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Body condition of three autochthonous canid species from Serbia

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Abstract: The body condition of three autochthonous canid species in Serbia (wolf– *Canis lupus*, jackal–*Canis aureus,* and fox–*Vulpes vulpes*) was assessed based on the value of Kidney Fat Index (KFI). A total of 1232 specimens (147 wolves, 711 jackals, and 375 foxes) were collected during a ten years period. Data were analyzed according to multiple factors such as sex, age, seasons, species, and genus. The results indicate that the adult members of the *Canis* genus have a significantly better body conditional status than the yearlings as well as the representatives of the *Vulpes* genus ($F (2, 900) = 71.465$, $p = 0.000$). Significant differences in KFI across seasons are recorded in jackal ($F (3, 542) = 6.912$, $p < 0.001$) and fox ($F (3, 371) = 2.675$, $p < 0.05$) populations. Jackals have the highest KFI during winter when females are in significantly better body condition than males. None of these three canid species has shown a significant difference in body condition between sexes, although females had slightly higher KFI. Among adult female jackals, a difference in KFI between nursing ones and other adult and pregnant females is observed ($H (2, n = 220) = 8.339$, $p = 0.016$). Food deprivation which occurs naturally during the winter months does not affect the body conditional status of these predators which are well prepared for the upcoming breeding season. Considered as a whole, in spite of the differences observed at the genus level, the body condition status of these autochthonous canids measured through KFI index indicates decent average fitness status in wolves, jackals, and foxes in Serbia.

Key words: Body condition, Kidney fat index, grey wolf, golden jackal, red fox

1. Introduction

Many animals, such as terrestrial carnivores, in cold-season environments, must deal with periodic food deprivation in addition to high thermoregulatory costs (Buskirk and Harlow, 1989). In order to adapt to the changing environment, they develop strategies such as torpor, good thermal insulation, and energy storage (Buskirk and Harlow, 1989). During vegetation season when the food sources are abundant, the excess dietary calories are stored in lipid depots (Allen, 1976). As general, mammals store energy primarily in the form of adipose tissue, which is both dense in energy and easily accessible (Lindstedt and Boyce, 1985). Unlike the vegetation season, the energy metabolism in mammals during winter switches to almost pure catabolism of lipids (Allen, 1976). For nonhibernating mammals, and primarily predators, it is extremely important to have a good body condition and enough fat reserves during wintertime when the energy demands are great and energy sources are scarce (Buskirk and Harlow, 1989).

Various methods are used in order to determine the status of the body condition of wild mammals. The most accurate is to determine the percentage of total body fat, but this technique is very time-consuming and demanding (Winstanley et al., 1998). Another, frequently used technique is bone marrow fat. When an animal is starving and its nutritional status declines, the body uses the fat from depots in the following order: subcutaneous, omental, renal, pericardial, and finally marrow (Harris, 1945; Sinclair and Duncan, 1972; Brooks et al., 1977). Therefore, the results obtained according to the bone marrow technique could be misinterpreted (Mech and Delgiudice, 1985), since the specimen with little marrow fat can be regarded as an extremely poor condition close to death (Bischoff, 1954; Franzmann and Arneson, 1976). The back fat index for estimating the amount of subcutaneous fat is also used but most commonly along with another index (Riney, 1982; Hillis and Mallory, 1996; Winstanley et al., 1998). Furthermore, Riney (1955) proposed the Kidney fat index (KFI) as an easy and fast method for determining the status of body condition. KFI is based on the assumption that the amount of perirenal fat is a reliable indicator of total body fat. Riney (1955) pointed out that KFI is easily estimated by weighing organs and surrounding fat after animal death. Moreover, according to Riney (1955), this index is suitable for the comparison

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of body conditions across seasons and between animals of different sizes. It is firstly used to assess the body condition among wild ungulates (Smith, 1970; Dauphine, 1975; Van Vuren and Coblentz, 1985; Takatsuki, 2000) and later on canids (Lajeunesse and Peterson, 1993; Cavallini 1996; Winstanley et al., 1998; Szabó, 2016) where it is considered as the best single index for predicting the total body fat (Winstanley et al., 1998) and the most useful and a workable technique which meets most of the requirements that researchers and wildlife managers have (Smith, 1970).

There are three autochthonous canid species in Serbia: grey wolf (*Canis lupus*), red fox (*Vulpes vulpes*), and golden jackal (*Canis aureus*). Jackals and foxes are widely distributed and they inhabit almost the whole country (Ćirović, 2000; Penezić, 2016), often living in sympatry. Commonly, both species occupy a variety of habitats-from natural to altered, humanized biotopes and from lowlands to hilly areas. The natural habitat of foxes is a dry, mixed landscape, with abundant "edge" of scrub and woodland (Macdonald and Reynolds, 2004). On the other hand, jackals in Balkan Peninsula prefer cultivated areas, wetlands in lower elevations, and heterogeneous habitats with adequate cover for hiding and breeding (Šálek et al., 2014). In Serbia, wolves prefer more forested, hilly, and mountainous areas. The main part of the distribution range is in the western, eastern, and southern parts of the country. There is also another small wolf population that inhabits the south Banat region, the south-eastern part of the Pannonian Plain (Milenković, 1997; Ćirović et al., 2019).

