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Carbon and nitrogen stable isotope analyses of mammal bone fossils from the Zhongba site in the Three Gorges Reservoir region of the Yangtze River, China

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Based on AMS ^{14}C dating data, carbon and nitrogen isotope analyses were conducted on mammal bone collagen of deer, cattle and pigs from the Zhongba site in the Three Gorges Reservoir region of the Yangtze River. These analyses were conducted to reconstruct palaeodiets of mammals, palaeoecology, palaeoenviroment and previous human activities in the study area. Results show that the collagen loss of bone did not change the *in vivo* isotopic composition of carbon and nitrogen stable isotopes, and most of the bone fossils were well preserved. The bone collagen of samples from deer had a mean $\delta^{13}C$ of –23.1‰ and a mean $\delta^{15}N$ of 4.7‰, suggesting that deer subsisted in a closed habitat and fed on branches and leaves. The bone collagen of cattle had a mean $\delta^{13}C$ of –19.6‰ and a mean $\delta^{15}N$ of 5.2‰, which indicates that cattle subsisted in an open habitat and fed on grasses and stems. The $\delta^{13}C$ values show that both deer and cattle fed on C_3 plants and lived in the same ecosystem, but the *t*-test results show that deer $\delta^{13}C$ and $\delta^{15}N$ values were both more negative than those of cattle, indicating that they inhabited different niches. The $\delta^{13}C$ and $\delta^{15}N$ values of cattle partially overlapped those of deer, suggesting some competition in diets between them. The *t*-tests show that the $\delta^{13}C$ and $\delta^{15}N$ values of pigs were more positive than those of cattle and deer, which signifies that pigs occupied a higher trophic level compared to cattle and deer. The wide range of pig $\delta^{13}C$ values demonstrates that pig trading had been taking place from early Neolithic Age to late Bronze Age. There were no significant differences in deer $\delta^{13}C$ and $\delta^{15}N$ values among different archaeological periods, making it clear that climatic, ecological and environmental conditions were kept relatively stable from 2200 to 4200 a BP. This stability may have been responsible for the extensive and complete cultural layers at the Zhongba site. The minimum number of samples required to estimate the mean $\delta^{13}C$ values of deer, pigs and cattle are 8, 73 and 16, respectively, and for mean $\delta^{15}N$ values of deer, pigs and cattle, the minimum numbers are 4, 5 and 6, respectively.

Zhongba site, bone collagen, $\delta^{13}C$, $\delta^{15}N$, palaeodiet, palaeoclimate, palaeoecology

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The ratios of C and N stable isotopes from animal tissues can be well preserved in collagen of bone fossils. The bone collagen $\delta^{13}C$ and $\delta^{15}N$ values reflect the mean C and N stable isotopic compositions in bone collagen during the mean lifetime, which provides information about the dietary preference of an individual. Climate may affect the $\delta^{13}C$ and

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δ^{15}N values of terrestrial mammals through its effect on plant δ^{13}C and δ^{15}N values [1–3]. Thus, analyses of C and N stable isotopic compositions in bone are used widely in archaeology [4,5]. Since the 1960s, many studies on palaeodiet, palaeoenviroment and human activities have been conducted using C and N stable isotopic compositions in bone collagen. For example, a set of mammal herbivore bones were studied for C and N stable isotopic compositions in bone collagen to reconstruct Pleistocene environment and ecology in Italy [6]. Moreover, climatic evolution of the last 5000 years in Europe was revealed by analysis of C and N stable isotopic compositions of cattle, deer and horse bone collagen [7]. Furthermore, the status, region and gender differences in diets were inferred by C and N isotopic analyses of human bones in the Mississippi River valley [8]. In China, relevant studies have been carried out since the 1980s. Many of these studies used analyses of bone elements and isotopes to determine the diet of ancient people [9, 10]. Recently, much progress has been made to reconstruct rice cultivation strategies and food source acquisition techniques for prehistoric societies [11,12].

The Zhongba site is located about 6 km distant from the confluence of the Ganjin and Yangtze rivers. The site is situated on an alluvial fan on the bank of the Ganjin River, and has an area of 8000 m^2. The central geographic coordinates of the site are 30°20′43″ N and 108°1′38″ E. The Zhongba site has yielded the thickest and most complete layers found in the Three Gorges region. This was mostly due to the rescue excavation carried out and the profile of the T0102 unit of the site, which has many cultural layers covering the past 5000 years. These include: the Neolithic Age, the Xia Dynasty, the Shang Dynasty, the Western Zhou Dynasty, the Spring and Autumn Period and the Warring States, the Han Dynasty, the Six Dynasty Period, the Tang Dynasty, the Song Dynasty, the Ming Dynasty and the Qing Dynasty [13]. Based on AMS^{14}C dating data, carbon and nitrogen isotope analyses on mammal bone collagen of deer, cattle and pigs from the Zhongba site in the Three Gorges reservoir of the Yangtze River were performed to reconstruct mammal palaeodiets, palaeoecology, palaeoenviroment and human activities. The minimum number of specimens that should be analyzed to estimate mean δ^{13}C and δ^{15}N values of deer, pigs and cattle are provided herein, based on the corresponding standard deviations of bone collagen δ^{13}C and δ^{15}N values.

