48 | 14.87±1.55 | 67.03±5.95 | 23.33±2.3 | 13.73±1.72 | 9.97±0.45 | 22.25±2.42 | 9.5±0.45 | 10.7±0.89 | 20.50±0.50 | 6.20±0.35 |
| | T | 23 | 41.75±1.34 | 68.57±3.87 | 63.79±2.89 | 21.2±1.28 | 15.65±1.46 | 69.33±3.97 | 24.5±1.95 | 14.03±1.52 | 9.78±0.32 | 23.35±2.08 | 9.75±0.5 | 11.8±1.76 | 20.59±3.42 | 6.51±0.67 |
| 12 months | M | 11 | 41.40±1.30 | 68.30±3.62 | 63.00±2.67 | 21.1±1.07 | 16.23±1.30 | 68.88±3.16 | 24.35±1.7 | 13.84±1.43 | 9.6±0.26* | 23.12±2.25 | 9.59±0.6 | 12.0±1.99 | 21.01±1.97 | 6.62±0.71 |
| | F | 12 | 42.13±1.46 | 68.83±2.75 | 63.67±1.76 | 21.5±1.56 | 15.36±1.84 | 69.63±5.01 | 24.73±2.4 | 14.23±1.72 | 10.1±0.39 | 23.57±2.57 | 9.8±0.67 | 11.6±1.76 | 20.27±3.54 | 6.37±0.63 |

Note: T: Total; M: Male; F: Female; Results of *t*-test, Comparison between males and females; **p*<0.05.

BODY WEIGHT

Body weights were routinely recorded at birth, 1 week, 2 weeks, a month interval from 1 to 6 months, and then 2 months intervals up to 12 months. All data were listed in Table 2. There were no significant differences in individual body weights between males and females of the same age class except at birth (t -test, $P>0.05$). As age increasing, a rapid increase of the average body weights was observed at the first 6 months, followed by a slight increase. The average daily gains were calculated and demonstrated to be consistent with the changes of average body weights, presenting a rapid increase at the first 6 months followed by a slight increase. The peaks of average daily gains were 137.33 g, 144.44 g, and 134.88 g for males, females, and total colony respectively at 6 months. Furthermore, relative growth rates of different ages were calculated out according to the following formula: $\text{relative growth rate} = (\ln W2 - \ln W1) / (D2 - D1)$; W1 and W2 were body weights at D1 and D2 respectively. Different from average daily gains, the peaks of relative growth rates were observed at 2 weeks.

BODY MEASUREMENT

Fourteen indices of body measurements were detected and analysed. The results were presented in Table 3. The variation tendency of body measurements was in keeping with that of body weight, giving a rapid increase in first 6

months following a slight increase up to 12 months. Comparisons between males and females showed that most indices were consistent for males and females and only 13 out of 140 results of different age classes emerged significant differences (t -test, $P<0.05$), indicating a relative stable pattern of growth for Jue-ma minipigs.

HEMATOLOGY AND BLOOD BIOCHEMISTRY

Hematology and blood biochemistry indices were determined and listed in Table 4 and 5. In 19 hematological parameters, NEUT, PDW, MPV, and P-LCR show significant differences between males and females (t -test, $P<0.05$). The main differences focus on indices of blood platelet. While there was only one index (TC) among 12 blood biochemical indices showing significant difference between males and females (t -test, $P<0.05$). When compared to human reference ranges, total values of 8 hematological parameters (HGB, HCT, MCH, PLT, RDW-SD, MPV, P-LCR, and NEUT) and 10 blood biochemical parameters (ALT, ALP, TP, ALB, GLB, BUN, CREA, GLU, TBILI, and TG) fall within human reference ranges.

ABSOLUTE AND RELATIVE ORGAN WEIGHT

Absolute and relative organ weights of heart, liver, spleen, lung, kidney, brain, bladder, and stomach were determined and presented in Table 6. Not only the absolute organ weights but also organ coefficients showed no significant differences between males and females (t -test, $P>0.05$).