In order to evaluate the physical condition of these three main apex predator species, we measured their KFI as we assume it is a good indicator of stored abdominal fat. Our objective was to determine the body condition of autochthonous canids, with special emphasis on the most unfavourable part of the year. Of particular interest was to test the difference in KFI among different age classes and seasons among jackals, since they have a continually increasing population trend and their range is expanding in Serbia as well as in Europe.

2. Materials and methods

Hunting of all three canid species is allowed in Serbia. Legally culled specimens were obtained in collaboration with local hunting organizations and to a lesser extent through cooperation with taxidermists or as roadkill. A total of 1232 specimens (147 wolves, 711 jackals, and 375 foxes) were collected over a wide area during the period 2008–2017. Generally, the hunting season for these predators is open during the whole year. The only exception is made for wolves when they rear young (from 15 of April to 1 of July) as well as for a small population in Vojvodina province where they are fully protected. However, the main hunting season for predators is during the winter and therefore most of the samples (n = 962) were obtained during that period. Regarding other seasons, 94 samples are collected during spring, 57 during summer, and 121 during autumn. By winter months are meant for December, January, and February; by spring-March, April, and May; summer months include June, July, and August while by autumn is considered September, October, and November. For each collected animal we noted the sex, culling date, location, and reproductive activity for females (pregnancy or lactation) whenever it was possible. Kidneys with all surrounding perirenal fat were removed in the field and frozen (–20 °C) for later analysis. Further processing of the material was carried out in the laboratory of the Faculty of Biology, University of Belgrade. The age was determined according to body size and tooth wear (juveniles < 0.5 years, subadults 0.5–1 year, and adults > 1 year). After all the available skulls were cleaned of muscle tissue, the shape and wear of maxillary incisors were examined to separate yearlings and adults using the procedure described by Lombaard (1971).

After thawing, kidneys with surrounding fat were placed and dried with paper to remove the excess moisture. The measurements of kidney fat were standardized by Riney (1982): the peritoneal fat is cut parallel to the kidney as viewed from the caudal pole, and then the rest of the fat is separated from the kidney. Firstly, each kidney and the associated fat are weighted to the nearest 0.1 g. Then, after the fat has been peeled off, the ("fat-free") kidney was measured again. The kidney fat index (KFI) is expressed as a percentage and defined as the weight of the fat that surrounds the kidney in relation to kidney weight multiplied by 100. As a final result, KFI is given as an average of the results obtained for both kidneys. The length and width of fat-free kidneys were measured with a vernier with a precision of 0.1 cm.

2.1. Statistical analyses

Descriptive statistics were used to present morphological measurements of the kidneys of adult specimens: length, width, and weight (Table 1). The differences in KFI among age classes were tested with one-way ANOVA, and among sexes and seasons with two-way ANOVA. Assumptions of the analyses were tested by examination of residuals. When a significant effect was detected, we used a post-hoc Tukey test to identify differences. Analyzing data, we first used one-way ANOVA to test differences in KFI between three age classes of jackals (adults, subadults, and juveniles) and found significant differences ($F (2, 705) = 24.529$, $p < 0.001$). However, as a post-hoc Tukey test did not show differences between subadults and juveniles ($p = 0.166$) and we decided to combine these two age classes together as a new category–yearlings. Further, among

Table 1. The average kidney size in adult foxes (n = 173), jackals (n = 434), and wolves (n = 79)

	length (cm)	width (cm)	weight (g)
Vulpes vulpes	46.2 ± 3.9	25.6 ± 3.1	16.7 ± 3.5
Canis aureus	56.2 ± 4.4	31.8 ± 3.6	28.8 ± 5.7
Canis lupus	84.1 ± 8.5	42.1 ± 5.2	85.2 ± 22

Table 2. Mean KFI values of three canid species in Serbia according to sex.

	Mean KFI	female	male
Vulpes vulpes	26.5%	27.4%	25.8%
Canis aureus	34.7%	34.4%	35.0%
Canis lupus	36.5%	38.2%	35.1%

adult jackal females, lactating females were excluded from further analyses due to significantly lower KFI as they could represent an important source of bias in our data. All the analyses were conducted using Statistica 5.1 (Statsoft, Tulsa, OK, USA[1]).

3. Results

The average kidney size of adult specimens of three canid species native to Serbia is given in Table 1. The mean KFI value according to species and sex is given in Table 2. Regarding the sex, male (n = 380) and female (n = 331) jackals did not show statistically significant difference in body condition ($F (1, 709) = 0.362$, $p > 0.05$). In terms of age classes, the difference in KFI between yearlings (29.5%) and adults (36.2%) is highly significant according to ANOVA ($F (1, 706) = 45.634$, $p < 0.001$). Furthermore, among adult females, five were pregnant and six were in lactation. We performed, therefore, the Kruskal-Wallis ANOVA test on females in lactation, gravid ones, and all other adult females, and found that nursing females have significantly lower KFI (23.47%) than the other two groups ($H (2, n = 220) = 8.339$, $p = 0.016$) (Supplementary material).