1 Rationale

1.1 Carbon isotopes

Based on photosynthetic pathways for fixing atmospheric CO_2 [14], the plants are divided into three categories. C_3 pathway and C_4 pathways are the two principal pathways that fractionate carbon isotopes to different degrees, resulting in plants with non-overlapping isotope compositions. C_3 plants typically are temperate plants, including trees, herbs, shrubs, and cool-climate grasses, which have a wide range in δ^{13}C values. Water-stressed ecosystems in arid environments are enriched in ^{13}C, and the pants have more positive values (as high as –21‰) than the mean C_3 δ^{13}C value of –27‰. Conversely, closed forest plants have lower δ^{13}C values (as low as –35‰) relative to the trees in open habitats because of the canopy effect [15]. In contrast, C_4 plants, including sedges and temperate and tropic grasses, grow in open environments and their δ^{13}C values are independent of the water stress, having mean δ^{13} C values of –12.5‰, with a restricted range of from –10‰ to –14‰ [16]. The isotopes of plants are fractionated in the tissues of consumers, resulting in positive shift in δ^{13}C values. This indicates the difference in δ^{13}C values between the animals and plants consumed. The fractionation factors are different depending on which tissues are analyzed. Compared with the δ^{13}C values of plants consumed, enrichments for animal muscle and bone collagen are about 1‰ and 5‰, respectively. For hydroxyapatite carbonate in bone, the enrichment can be up to 12‰. Thus, by analyzing the δ^{13}C values of herbivore bone collagen, we can infer the plants upon which the animals fed, and hence the contemporary climate and environment in which they lived [17].

1.2 Nitrogen isotopes

The δ^{15}N values in soil range considerably, from about –7‰ to 18‰. The nitrogen fixation progress is influenced by the climate, with high temperatures and arid environments affecting the progress negatively. The depth of plant roots and seasonal changes also impact on δ^{15}N values in the soil [18]. Hence N isotopic signatures may indicate the trophic level that the animals occupied and the protein sources of consumers. The animals enrich the δ^{15}N values of plants by about 3‰ to 5‰, although some studies have shown that consumers may be 6‰ more positive than the diets they consume. There is still no accepted theory that can model the δ^{15}N values in plants. The plants that can directly fix nitrogen usually are leguminous plants, such as beans, and yielding about 0 in their δ^{15}N values. The herbivore animal δ^{15}N will be elevated from 3‰ to 4‰ over the plants, and the effect is such that the same enrichment over dietary protein occurs at every trophic level of the food chain [19]. Marine food chains have much longer trophic levels relative to terrestrial food chains. This could be responsible for the elevated marine fish δ^{15}N that is more positive than 10‰, and the same applies to the freshwater food chains, which is demonstrated by freshwater fishes also having enriched δ^{15}N [20]. Animals in arid environments have more positive δ^{15}N values because of excretion of urine and sweat of animals, which enriches δ^{15}N.

2 Materials and methods

2.1 Profile characteristics of the site

The bone samples collected were from levels 17–68. Level 17, together with the upper levels, dates to the Qin and Han dynasties. Levels 17–33, 34–43, 44–53, 49B–52B, 54–68 and 69 correspond to the Warring States, the Spring and Autumn Period, the Western Zhou Dynasty, the Shang Dynasty and the Xia Dynasty, the early of Neolithic Age and raw soil disturbed by ashpits of the Neolithic Age, respectively. Excavations conducted at the archaeological site resulted in at least 33 species of mammals, 18 species of fishes, and numerous unidentified birds, amphibians and reptiles. The most common mammals belonged to *Artiodactyla*, *Carnivora*, *Lagomorpha*, *Perissodactyla*, *Primate*, *Rodentia*, *Artiodactyla* including *Bovidae* (*Bos* sp., *Bubalus* sp.), *Cervidae* (*Cervus albirostris*, *Cervus elaphus*, *Cervus* sp., *Elaphurus davidianus*, *Hydropodes inerm*, *Muntiacus reevesi* and *Muntiacus* sp.), Suidae (*Sus scrofa*) and other indeterminate taxa. Primates included *Homo sapiens*, *Macaca mulatta*, *Macaca* sp., *Rhinopithecus* sp., *Presbytis* sp. and other indeterminate taxa. Here bone samples of cattle, deer and pigs, ranging from early Neolithic Age to late Bronze Age, were analyzed to reconstruct paleoenvironments and the paleodiets of animals.

2.2 AMS^{14}C dating

The layer ages of the Zhongba site were determined by AMS^{14}C dating of 27 animal bones and 5 charcoals. The analyses were conducted at the Institute of Heavy Ion Physics, Peking University. The corresponding ages are shown in Figure 1 and Table 1 [21].

2.3 Carbon and nitrogen isotope analyses

Bone samples were pretreated according to the method of Koch et al. [22]. Surface contamination of the bones was removed with a drill and a scalpel. Then, a piece of bone an was finely ground in an agate mortar to a size of less than 75 μm. Bone powder was collected and accurately weighed in an electronic balance. Next, the powder was decalcified in 0.1 mol/L hydrochloric acid for several days, with fresh acid replacement once a day until the demineralization was complete. The solution was then rinsed to natural PH with distilled water, and the gelatin was soaked in 0.125 mol/L NaOH for 20 h to eliminate non-collagenous organic residues and humic acids. Then, samples were rinsed to neutrality again. Samples were gelatinised in 0.001 mol/L HCl at 95°C over night and filtered. The resultant residues were freeze-dried to obtain collagen. The collagen samples were weighed in a balance, and the collagen extraction yields are presented in Table 2. The stable isotope ratios for carbon and nitrogen from the resultant CO_2 and N_2 were measured

