Dietary enrichment and feed preferences of threatened goitered gazelle (*Gazella subgutturosa*) in captivity

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Abstract

Arid-adapted ungulates species, such as gazelles, have faced tremendous population declines in recent decades. Numerous breeding stations were established across Asia and the Middle East to facilitate the captive breeding of endangered gazelle species. We intended to improve the feed quality of goitered gazelle (*Gazella subguttorosa*) in captivity at the Kızılkuyu Gazelle Breeding Station in Şanlıurfa Province in southern Turkey. In a feed choice experiment, five different types of supplementary feed were offered to the gazelles (black barley, white barley, forage wheat, white barley flake and a combination of black barley and white barley flake). Statistical analysis revealed a significant difference between the amount of feed types consumed. The mixture of black barley and white barley flake was the most preferred feed type. Black barley alone was the least preferred feed type, while white barley flake was the second most consumed feed, suggesting that white barley flake was particularly attractive to the gazelles. However, nutrient content analysis revealed no significant difference between feed types. When feed consumption was examined between sampling days, a significant difference was unravelled between day 1, 2, 8 and day 3 to 7. Results were discussed with respect to improving the gastro-intestinal health of captive gazelles, which is imperative when choosing

suitable individuals for reintroduction, which should be in good physical condition to increase the chances for survival in the wild.

Keywords: captive breeding, dietary preference, supplementary feed, white barley flake, reintroduction

Introduction

Desert dwelling ungulates, such as Arabian oryx (*Oryx leucoryx*), addax antelope (*Addax nasomaculatus*), or many gazelle species (Antilopini), are iconic inhabitants of North Africa and the Middle East and are adapted to some of the harshest environments on Earth (Batanouny, 2000). Most ungulate species occurring in the Middle East, (11 out of 13; Mallon et al., 2023) are threatened or endangered due to illegal hunting, habitat loss and climate change (Newby, 1990; Thouless et al., 1991; Durant et al., 2014). To combat the ongoing population decline, several countries established specialized *ex situ* breeding centres for endangered desert ungulates (e.g., King Khalid Wildlife Research Centre in in Saudi Arabia, or Bukharsky Breeding Station in Usbekistan; Sludsky, 1956a; Kichenside and Lindsay, 1997; Prisyazhnyuk and Soldatova, 1984). Such breeding centres play a crucial role in the conservation of threatened ungulates, utilizing advanced reproductive technologies and veterinary care to breed and maintain healthy populations in captivity (Thouless et al., 1991; Dunham, 1995, 1997; Soares et al., 2021). By preserving genetic diversity and facilitating reintroduction programs, such facilities aim to restore endangered ungulate populations to their natural habitats. In the Şanlıurfa Province of southern Turkey, the Kızılkuyu Gazelle Breeding Station is dedicated to breeding goitered gazelles (*Gazella subgutturosa*) for reintroduction into the Kızılkuyu Wildlife Development Area. The goitered gazelle is a desert-adapted, migratory gazelle species, inhabiting the steppes of central Asia reaching from southern Turkey (here and along the

Euphrates Valley hybridizing with the Arabian sand gazelle, *G. marica*; see below) to northern China and Mongolia. Historically widespread, its range was markedly reduced, prompting the IUCN (International Union for the Conservation of Nature) Red List to classify the species as 'Vulnerable' (IUCN, 2017).

For *ex situ* breeding centres, it was recommended to carefully manage the nutrition of gazelles and to ensure their health and successful reproduction. If financially and practically feasible, the gazelles' diet should closely mimic what would be consumed in the wild (IUCN/SSC, 2014). However, in captivity nutrition is often based on dried, baled alfalfa (*Medicago sativa*) supplemented with specifically formulated pellets (e.g., Superlac concentrate) or pelleted grain (Reed, 1977; Mardonov, 1997) without which health issues such as malnutrition, diarrhoea or other digestive problems can occur (Soares et al., 2021). A balanced feed allows better digestibility and reduces the risk of nutrient imbalances, often followed by an increased risk of diseases and mortality (Lynch et al., 1992). Small domestic ruminants, such as sheep *(Ovis aries*), tend to choose high quality feeds with high calorific value and good taste (e.g., avoiding bitter plants with high alkaloid content; Baumont et al., 2000). When sheep were offered roughage (alfalfa), concentrate feed and forage grains, they preferred roughage but enriched their diet taking concentrate or grains to balance their gastro-intestinal microbiome (Cooper et al., 1995) and to alleviate illness (Villalba and Provenza, 2007, Villalba et al., 2010).

Although requiring a relatively simple diet—ruminants prefer energy-rich food with sufficient fibre content (Provenza, 1995)—sudden changes of the diet should be avoided due to increased risk of rumen acidosis (Allen, 1997), and new food items should be introduced gradually (Kichenside, 1998). In Turkish breeding centres (i.e., Kızılkuyu, Ceylanpınar, Ali Pınar and Casım Felhan Gazelle Breeding Stations), goitered gazelles are mainly fed on dried alfalfa,

while pellets and salt licks are occasionally offered as supplements (Uztemur and Orman, 2022; 2024; Uztemur et al., 2024).