On an annual basis, significant differences ($F (3, 542) = 6.912$, $p < 0.001$) also occur in body condition in adult jackals (Supplementary material). Mean KFI values for adult jackals are 31.32% in spring, 30.79% in summer, 35.69% in autumn, and 37.36% in winter. Post-hoc Tukey test indicated statistically significant differences in body condition between winter and spring ($p = 0.003$) as well as winter and summer ($p = 0.007$).

Furthermore, a two-way ANOVA indicates differences between sexes and seasons ($F (3, 538) = 4.866$, $p = 0.002$) in adult jackals (Supplementary material). Post-hoc Tukey test pointed to significant differences in KFI in adult female jackals during winter compared to females in the other three seasons ($p < 0.05$), and compared to males in winter ($p < 0.001$) and spring ($p = 0.002$).

For age classes in wolves, a difference between subadults and adults ($F (1, 123) = 4.999$, $p < 0.05$) is recorded. The KFI values obtained are 31.9% and 37.7% respectively for each group. ANOVA showed no significant differences between sexes regarding the seasons ($F (3, 94) = 0.249$, $p > 0.05$), neither between seasons ($F (3, 94) = 0.876$, $p > 0.05$) nor between sexes ($F (1, 94) = 0.784$, $p > 0.05$).

ANOVA showed no significant difference ($F (1, 373) = 2.27$, $p > 0.05$) between the sexes in foxes. Moreover, no difference between three age classes have been recorded ($F (2, 336) = 0.332$, $p > 0.05$). According to ANOVA, a border line of significance is recorded ($F (3, 371) = 2.675$, $p = 0.047$) in KFI across seasons (Supplementary material) in fox populations.

When KFI of only adult jackals, wolves, and foxes are compared, a highly significant difference ($F (2, 900) = 71.465$, $p = 0.000$) is observed (Supplementary material). A post hoc Tukey test and comparisons of means showed that foxes are in poorer body conditions than the other two canid species ($p < 0.001$ for both interactions).

A comparison of KFI between adult specimens of three canid species in Serbia across four seasons (Figure), according to ANOVA, did not show significant differences ($F (6, 891) = 0.572$, $p > 0.05$).

4. Discussion

This paper gives for the first time an insight into the body conditional status of wild canids in Serbia with reference to seasonal aspects, sex, age, and species level. Results showed that these predators accumulate significant fat reserves in winter months when food deprivation occurs in natural habitats. In Serbia, jackals have the highest KFI during this most unfavourable part of the year. This result could be linked with the feeding habits of this mesopredator species. Jackals are known as omnivorous and opportunistic foragers and it is common that their diet varies across habitats and seasons (Jhala and Moehlman, 2004). In some MSOM models, the carnivore community did respond to environmental covariates, although the coefficients did not show a consistent response between seasons or years (Bohnett et al., 2021). Furthermore, jackals are social canids commonly living in large groups (Macdonald, 1979). In terms of foraging behaviour, when alone or in pairs jackals typically look forward to small or medium-

[1] StatSoft Inc (1996) STATISTICA (data analysis software system), version 5.1. Website www.statsoft.com [accessed 17 December 2021].