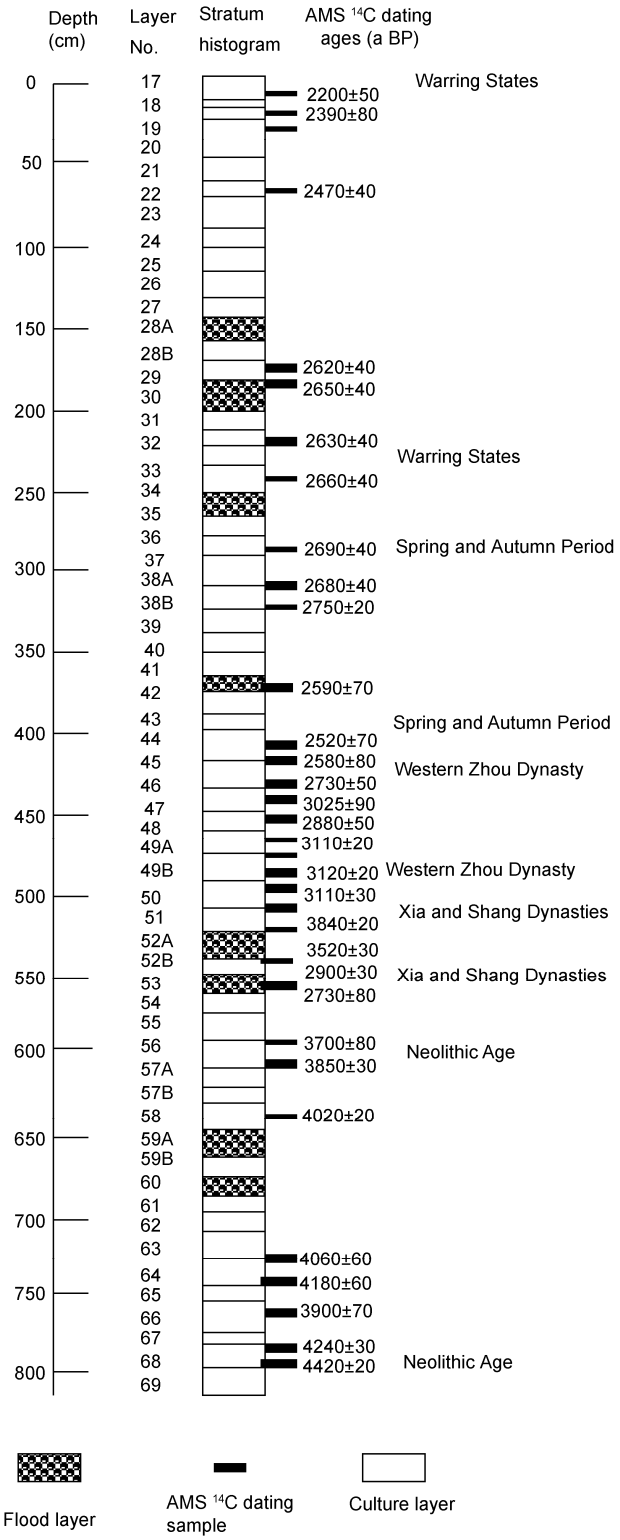

Figure 1 Profile of unit T0202 and ages of the Zhongba site.

on a Delta plus Advantage (produced by Finnigan) isotope ratio mass spectrometer at Key Laboratory of Lake Sediment and Environment, CAS. The C/N ratios were determined in a VARIOEL III (produced by Elementar) element analyzer. The isotopic ratios of carbon and nitrogen

Table 1 AMS^{14}C dating and its calibrated ages of layers in the excavation unit T0202 of the Zhongba site

AMS^{14}C dating No.	Material	Layer	Yield sample No.	AMS ^{14}C dating ages (a BP)	Calibrated ages (OxCal 3.0) (68.2% probability, cal a BP)
BA01357	bone	18	FCN 0006	2380±70	2470–2310
BA01361	bone	18	FCN 0104-2	2390±70	2150–2250
BA01409	bone	22	FCN 0643	2430±60	2430–2510
BA01362	bone	29	FCN 0981-1	2430±80	2600–2640
BA01367	bone	29	FCN 1082	2450±60	2610–2690
BA01419	bone	32	FCN 2094	2460±60	2590–2670
BA01420	bone	33	FCN 2136	2460±60	2620–2700
BA01424	bone	37	FCN 2229	2480±80	2660–2720
BA01368	bone	38b	FCN 2219-1	2490±70	2730–2770
BA01373	bone	38b	FCN 2275	2520±70	2640–2720
BA01429	bone	43	FCN 2379	2540±60	2550–2660
BK2002044	charcoals	46	FCN 2050	no result	no result
BA01374	bone	46	FCN 2513-1	2600±60	2450–2590
BK2002045	charcoals	46	FCN 2514	2640±60	2680–2780
BA01380	bone	46	FCN 2527-1	2680±70	2510–2650
BK2002046	charcoals	46	FCN 2528	2730±80	2985–3065
BA01433	bone	48	FCN2578	2730±85	2830–2930
BA01382	bone	49b	FCN 2613-1	2780±60	3090–3130
BA01384	bone	49a	FCN 2613-3	3025±90	3100–3130
BA0143	bone	49a	FCN2728	3100±60	3080–3140
BK2002047	charcoals	50	FCN 2658	3110±100	3460–3500
BA01435	bone	50	FCN 2675	3110±120	3520–3550
BA01437	bone	52a	FCN 2699	3210±120	2870–2930
BA01439	bone	53	FCN 2842	3240±100	2730–2810
BA01390	bone	56	FCN 2958-1	3540±60	3820–3880
BA01397	bone	56	FCN 2975-4	3590±60	3620–3780
BA02018	bone	58a	FCN 3142	3640±100	4000–4040
BK2002048	charcoals	64	FCN 3320	3660±100	4120–4240
BA02028	bone	64	FCN 3329	3800±70	4000–4120
BA02030	bone	65b	FCN 3498	3800±80	3830–3970
BA01398	bone	68	FCN 3582-1	3840±60	4210–4270
BA01403	bone	68	FCN 3582-6	3880±90	4400–4440

are expressed in the standard per mil (‰) notation as δ^{13}C and δ^{15}N (δ=[($R_{sample}/R_{standard}$)−1] × 1000, where R=^{13}C/^{12}C or ^{15}N/^{14}N) relative to the international standards V–PDB and N_2, respectively. The results are shown in Table 2 and Figure 2.

2.4 Statistical analyses

Statistical analyses were conducted with SPSS 11.0 software. We used *t*-tests to determine whether the differences in mean values between two species were significant. If the probability value was larger than the hypothesized value 0.05, the differences in mean values between the two groups were significant [23].