Table 4: Determination of hematology parameters in the Jue-ma minipigs

| Items | Units | Human Reference ranges | Jue-ma minipigs | | |
|--------|------------------------|------------------------|-----------------|--------------|---------------|
| | | | Total (n=54) | Male (n=11) | Female (n=43) |
| WBC | $1 \times 10^9 / L$ | 4.00~10.00 | 17.05±4.96 | 19.06±5.14 | 16.54±4.78 |
| RBC | $1 \times 10^{12} / L$ | 3.90~5.90 | 8.17±0.72 | 8.19±0.49 | 8.16±0.77 |
| HGB | g/L | 116.0~179.0 | 151.78±18.66 | 147.91±16.56 | 152.77±19.05 |
| HCT | % | 37.0~52.0 | 48.79±6.01 | 47.83±6.07 | 49.03±5.97 |
| MCV | fL | 80.0~98.0 | 59.82±5.98 | 58.61±7.88 | 60.13±5.33 |
| MCH | pg | 27.2~34.3 | 18.59±1.68 | 18.12±2.25 | 18.70±1.47 |
| MCHC | g/L | 27.2~34.3 | 311.31±12.68 | 310.09±13.21 | 311.63±12.51 |
| PLT | $1 \times 10^9 / L$ | 99.0~303.0 | 281.22±101.67 | 314.82±85.84 | 272.63±103.65 |
| RDW-SD | fL | 40.0~53.0 | 40.97±3.55 | 41.17±5.64 | 40.92±2.83 |
| RDW-CV | % | 10.0~14.5 | 21.63±2.66 | 22.67±4.67 | 21.37±1.69 |
| PDW | fL | 10.0~18.0 | 138.00±17.35 | 157.00±26.00 | 135.76±14.27* |
| MPV | fL | 6.5~12.5 | 10.62±0.83 | 11.45±0.95 | 10.52±0.76* |
| P-LCR | % | 13.0~43.0 | 30.63±5.46 | 35.45±5.85 | 30.06±5.10* |
| PCT | % | 0.12~0.24 | 0.30±0.08 | 0.33±0.001 | 0.30±0.09 |
| NEUT | % | 1.8~6.4 | 5.59±3.13 | 7.81±4.40 | 5.02±2.37* |
| LYMPH | % | 1.9~8.0 | 10.66±3.04 | 10.58±2.94 | 10.68±3.06 |
| MONO | % | 0.16~1.00 | 0.12±0.12 | 0.10±0.10 | 0.13±0.12 |
| EO | % | 0.02~0.30 | 0.50±0.45 | 0.37±0.22 | 0.54±0.49 |
| BASO | % | 0~0.1 | 0.19±0.12 | 0.19±0.09 | 0.18±0.13 |

Note: Results of t -test, Comparison between males and females; * $p<0.05$.

Table 5: Determination of blood biochemical parameters in the Jue-ma minipigs

| Items | Units | Human Reference ranges | Jue-ma minipigs | | |
|-------|--------|------------------------|-----------------|--------------|---------------|
| | | | Total (n=51) | Male (n=11) | Female (n=40) |
| ALT | U/L | 0~40 | 37.41±7.02 | 40.00±7.91 | 36.70±6.68 |
| AST | U/L | 0~40 | 52.61±22.08 | 56.00±16.66 | 51.68±23.45 |
| ALP | U/L | 42~140 | 101.0±56.55 | 114.18±52.22 | 97.38±57.77 |
| TP | g/L | 60.0~85.0 | 77.24±7.67 | 79.51±6.80 | 76.62±7.85 |
| ALB | g/L | 30.0~55.0 | 41.91±5.63 | 43.33±4.64 | 41.52±5.87 |
| GLB | g/L | 20.0~40.0 | 35.33±7.43 | 36.18±7.87 | 35.10±7.39 |
| BUN | mmol/L | 2.10~7.90 | 4.40±1.05 | 4.45±1.17 | 4.38±1.03 |
| CREA | μmol/L | 35~124.00 | 101.49±19.8 | 108.55±17.49 | 99.55±20.19 |
| GLU | mmol/L | 3.9~6.1 | 4.92±1.24 | 5.14±0.98 | 4.86±1.31 |
| TC | mmol/L | 2.80~5.70 | 2.10±0.52 | 1.73±0.57 | 2.20±0.46* |
| TBILI | μmol/L | 0~20.5 | 0.70±0.35 | 0.81±0.44 | 0.67±0.33 |
| TG | mmol/L | 0.40~1.81 | 0.58±0.23 | 0.56±0.25 | 0.58±0.23 |