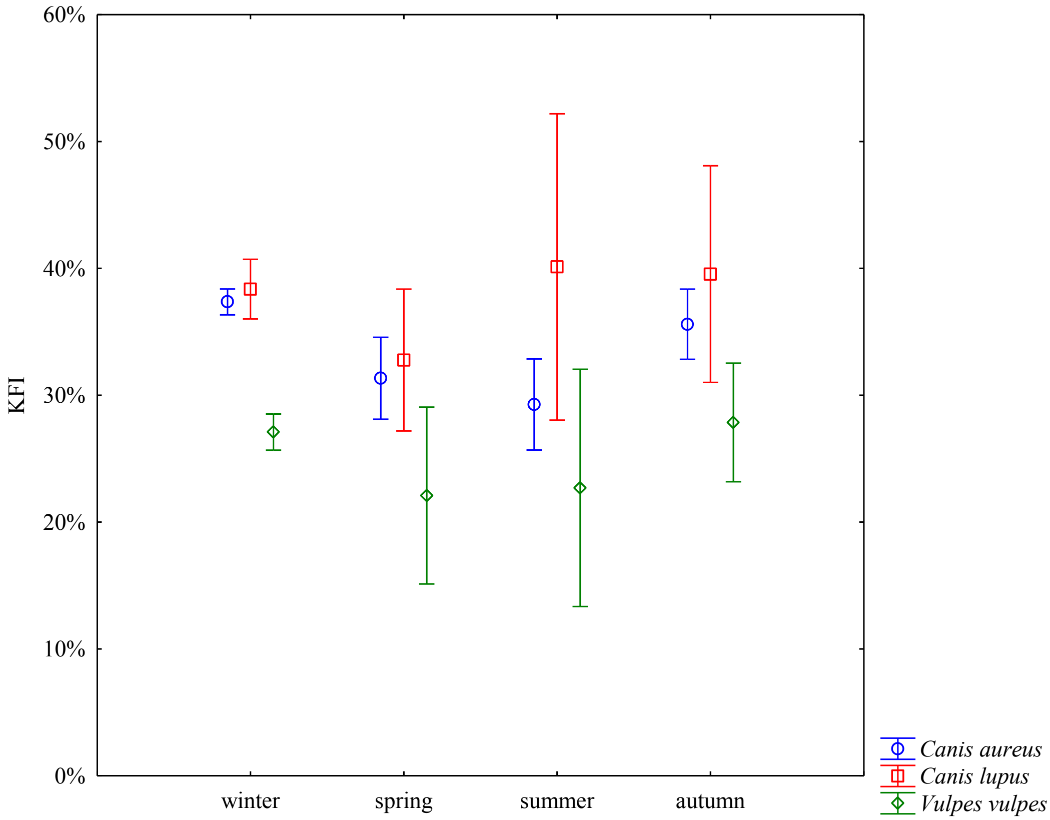

Figure. Differences in KFI between adult jackals, wolves, and foxes across four seasons.

sized prey (like rodents or hares) as opposed to group hunts when a larger catch is more certain (Lamprecht, 1978; Stanford, 1989). In some parts of its global range, if abundant, anthropogenic food sources can play an important role in its diet (Borkowski et al., 2011; Raichev et al., 2013; Ćirović et al., 2014, 2016). Previous studies on the dietary habits of jackals in Serbia showed that they significantly exploit anthropogenic food sources mainly in form of slaughter waste (Ćirović et al., 2014; Penezić and Ćirović, 2015; Ćirović et al., 2016). In fact, during winter, slaughter waste with other forms of anthropogenic food is the main food source for jackals in Serbia (Ćirović et al., 2014; Penezić, 2016). Results of this research show that jackals in Serbia are in adequate body condition, and prepared for the breeding season. The results of more or less continuous successful mating seasons are visible nowadays when the number of jackals has increased in comparison to the 1980s when they were present only in the form of two small relict populations (Milenković 1983, 1987; Ćirović et al., 2016). At present, jackals inhabit the whole territory of Serbia and local densities have become among the highest in Europe with 4.8 territorial groups/10 km^2 as recorded in eastern Serbia (Šálek et al., 2014). Reproduction is energetically expensive in mammals, and in order to support that high energetic cost of late pregnancy and lactation, maternal food intake in these carnivores must increase and/or the energy accumulated prior to reproduction or during early pregnancy (Loveridge, 1986; Gittleman and Thompson, 1988; Oftedal and Gittleman, 1989). In the European part of the jackal range, the oestrus period occurs once a year, generally in late January until March while parturition occurs 63 days after (Giannatos, 2004). Results of this research show that nursing female jackals had significantly lower KFI than pregnant and other adult females. This result could be expected since lactation involves a great drain of nutrients and energy, but it is for the first time recorded in golden jackals according to the best of our knowledge. Results of this research also indicate that jackals are in the poorest fitness status during the summer months. This is also in line with the studies on jackal dietary habits conducted in Serbia so far, showing that small mammals and fruit are dominant during summer (Penezić and Ćirović, 2015; Ćirović et al., 2016; Penezić, 2016) and represent food of lesser caloric value than slaughter waste consumed during colder periods of the year. Variations in dietary habits could result in a change of KFI value towards a lesser one. In order to compare the results of body condition status with other studies, we found it more accurate to compare trends than actual values. In Hungary, the same trend, with higher KFI values in autumn and winter in relation to summer is recorded by Szabó (2016). KFI was also used to estimate the body condition of hares (Flux, 1971, Bonino and Bustos, 1998). In New Zealand, a similar trend in KFI is observed: regardless of the availability of food, fat reserves in hares are highest in winter when the breeding season starts (Flux, 1971). In northwestern Patagonia, in the European hare population, a similar KFI pattern has been described: reproductive male and female hares have a gradual increase starting in autumn