3 Explanation and discussion

3.1 The preservation quality of bone

C/N rations are an important indicator of bone preservation quality, with an acceptable value of well preserved collagen ranging from 2.9 to 3.6. Furthermore, the C/N rations of fresh bone collagen of the modern animals fall in this same range [24]. Collagen samples with C/N values outside this range are probably due to contamination, or to the loss of collagen C and N isotopes to the surrounding environment

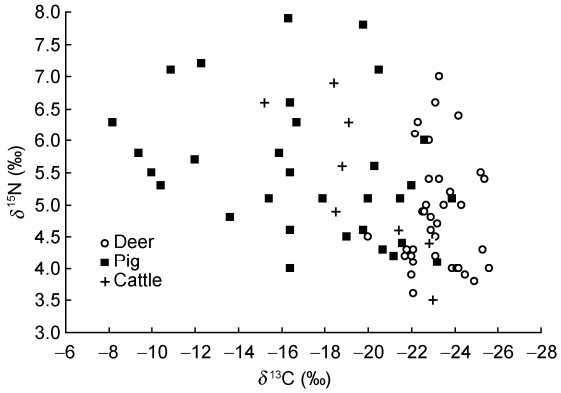

Figure 2 δ^{13}C and δ^{15}N values of animal bone collagen.

Table 2 Collagen extraction yields and collagen $\delta^{13}C$ and $\delta^{15}N$ values of animal bones in the layers of the Zhongba site[a)]

Layer	No.	Animal	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C%	N%	C∶N	Collagen extraction yields (mg g^{-1})
18	**104a**	**deer (left scapula)**	**–24.2**	**2.8**	**11.1**	**3.7**	**3.4**	**4.2**
18	**104b**	**deer (left scapula)**	**–23.8**	**5.1**	**6.0**	**1.8**	**3.7**	**21.4**
19	112a	deer (right scapula)	–23.9	4.0	22.1	7.9	3.2	19.1
20	296	deer (left scapula)	–25.6	4.0	21.4	7.6	3.2	20.7
22	643a	deer (humerus)	–23.1	4.2	11.2	4.0	3.2	23.3
22	643b	deer (humerus)	–23.1	4.2	16.8	6.0	3.2	8.4
22	643c	deer (femur)	–22.9	4.8	23.8	8.4	3.2	79.3
22	643d	deer (metacarpus)	–22.7	5.0	13.7	4.9	3.2	10.9
22	643e	deer (femur)	–22.6	4.9	20.2	7.4	3.1	4.1
23	715	deer (left scapula)	–22.0	3.9	27.7	9.8	3.2	19.2
25	809	deer (left scapula)	–24.9	3.8	31.5	11.4	3.2	16.8
27	909	deer (right tibia)	–25.2	5.5	20.1	7.1	3.2	20.6
27	912	deer (left scapula)	–22.5	4.9	30.9	11.2	3.2	64.5
28b	**975**	**deer (left radius)**	**–24.8**	**3.7**	**5.2**	**1.7**	**3.5**	**4.6**
29	981	deer (scapula)	–23.5	5.0	26.1	9.5	3.2	46.8
31	2082	deer (left tibia)	–23.0	3.5	18.6	6.6	3.2	7.1
32	2094a	deer (scapula)	–22.1	3.6	31.4	11.1	3.2	33.2
33	2016a	deer (hipbone)	–22.1	4.3	21.7	7.9	3.2	23.4
33	**2016b**	**deer (radius)**	**–22.0**	**2.3**	**0.9**	**0.2**	**4.8**	**55.3**
33	2136a	deer (phalanx)	–24.1	4.0	32.3	11.5	3.2	41.4
33	**2136b**	**deer (radius)**	**–19.7**	**4.7**	**13.6**	**4.4**	**3.6**	**34.8**
33	2136c	deer (phalanx)	–25.3	4.3	28.7	10.3	3.2	10.5
33	2136d	deer (tibia)	–22.3	6.3	29.7	10.5	3.2	23.2
34	2151a	deer (right calcaneus)	–22.8	5.4	19.5	7.1	3.2	34.1
34	2128	deer (hipbone)	–20.0	4.5	24.1	8.7	3.2	23.4
34	2151b	deer (phalanx)	–22.9	4.6	21.8	7.8	3.2	44.7
36	2225a	deer (right humerus)	–22.0	5.3	33.2	11.9	3.2	27.9
38a	2244	deer (hipbone)	–23.1	4.5	27.5	10.0	3.2	45.5
38b	2219	deer (phalanx)	–21.8	4.3	26.4	9.5	3.2	20.9
40	2316g	deer (right tibia)	–23.1	6.6	18.7	6.6	3.2	32.3
42	2345a	deer (metacarpus)	–24.3	5.0	28.7	10.5	3.1	28.7
42	2345b	deer (left humerus)	–23.3	7.0	29.1	10.3	3.2	33.4
43	2379a	deer (radius)	–25.4	5.4	22.5	8.1	3.2	59.2
43	2379b	deer (talus)	–22.0	4.2	29.2	10.5	3.2	41.1
45	2053	deer (radius)	–22.1	4.1	30.6	10.7	3.3	53.2
46	2513	deer (femur)	–23.2	4.7	33.0	11.7	3.2	18.2
46	2527a	deer (metacarpus)	–22.8	6.0	17.1	6.1	3.2	77.1
47	2563	deer (right tibia)	–24.2	6.4	25.8	9.0	3.3	16.6
48	**2578**	**deer (cervical vertebra)**	**–23.1**	**3.4**	**2.7**	**0.6**	**4.6**	**6.2**
50	2645c	deer (right tibia)	–24.5	3.9	20.8	7.6	3.1	50
50	2642	deer (humerus)	–23.3	5.4	14.9	5.4	3.2	70
50	2679a	deer (scapula)	–23.8	5.2	14.8	5.2	3.2	56.5
50	2679b	deer (tibia)	–22.6	4.9	6.8	2.2	3.5	7.7
51b	**2735**	**deer (left hipbone)**	**–26.0**	**4.6**	**9.6**	**3.3**	**3.3**	**22.1**
52a	2914	deer (scapula)	–21.7	4.2	27.6	9.8	3.2	21.7
55b	2942	deer (cervical vertebra)	–22.2	6.1	31.7	11.4	3.2	76.3
60	**3211**	**deer (right tibia)**	**–22.6**	**5.0**	**9.8**	**3.4**	**3.3**	**11.2**
63	**3298d**	**deer (left talus)**	**–23.4**	**6.2**	**1.3**	**0.7**	**2.2**	**6.1**
64	3329c	deer (hipbone)	–24.2	4.0	15.7	5.9	3.1	36.7