Note: Results of *t*- test, comparison between males and females; * *p*<0.05.

Table 6: Absolute and relative organ weights in Jue-ma minipigs

| Items | Total (n=21) | | Male (n=9) | | Female (n=12) | |
|------------------|--------------|--------------|---------------|--------------|---------------|--------------|
| | Absolute (g) | Relative (%) | Absolute (g) | Relative (%) | Absolute (g) | Relative (%) |
| Heart | 93.14±17.79 | 0.39±0.04 | 90.03±15.79 | 0.39±0.02 | 95.48±19.50 | 0.39±0.04 |
| Liver | 425.24±95.52 | 1.80±0.37 | 434.74±113.76 | 1.91±0.43 | 418.12±83.94 | 1.71±0.32 |
| Spleen | 44.65±13.84 | 0.19±0.05 | 44.17±15.74 | 0.19±0.05 | 45.02±12.96 | 0.18±0.05 |
| Lung | 243.39±78.40 | 1.03±0.34 | 248.33±102.25 | 1.08±0.40 | 239.68±59.46 | 0.99±0.30 |
| Kidney (L) | 38.31±9.04 | 0.16±0.02 | 37.99±11.45 | 0.16±0.03 | 38.60±6.85 | 0.16±0.02 |
| Kidney (R) | 38.02±6.40 | 0.16±0.02 | 37.73±8.49 | 0.17±0.03 | 38.28±4.46 | 0.16±0.02 |
| Brain | 68.98±6.42 | 0.32±0.03 | 65.60±5.49 | 0.32±0.09 | 68.08±7.03 | 0.32±0.01 |
| Bladder | 28.40±13.21 | 0.12±0.05 | 32.82±15.06 | 0.14±0.05 | 25.09±11.16 | 0.10±0.05 |
| Stomach | 227.74±42.69 | 0.98±0.25 | 233.52±42.40 | 1.04±0.26 | 223.40±44.25 | 0.93±0.25 |
| Body weight (kg) | 24.11±4.87 | — | 23.11±4.84 | — | 24.86±4.96 | — |

Note: Results of *t*- test, comparison between males and females; * *p*<0.05.

DISCUSSION

In recent years, minipigs began to be used in pharmacology, toxicity and basic researches for its anatomical and functional similarities to humans (van der Laan, et al., 2010; Bode et al., 2010; Hulet et al., 2014; Okazaki et al., 2014). Even in the field of medical devices, the pig is regarded as a suitable animal model indicated by the ISO Guidelines (van der Laan, et al., 2010). Take animal welfare and tenet of “3Rs” into consideration, minipig is regarded as one of the suitable laboratory animals to substitute dogs or monkeys in preclinical pharmacology and toxicology studies. However, the use of minipigs is far behind that of dogs or monkeys. The famous minipig species used in developed countries have clear background information, but most of them are known as multi-species hybrid bred (Bollen and Ellegard, 1996; Panepinto et al., 1981; Simianer et al., 2010). Different from the above minipig species, Jue-ma minipigs are formed by natural selection with high genet-

ic homozygosity and stable phenotype (Shang and Wei, 2007), which can satisfy special needs of biomedical researches.