and reaching a peak in late winter followed by a gradual decline as the breeding season starts (Bonino and Bustos, 1998). Furthermore, results obtained in this research showed no statistically significant difference in body condition between sexes in jackals. The same results were obtained Szabó (2016) for the Hungarian population. KFI in wolves did not show significant oscillations during the year. Its diet is not as diverse as jackals. Wolves have 5 to 10 broad food categories which essentially comprise their staple diet (Newsome et al., 2016) which are more or less available during the whole year. Wolf is also apex predator in ecosystems they inhabit and studies showed that even their ranging behaviour, activity, and diet are altered by subsidies from anthropogenic resources (Petroelje et al., 2019), so their functional ecological roles are altered and consequently derives alteration of many other ecosystem processes (Newsome et al., 2015). In Europe, the wolf diet is dominated by medium-sized wild ungulates (especially wild boar and roe deer) although the consumption of domestic species is frequent and much higher than in North America (Newsome et al., 2016). In comparison to North America, grey wolves in Europe consume fewer medium-sized mammals but garbage and fruit feature in three times as many studies (Newsome et al., 2016). Similar findings are also obtained in Serbia. During the winter months, besides medium-sized wild ungulates, slaughter waste has an important role in the wolf's diet (Ćirović and Penezić, 2019), providing these predators enough energy to be in good body condition and prepared for mating season. Moreover, 80 wolf carcasses whose stomach content was analysed (Ćirović and Penezić, 2019) entered this research. The outcomes of this research also suggest that KFI in foxes is significantly lower than in wolves and jackals. The smallest native canid in Serbia showed no differences in KFI among sexes, the same as Szabó (2016) obtained in his research on foxes in Hungary. Moreover, we did not record significant differences in KFI among age classes, which is in line with the results of Cavallini's research conducted on Italian foxes (1996). In this research a weak, but still significant, the difference in KFI between seasons is recorded in foxes. Like jackals, fox dietary niche breadth in Europe is wide and studies show that it depends mostly on latitude, season, and anthropogenic influence in the habitat (Dell'Arte et al., 2007; Soe et al., 2017). In Europe, the primary food categories consumed by red foxes are rodents, lagomorphs, and ungulate carrion (Jędrzejewski and Jędrzejewska, 1992; Soe et al., 2017). Voles are considered a staple food in Poland (Kidawa and Kowalczyk, 2011) while Goszczyński (1986) found that high densities of voles reduce seasonal differences in the food composition of this predator species. When the prey densities are at their highest, then the predators are most efficient in their catch and this is at the same time the most energy-consuming activity (Norberg, 1977). Therefore, it could be expected to have the lowest KFI values during the period of the year when prey species are most abundant and when the hunt is frequent. Foxes also respond well to seasonal variation in the availability of food (Jędrzejewski and Jędrzejewska, 1992; Kidawa and Kowalczyk, 2011). During winter in Belarus, when many other food categories become scarce or less accessible due to snow cover, fox consumption of carrion increases (Sidorovich et al., 2006).