(To be continued on the next page)

(Continued)

67	**3559**	**deer (scapula)**	**–23.5**	**1.7**	**1.1**	**0.2**	**5.2**	**17.4**
68	**3582c**	**deer (scapula)**	**–19.8**	**4.9**	**7.6**	**2.6**	**3.3**	**15.1**
18	**104c**	**pig (humerus)**	**–21.4**	**2.9**	**2.1**	**0.7**	**3.5**	**32**
18	**104d**	**pig (calcaneus)**	**–16.4**	**1.8**	**0.8**	**0.1**	**5.1**	**72**
19	112b	pig (humerus)	–21.2	4.2	34.7	12.0	3.3	41.4
19	112c	pig (mandible)	–16.3	7.9	54.7	19.2	3.3	27.7
21	492a	pig (scapula)	–22.0	5.3	17.6	6.2	3.2	13.8
21	492b	pig (humerus)	–20.5	7.1	27.7	9.9	3.2	54.8
22	**643f**	**pig (metacarpus)**	**–22.1**	**1.8**	**1.5**	**0.4**	**3.8**	**30.3**
26	869a	pig (hipbone)	–23.9	5.1	21.5	7.5	3.3	60.5
26	**869b**	**pig (calcaneus)**	**–21.5**	**3.8**	**2.9**	**0.8**	**4.1**	**77.3**
29	981b	pig (phalanx)	–19.8	7.8	23.8	7.8	3.5	19.5
31	2073	pig (vertebra)	–21.5	5.1	20.4	7.4	3.2	46
33	2136e	pig (vertebra)	–20.7	4.3	34.9	12.2	3.3	31.2
36	2225b	pig (ulna)	–12.0	5.7	17.9	6.4	3.2	16
38b	2219a	pig (calcaneus)	–19.8	4.6	14.4	5.1	3.2	10.9
38b	2219b	pig (humerus)	–21.6	4.4	27.4	9.8	3.2	87.4
38b	2219c	pig (humerus)	–20.3	5.6	30.9	11.0	3.2	20.6
46	2527b	pig (calcaneus)	–12.3	7.2	31.5	10.9	3.3	47.2
46	2527c	pig (vertebra)	–10.9	7.1	22.4	8.1	3.2	78.4
50	2645a	pig (tibia)	–16.4	4.6	21.3	7.6	3.2	58.1
50	2645b	pig (scapula)	–17.9	5.1	12.5	4.4	3.2	51.5
52a	**2699a**	**pig (hipbone)**	**–22.5**	**11.8**	**0.8**	**0.6**	**1.5**	**2.7**
53	**2842b**	**pig (tibia)**	**–21.0**	**7.0**	**3.8**	**1.3**	**3.3**	**44.9**
53	**2842c**	**pig (radius)**	**–16.4**	**6.6**	**25.3**	**9.0**	**3.2**	**53.4**
56	2975	pig (ulna)	–15.4	5.1	16.9	6.0	3.2	35.4
58	3142a	pig (hipbone)	–9.4	5.8	16.5	5.8	3.2	60.2
58	3142b	pig (tibia)	–19.0	4.5	17.0	6.2	3.2	38.4
62	3487a	pig (metacarpus)	–20.0	5.1	18.2	6.6	3.1	34.4
62	**3487b**	**pig (humerus)**	**–10.0**	**5.5**	**19.5**	**7.0**	**3.2**	**51.8**
62	3265	pig (radius)	–18.9	5.8	3.0	0.9	3.6	36.6
62	3487c	pig (femur)	–8.2	6.3	39.2	14.0	3.2	30.7
63	3298a	pig (maxilla)	–16.7	6.3	19.8	7.1	3.2	59
63	3298b	pig (humerus)	–16.4	5.5	18.3	6.5	3.2	71.1
63	3298c	pig (ulna)	–23.2	4.1	23.8	8.5	3.2	7.8
64	**3329a**	**pig (ulna)**	**–13.1**	**6.1**	**12.3**	**4.5**	**3.1**	**8.6**
64	3329b	pig (radius)	–10.4	5.3	16.9	6.3	3.1	37.2
65	3456a	pig (talus)	–13.6	4.8	14.0	5.3	3.0	85
65	3456b	pig (vertebra)	–22.6	6.0	14.3	5.1	3.2	27.6
65	**3456f**	**pig (talus)**	**–14.2**	**5.6**	**5.2**	**2.2**	**2.7**	**27.9**
65	3456g	pig (femur)	–15.9	5.8	33.9	12.0	3.2	32.8
65	2472a	pig (humerus)	–16.4	4.0	19.9	7.4	3.1	18
65	2472b	pig (calcaneus)	–17.9	6.7	3.3	1.6	2.3	4.4
30	2016c	cattle (rib)	–23.0	3.5	18.6	6.6	3.2	41.9
50	2679c	cattle (carpus)	–18.8	5.6	13.9	5.1	3.1	70.3
52a	**2699b**	**cattle (talus)**	**–20.3**	**4.6**	**2.4**	**0.8**	**3.3**	**40.8**
52a	**2699c**	**cattle (scapula)**	**–20.2**	**5.4**	**4.9**	**1.7**	**3.3**	**13.2**
53	2842a	cattle (tarsus)	–22.8	4.4	18.3	6.5	3.2	35
56	2958a	cattle (scapula)	–21.4	4.6	14.1	5.1	3.2	81.8
56	**2958b**	**cattle (carpus)**	**–20.1**	**4.1**	**8.3**	**3.0**	**3.2**	**20.9**