Jue-ma minipig is a special pig in China initially grazing in south Gansu province. Our work of selection has begun since 2009. After more than 5 years selection, the captive Jue-ma minipigs tends to be gentle as farming pigs with stable reproduction performances, growth performances, and physiological characteristics. In original area, the average values of litter sizes, individual weights, and litter weights at birth are 3.74±1.40, 0.41±0.04 kg, and 1.76±0.46kg respectively (Lang and Wang, 2008). The litter performances of captive Jue-ma minipigs in this study were significant higher than grazing Jue-ma minipigs in original area (*t*-test, *P*<0.05). Though the growth performances at birth were higher than those of grazing minipigs, as age increasing, the daily gains and relative growth rates of body weights were slowed down. When compared

to grazing Jue-ma minipigs, there were only 2 body measurements (HAW and BL) showing significant differences (Cai, 2006), which might be caused by different measuring methods. Generally, the growth performances of captive Jue-ma minipigs resembled grazing ones. Hematology and blood biochemistry analyses are important for evaluation of health status in animals, which may also provide needed information in nonclinical drug safety evaluation. In 19 hematological and 12 blood biochemical indices, only 5 indices presented significant differences between males and females, which were NEUT, PDW, MPV, P-LCR, and TC. Besides, there were 8 hematological parameters and 10 blood biochemical parameters fall within human reference ranges, indicating the potentials of Jue-ma minipigs in biomedical researches. However, species differences significantly affected the hematological traits; there were nearly half of the parameters of blood that did not come up within human reference values. So, it is particularly important to generate the background information. The absolute and relative organ weights are important biological characteristics for genetic quality monitoring. The consistency of absolute and relative organ weights in males and females further confirmed the genetic stability of Jue-ma minipigs.

As we know, the Gottingen minipig is one of the most ideal laboratory animals for medical research (Lang and Wang, 2008), which is easy and comfortable handling in experiments with low body weight and minimizing costs of breeding and reagents. Though the work of selection of Jue-ma minipigs is still far behind that of Gottingen minipigs, the existing evidences indicate that Jue-ma minipigs are suitable laboratory animals after further cultivation. The temperament of Jue-ma minipigs tends to be docile, which makes it easier to handle animals. The growth performances of Jue-ma minipigs generally resemble Gottingen minipigs showing a rapid to slight increase (Köhn et al., 2007a, b). And only the body weights of birth and 5~6 month for Jue-ma minipigs are a little higher than Gottingen minipigs. There are differences in some hematological and biochemistry indices between Gottingen and Jue-ma minipigs, which are mainly caused by species variation and age class. The absolute and relative organ weights of Gottingen minipigs weighed about 14kg can be found at the following website: http://minipigs.dk/uploads/media/Organ_weights_01.pdf. Compared to the Gottingen minipigs, the absolute organ weights of Jue-ma are significant higher than Gottingen minipigs, while most relative organ weights are lower than Gottingen minipigs. Previous reports demonstrated that the absolute and relative organ weights are affected by many factors, such as sex, age class, body weight, and nutritional status (Marino, 2012; Lessard-Beaudoin et al., 2015). The differences between Jue-ma and Gottingen minipigs might be caused by body weights. As age or body weight increasing, the absolute organ weights increase and relative organ weights decrease gradually.

CONCLUSIONS

For Chinese minipig species, lack of background data is the main weakness for laboratory use. This study showed partial data of external characteristics, reproduction performances, growth performances, and physiological characteristics of Jue-ma minipigs gathered over the past period of selection. Though this study is limited by sample quantity and animal genetic quality, the data can preliminarily be the base reference values for Jue-ma minipigs. Further efforts will be conducted to optimize minipig breeding programmes and generate more background information contributing to future physiology and biomedical researches.

COMPETING INTERESTS

The authors declare that there are no competing interests regarding the publication of this paper.

AUTHORS' CONTRIBUTIONS

Fangui Min, Jinchun Pan, and Xilong Wang conceived and designed the study. Jinchun Pan, Fangui Min, Xilong Wang, and Qixiong Wei performed the protocol. Fangui Min wrote the paper. All co-authors read and approved the final manuscript.

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