This research shows that three autochthonous canid species are in good body condition and the winter months do not represent nutritional bottlenecks for them. During the research period, an average of 2.36 t of communal waste was made and deposited into or onto land, including specially engineered landfills, temporary storage, and more than 2300 registered illegal dumps (Statistical Office of the Republic of Serbia, 2012[2]; Environmental protection Agency, Ministry of environmental protection 2020[3]) whose actual number is far higher. Leftovers of domestic animals in these illegal dumps represent the main food category in the diet of wolves and jackals during the winter months and not just the subsidies. Therefore, these canids exploit to a large extent available anthropogenic resources and this kind of feeding habit provides increased calories with decreased energy expenditure which results in good fitness status. More work is needed, particularly to compare the KFI of these predators living in areas with no anthropogenic food sources available.

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Supplementary material-figure captions

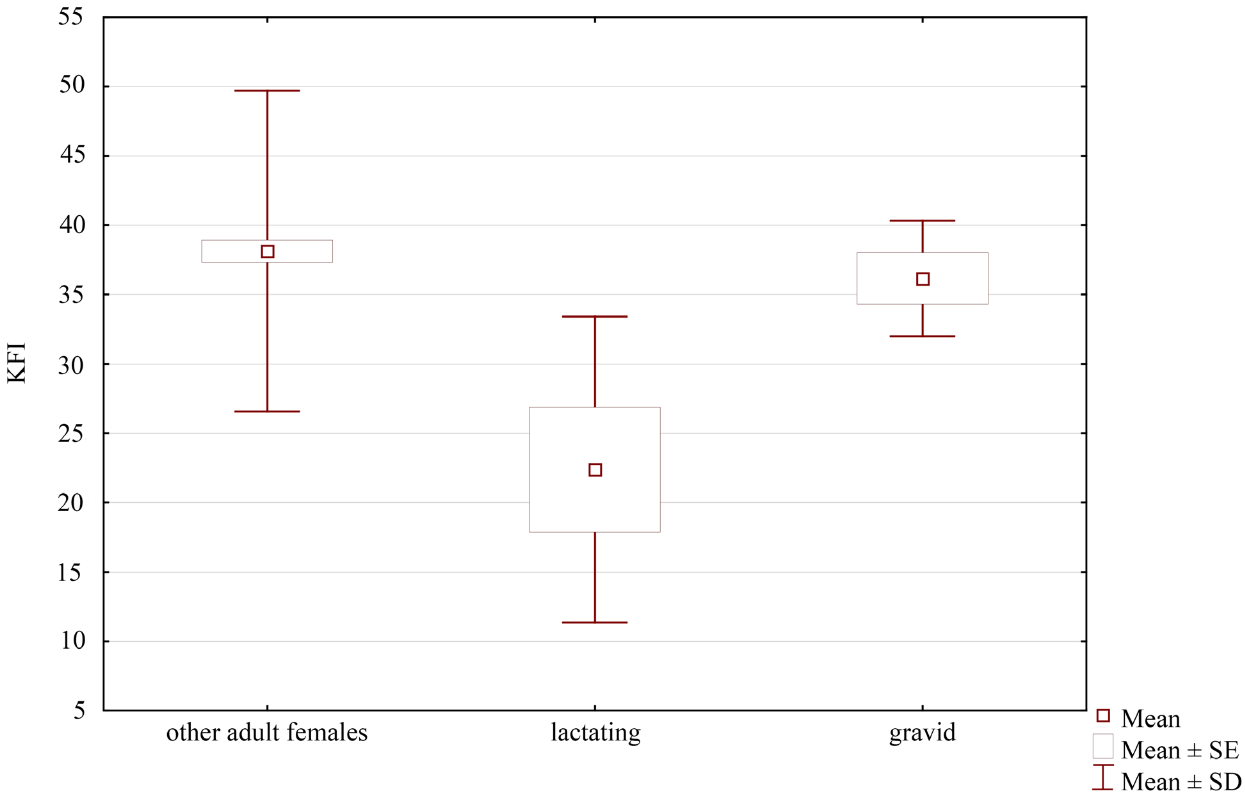

Figure 1. Boxplot showing the differences in KFI between lactating, pregnant, and all other adult female jackals.

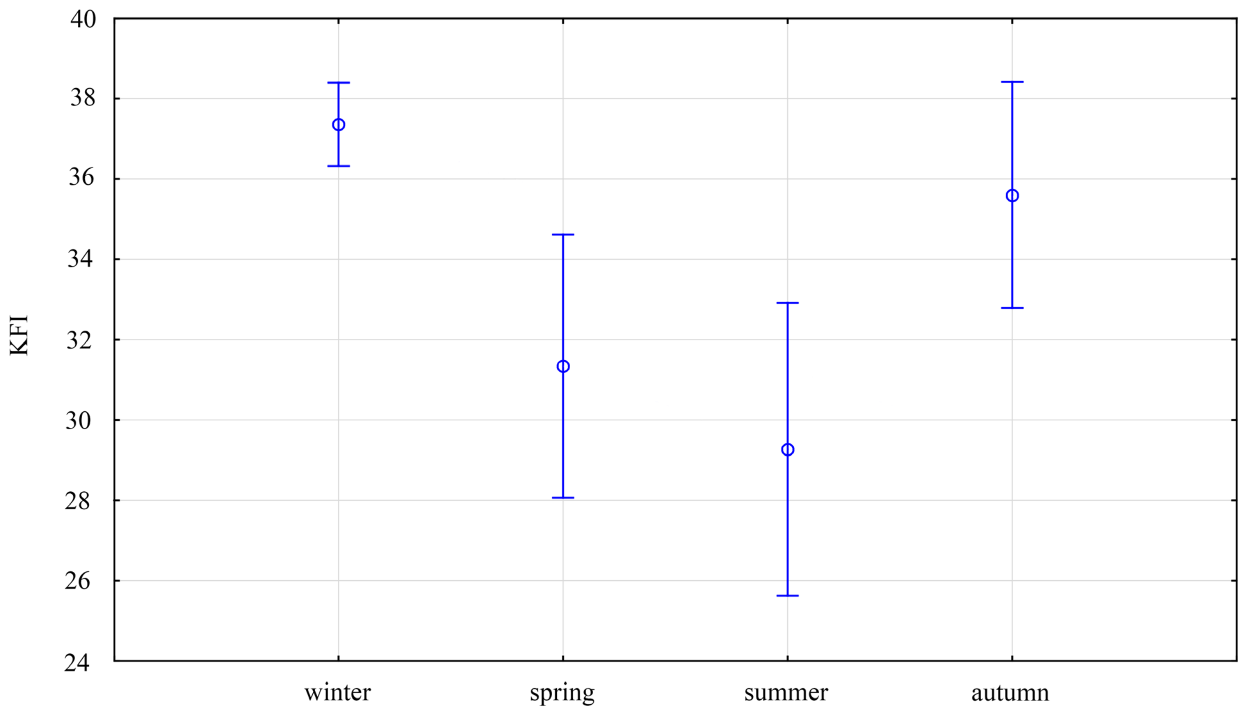

Figure 2. Differences in KFI values across all seasons in adult jackals.