(Continued)

61	3250	cattle (scapula)	–15.2	6.6	25.0	9.0	3.2	19.1
64	**3329d**	**cattle (scapula)**	**–17.8**	**5.4**	**8.3**	**3.3**	**2.9**	**32.8**
65	**3456c**	**cattle (calcaneus)**	**–19.5**	**4.8**	**10.9**	**4.3**	**2.9**	**36.8**
65	3456d	cattle (metacarpus)	–18.4	6.9	15.8	5.8	3.1	44.2
65	3456e	cattle (mandible)	–19.1	6.3	17.4	6.8	2.9	34.8
68	**3582a**	**cattle (limb)**	**–20.5**	**6.0**	**9.4**	**3.4**	**3.1**	**70.8**
68	3582b	cattle (rib)	–18.5	4.9	16.8	6.3	3.1	39.3

a) The boldface means the sample has been contaminated.

[25]. Another important criterion to estimate collagen preservation is whether the C and N mass contents of collagen account for over 13.0% and 4.8%, respectively [24]. Based on these criteria, we found 11 contaminated pig bones, and 11 contaminated deer bones. For pigs and cattle, 31 out of 41 samples and 8 out of 14 samples were well preserved, respectively. Sample preservation results are shown in Table 2, with bolded samples indicating contaminated bone collagen. Overall, the animal bones of the Zhongba site were well preserved and over 50% of the isotopic analyses were conducted successfully. However, several samples of each kind of animal were found to be contaminated. Generally, bones from a cold and arid area can be well preserved, but there were high temperatures and humidity in the Zhongba area. Even so, animal samples from the Zhongba site also can be well preserved possibly due to the muscles and guts having been peeled off the skeletons by humans.

Another important issue that should be taken into account is whether the loss of bone collagen has changed the pristine biological isotopic information. Beside the contaminated bone samples, the collagen extraction yielded a wide range with a maximum of 87.4 mg g^{-1}, and a minimum of 4.1 mg g^{-1}. The mean was 38.4 mg g^{-1}, and the standard deviation was 21.29 mg g^{-1}. In addition, the samples from the same layer also had wide ranges in collagen extraction yields. Figure 3 shows the bone collagen extraction yields for Zhongba deer, cattle and pigs [26].

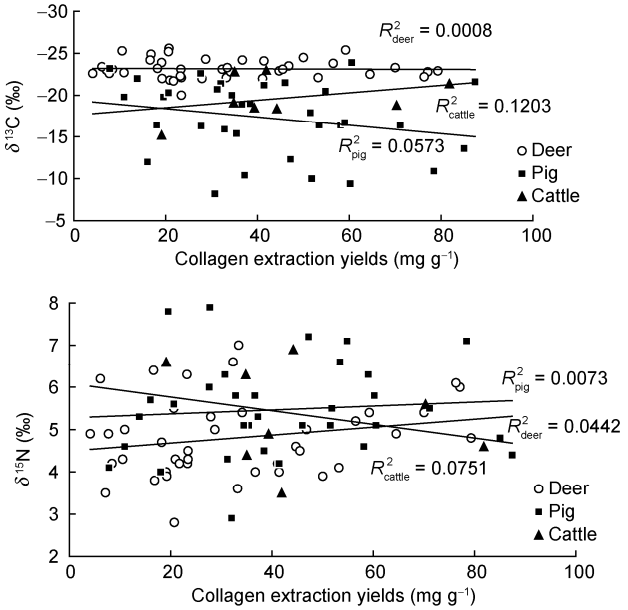

Figure 3 δ^{13}C, δ^{15}N values of bone collagen plotted as a function of collagen extraction yields.

3.2 Carbon isotope explanation

The mean δ^{13}C values of deer, cattle and pigs were –23.1‰, –19.6‰ and –17.1‰, respectively, and the differences in mean δ^{13}C values between deer and cattle, deer and pigs and cattle and pigs were all significant (Student's *t*-test, $P<0.01$). This means that there were differences in food sources among the animals. Given that animals will enrich by 5‰ their tissue δ^{13}C value over their food source, the food consumed by deer, cattle and pigs likely had mean δ^{13}C values of –28.1‰, –24.6‰ and –22.1‰, respectively. The δ^{13}C values of deer ranged from –25.6‰ to –20.0‰, and the corresponding δ^{13}C values of plants consumed by deer ranged from –30.6‰ to –25.0‰, indicating that deer subsisted in closed habitats and fed only on C_3 plants. The possible reason why these plants have such negative δ^{13}C values is the canopy effect of forests [27]. In addition, the δ^{13}C values of cattle ranged from –23.0‰ to –15.2‰, and the corresponding δ^{13}C values of plants they consumed likely ranged from –28.0‰ to –20.2‰, indicating that cattle also ate only C_3 plants. The fact that mean δ^{13}C values of cattle were more positive than those of deer suggests that the former lived in open habitats. We can conclude from Figure 4 that the standard deviation of deer values was the smallest (1.18‰), demonstrating that the food sources of deer were similar. Cattle had a larger standard deviation of δ^{13}C values than that of the deer. Hence, they had a wider food source range than deer. The omnivorous pigs had a considerably large standard deviation (up to 4.46‰), with δ^{13}C values ranging from –23.9‰ to –8.2‰. The corresponding likely δ^{13}C values of food consumed by pigs ranged from –28.9‰ to –13.2‰, indicating that the diets of pigs ranged from C_4-dominated plants to only C_3 plants. The clear differences in pig food and C_3 food content percentages at the Zhongba site may be the results of different pig breeding strategies [28]. At that time, the Zhongba economy was based on salt produced rather than agriculture or animal husbandry [22]. The ancient people in the Zhongba area exchanged salt for food, such as pigs. This means that it is feasible that pigs trading was carried out in

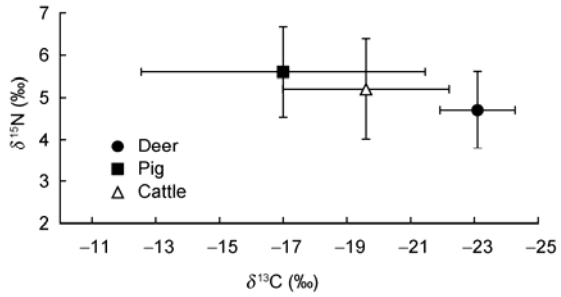

Figure 4 Means and standard deviations (2σ) for δ^{13}C and δ^{15}N values of animal bone collagen.