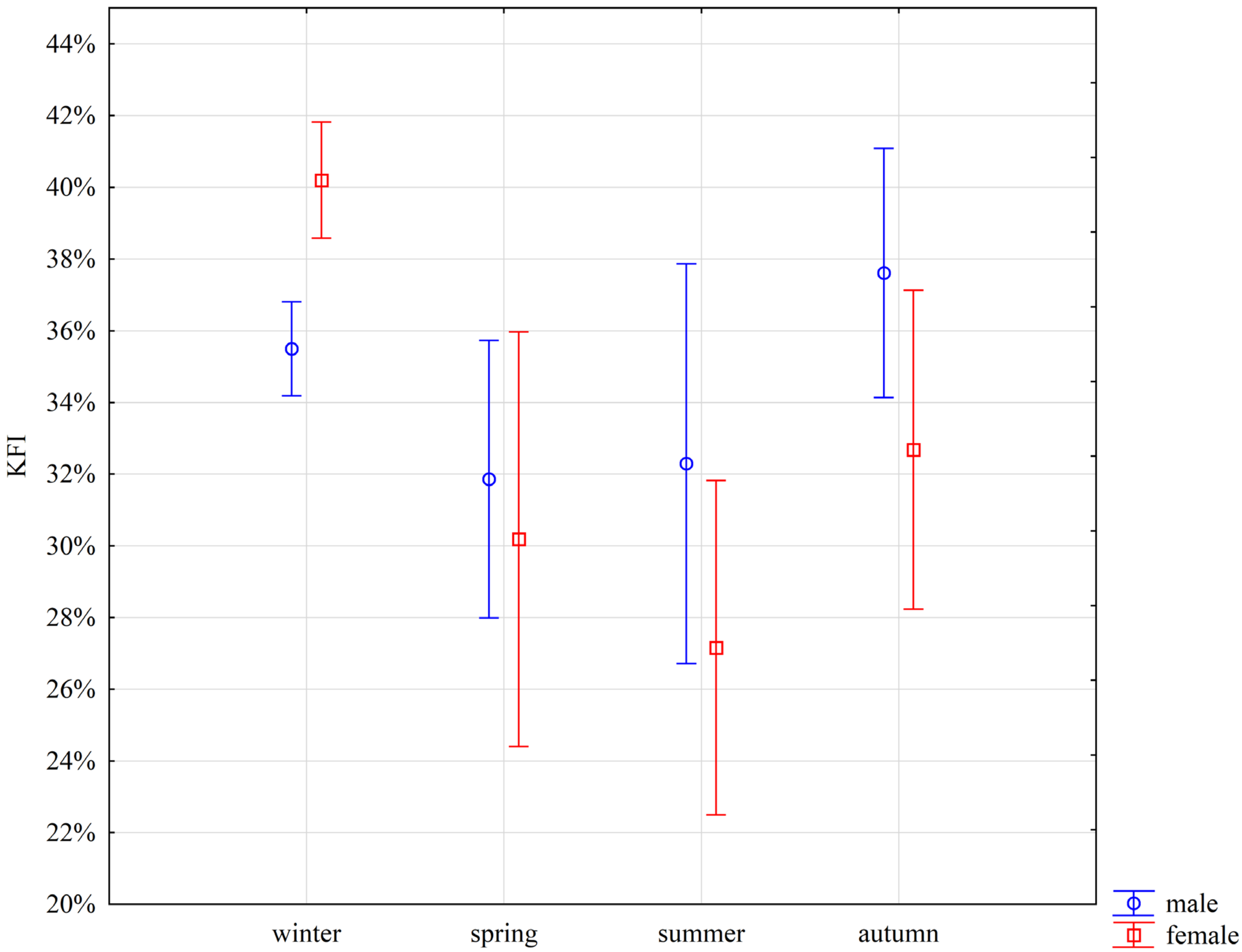

Figure 3. Two-way ANOVA shows differences in physical condition between sexes and across seasons for adult jackals in Serbia.