China from about 4000 years ago [29,30].

3.3 Nitrogen isotope explanation

The mean δ^{15}N values of deer, cattle and pigs were 4.7‰, 5.2‰ and 5.5‰, respectively, and the differences in mean δ^{15}N values between deer and cattle, deer and pigs and cattle and pigs were all significant (Student's *t*-test, $P < 0.01$). The mean δ^{15}N value of deer was more negative than that of cattle. This is because deer fed on foliage and shrubs in closed habitats and cattle fed on grasses in open habitats, and foliage and shrubs have more negative δ^{15}N values than grasses [30]. Pigs often ate leftovers of ancient peoples, which therefore would have included offal of deer and cattle [31,32]. Thus, pigs lived at a higher trophic level and had more positive δ^{15}N values than cattle and deer, as seen in their nitrogen isotope enrichment. However, the differences among pigs, cattle and deer were less than a trophic level difference of 3.0‰, hence the diets of these particular pigs probably did not included much animal meat. The δ^{15}N values of herbivorous cattle and deer are close to those of modern European herbivores, and are markedly different from those of modern African herbivores. This indicates that the moisture in the atmosphere in the ancient Zhongba area was similar to that of modern Europe, but different to that of modern Africa [33].

3.4 Palaeoclimate and palaeoenviroment

Generally speaking, the chemical and physical indices from site were disturbed by human activities and can not fully reflect environmental evolution. However, the bone collagen δ^{13}C and δ^{15}N of wild herbivore mammals can be used as indices of palaeoclimate and palaeoenvironment for the reasons mentioned above [34,35]. Most cattle samples in the Zhongba layers have not been analyzed in this study. Thus, the sample number of cattle bone collagen is not statistically robust because of the limited cattle samples. However, a high number of deer bone collagen samples were analyzed. Thus, we selected deer bone collagen δ^{13}C and δ^{15}N as environmental indices to reconstruct palaeoclimate and palaeoenvironment of the Zhongba area. In order to study the environment of different ages in this area, unit T0202 was initially divided into three phases: Phase I, Phase II and Phase III based on AMS^{14}C dating and archaeology (layers containing contaminated bone samples excluded). Phase I comprised levels 50–64 and dated to approximately 3500–4200 a BP (the Xia Dynasty, early Neolithic Age). Phase II included levels 34–49 and dated to approximately 2800–3500 a BP (the Western Zhou Dynasty). Lastly, Phase III included levels 18–33 and dated to approximately 2200–2800 a BP (the Warring States and Spring and Autumn Periods). Table 3 shows the descriptive statistics of deer collagen δ^{13}C and δ^{15}N values of the different periods. Figure 5 shows the stratigraphic and temporal variation of collagen δ^{13}C and δ^{15}N values for deer. Herbivore bone collagen δ^{13}C values related to the corresponding eaten plant δ^{13}C values, and the plant δ^{13}C values related to climate and forest canopy closure. A temperature decrease, a precipitation increase and a closed canopy caused more negative plant ^{13}C values [36]. The *t*-tests showed that there were no differences in collagen δ^{13}C between Phase I and Phase II, Phase II and Phase III, Phase I and Phase III (Student's *t*-test, $P< 0.05$). This indicates that the climate, temperature and

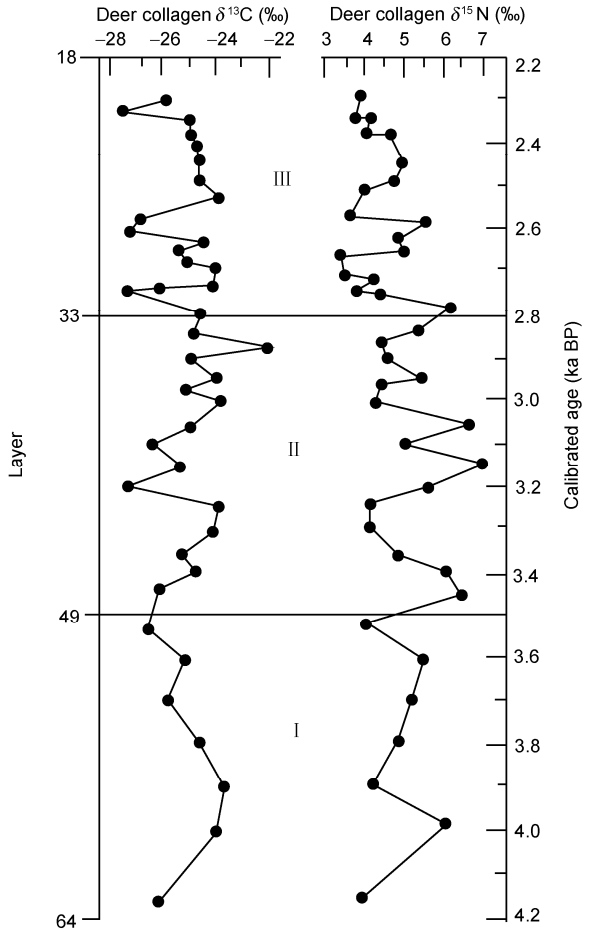

Figure 5 Stratigraphic variation of collagen δ^{13}C and δ^{15}N values for deer.

Table 3 The descriptive statistics of deer collagen $\delta^{13}C$ and $\delta^{15}N$ values of different periods

Isotope δ value	Period	N (Sample number)	Minimum	Maximum	Mean value	Standard deviation
$\delta^{13}C$	I	7	–24.5	–21.7	–23.2	1.0
	II	15	–25.4	–20.0	–22.8	1.2
	III	18	–25.6	–22.0	–23.3	1.3
$\delta^{15}N$	I	7	3.9	6.1	4.8	0.8
	II	15	4.1	7.0	5.2	0.9
	III	18	3.5	6.3	4.5	0.7

vegetation were relatively stable from 2200 to 4200 a BP. The plant $\delta^{13}C$ signals were passed on to herbivore bone collagen through the food web. Plants in warmer and drier areas have more positive $\delta^{15}N$ values. Thus, bone collagens of herbivores living in warmer and drier habitats consequently have more positive $\delta^{15}N$ values [37]. Similarly, the *t*-tests showed that there were no differences in collagen $\delta^{15}N$ between Phase I and Phase II, Phase II and Phase III, Phase I and Phase III (Student's *t*-test, $P< 0.05$). This indicates that there were no large oscillations in relative humidity and temperature from 2200 to 4200 a BP. Pollen analysis for this area shows that the predominant pollen of trees and shrubs were high, and the pollen of aquatic herbs, ferns, Gramineae and Raununculaceae also were high between 3200 BC and 500 BC. This indicates that it was warm and moist, and that there was no large oscillation in climate during this period [38]. The Zhongba site belongs to the middle subtropics, and is in the valley of the upper course of the Yangtze River. In addition, the Daba Mountains and the Wushan Mountains are situated northwest and southeast of the study area, respectively. Thus, the influence of low temperature is not clear in the Zhongba area [39]. The two environmental proxies of collagen $\delta^{13}C$ and $\delta^{15}N$ together indicate that the ecology, climate and vegetation in the area were relatively stable from 2200 to 4200 a BP, which may be responsible for the thickest and most complete cultural layers observed in the Zhongba area.

3.5 Minimum sample number

Generally, the animal fossils in one site are not large in number, and the usable number often decreases after the fossils have been prepared. Thus, it is important to determine if enough samples have been collected to allow reconstruction of palaeodiets using C and N isotopic ratios. Thus, studies on the minimum sample number bear a certain meaning in environmental archaeology. For example, given the standard deviation of 1.18, at least 9 samples should be analyzed to estimate the mean $\delta^{13}C$ value of deer within 1‰ with 95% confidence (not including the contaminated samples). As shown in Table 4, the minimum sample numbers need to obtain mean $\delta^{13}C$ and $\delta^{15}N$ values of deer, cattle and pigs within 1‰ with 95% confidence can be suggested based on the fossil collagen standard deviations of $\delta^{13}C$ and $\delta^{15}N$ [40]. Because of the wide range of standard deviations of $\delta^{13}C$, the corresponding minimum sample numbers of pigs and cattle are 73 and 16, respectively. Thus, the valid samples of pigs (number = 31) and cattle (number = 8) were not sufficient for calculating mean $\delta^{13}C$ values in this study. However, valid sample numbers of deer were sufficient to calculate mean $\delta^{13}C$ and $\delta^{15}N$ values.

Table 4 Minimum sample numbers substituting mean $\delta^{13}C$ and $\delta^{15}N$ values of deer, cattle and pigs

Isotope	Deer	Pigs	Cattle
SD ($\delta^{13}C$)	1.18	4.46	2.61
$\delta^{13}C$ minimum number (95% confidence)	8	73	16
SD ($\delta^{15}N$)	0.91	1.07	1.19
$\delta^{15}N$ minimum number (95% confidence)	4	5	6

4 Conclusions

Information on palaeodiets, palaeoclimate, palaeoecology and human activities was extracted based on carbon and nitrogen stable isotope ratio analyses of deer, cattle and pig bone collagen from the Zhongba site in the Three Gorges Reservoir region of the Yangtze River. The main conclusions are as follows:

(1) Most mammal fossil bone collagen from the Zhongba site was well preserved and not contaminated. The loss of bone collagen did not alter the original information that could be derived from the biological isotopes.

(2) Both deer and cattle fed only on C_3 plants, indicating that they subsisted in the same ecosystem, but utilized different niches. Deer inhabited closed forests and cattle inhabited open areas. Thus, deer had more negative $\delta^{13}C$ values because of canopy effects. $\delta^{13}C$ values of pigs ranged widely from –23.9‰ to –8.2‰, and the corresponding plants consumed were estimated to have carbon isotopic signatures of –28.9‰ to –13.2‰. The diets among pigs were very different, which may be a result of the fact that pigs were brought to the site from other places by trading in stead of them having been reared locally. The mean $\delta^{15}N$ value of deer was more negative than that of cattle, because $\delta^{15}N$ foliage and shrubs eaten by deer were more negative than those of herbaceous plants eaten by cattle. The diets of pigs included deer and cattle, and thus lived at a higher trophic level, which is the likely reason for the more positive $\delta^{15}N$ values of pig than those of deer and cattle.

(3) The results of bone collagen carbon and nitrogen isotope analyses show that the differences in deer $\delta^{13}C$ and $\delta^{15}N$ among different archaeological periods were not significant. This indicates that there were no large oscillations in climate, environment or ecology from 2200 to 4200 a BP, which explains well the complete cultural layers found at the Zhongba site.

(4) The minimum samples that can estimate mean $\delta^{13}C$ values within 1‰ with 95% confidence based on standard deviation of $\delta^{13}C$ are 8, 73 and 16 for deer, pigs and cattle,

respectively, in the Zhongba area, and 4, 5 and 6 for mean δ^{15}N values of deer, pigs and cattle, respectively.

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