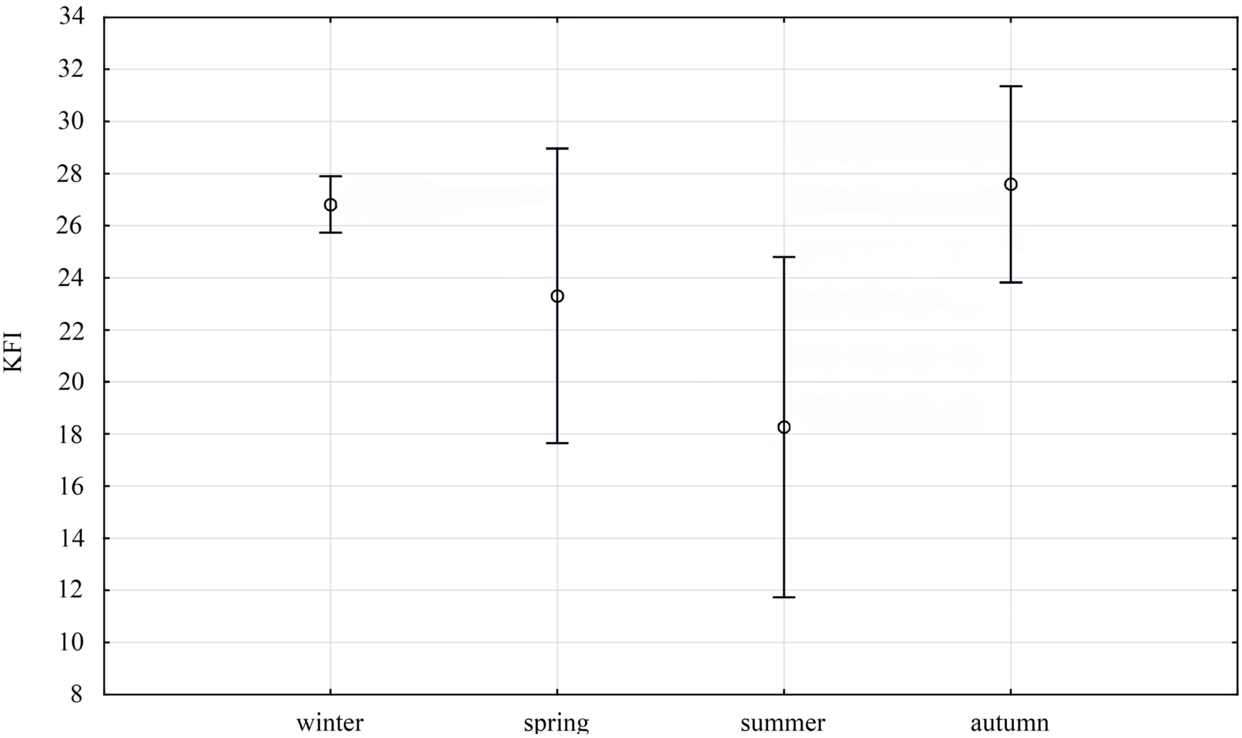

Figure 4. Differences in KFI values across all seasons in foxes.

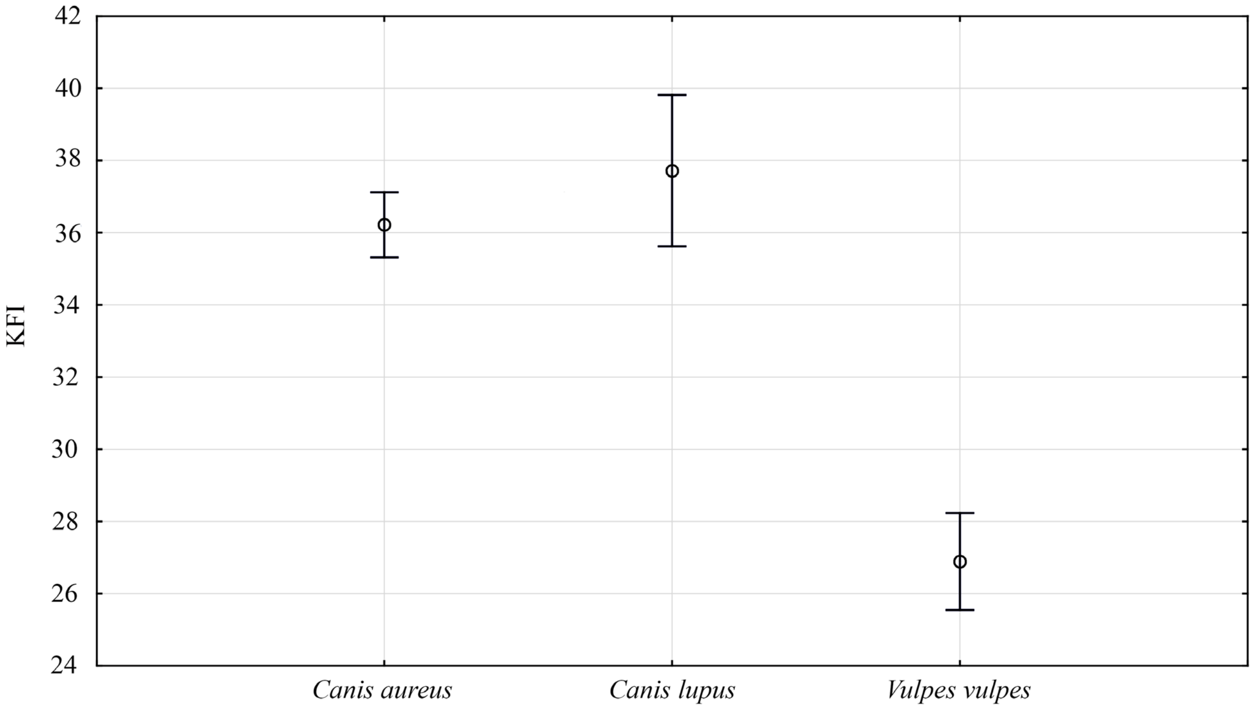

Figure 5. Differences in KFI between adult jackals, wolves, and foxes in Serbia.