To improve the nutritional regime at Turkish breeding centres it was recommended to test five supplementary grains to enrich the nutritional stage of gazelles. Our study aimed to pioneer the preparation of a more economical and more nutritious feed for gazelles in captivity and to enhance their health and well-being. We used choice tests (i.e., choice feeding or cafeteria experiments *sensu* Meier et al., 2012 or Olowu and Yaman, 2019; Kahraman et al., 2022.) to test the dietary preferences of goitered gazelles in the Kızılkuyu Gazelle Breeding Station, offering five supplementary feed types (i.e., black and white barley grain, forage wheat, white barley flake and a combination of black and white barley flake). In a first step we established the nutritional value of each feed type (i.e., dry matter, crude ash, crude protein, crude fat, starch and neutral detergent fibre [NDF]), and we determined whether the feed composition had an impact on the dietary preferences of gazelles. In a second step, we tested whether one of these feed supplements was preferred or avoided by the gazelles and whether their choice changed over time (i.e., a test period of two weeks).

Material and methods

Study area

The study was conducted at the Kızılkuyu Gazelle Breeding Station (37˚02' N, 38˚42' E) in Şanlıurfa Province of southern Turkey. The climate is semi-arid and subcontinental with extremely hot and dry summers, but cold and rainy (sporadically snowy) winters (WorldClim: Hijmans et al., 2005). The breeding station comprises an enclosure of 1.1 km^2 encompassed by a wired fence. At the time of our study, a total of about three hundred gazelles of different age and sex classes were kept in the enclosure. Daily feed requirements (dried alfalfa) for the entire

herd were determined to be 90 kg, with an average of 300 g per gazelle/day. Dried alfalfa was routinely offered *ad libitum*—also during the experiments (see below)—and occasionally gazelles nibbled on natural vegetation growing inside the enclosure.

Study species

Gazelles held at the Kızılkuyu Gazelle Breeding Station are considered natural hybrids, morphologically assigned to the goitered gazelle, *G. subgutturosa*, but with maternal haplotypes of the Arabian sand gazelle (*G. marica*; Murtskhvaladze et al., 2012, Bozkaya and Gűrler, 2018; Figure 1). Goitered gazelles are adapted to Asian arid lands, capable of surviving in extreme temperatures and with limited access to free water, largely depending on hygroscopic water from food (Blank and Li, 2022). They typically live in small herds composed of females and their young, with a dominant male during the breeding season. Outside the breeding season males are solitary or form bachelor groups (Heptner et al., 1988; Baskin and Danell, 2003). During autumn and spring, goitered gazelles migrate over large distances to follow rains and green pasture (Zhevnerov, 1984). However, remaining wild populations were forced out of their natural habitats or shortened their migrations (Sludsky, 1956b; Zhevnerov et al., 1983). Details on the natural diet of *Gazella subgutturosa* were mainly reported from the former Soviet republics (Zhevnerov et al., 1983; Zhevnerov and Bekenov, 1983; Prisyazhnyuk and Soldatova, 1984; Heptner et al., 1988; Sokov, 1993) and more recently from China (Yang et al., 2010; Xu et al., 2012; Zheng et al., 2012; Zhang et al., 2024). Forbs dominate the diet throughout the year, accounting for 50.4% of their forage. Tree intake peaked in autumn (36.7%), while shrubs (22.0%) and graminoids (14.8%) were mostly consumed during winter, suggesting that goitered gazelles seasonally adjust their foraging behaviour to thrive in arid and semi-arid environments (Zhang et al., 2024).

In contrast to the extensive knowledge on the diet of domestic ungulates, little is known about suitable rations of browse and dry fodder for rare, wild ungulates in captivity (Clauss et al. 2003). Arabian sand gazelles and Arabian mountain gazelles (*Gazella arabica*) at King Khalid Wildlife Research Centre in Saudi Arabia are fed *ad libitum* on dried, baled alfalfa and 200–300 g Superlac concentrate per animal and day (for details see Kichenside and Lindsay, 1997; Kichenside, 1998; Mohammed et al., 2002). By contrast, the Breeding Centre for Endangered Arabian Wildlife (BCEAW) in Sharjah, UAE, recommends feeding a range of locally cultivated, indigenous (e.g. *Acacia* spp.) and non-indigenous plants (e.g. *Prosopis* ssp.) to supply Arabian mountain and sand gazelles with fresh browse, together with commercially produced pellets designed for sheep and gazelle (Whitehouse-Tedd et al., 2016).

Data collection

Experiments were conducted between 17 to 30 August 2023, i.e., during the hottest month of the year. To ensure independence of daily choices, experiments were carried out irregularly and non-consecutively on eight days, with one to two days break between each choice tests. Five types of feed were tested, including black barley grain, white barley grain, white barley flake, forage wheat grain and a combination of black barley grain and white barley flake (50% each). Per feed, 20 kg of each feed type were offered at the same time in five different feeder troughs located in a central position within the enclosure. Feeder troughs were placed close to each other, allowing gazelles to view all feed types at the same time and to freely choose the feed they prefer. All gazelles were able to access the feeders and assess the feed within five minutes after the experiment started. Choice experiments were conducted at the usual feeding times, i.e., at 15:00 in the afternoon and were terminated after 1.5 hours, i.e., to prevent one feed type to get finished, prompting gazelles to switch to another feed type. Subsequently, all feeders were removed from the enclosure and the remaining feed was weighed to determine the amount of

feed consumed using a portable electronic scale (PLT Power A08). Ethical approval was obtained from the respective authorities in Turkey and the UK (see below).

Feed content analysis

One kilogram of each feed type was sampled and sent to the University of Bursa Uludag (Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases) to establish the nutritional value, namely percentage proportion of dry matter, crude ash, crude oil and crude protein using Weende analysis (Henneberg and Stohman, 1860), crude fibre and neutral detergent fibre (NDF) using Van Soest analysis (Van Soest and McQueen, 1973; Van Soest et al., 1991), and crude starch applying Carrez I and II clarifications as described in Carrez (1909) and Dougall (1956).

Statistical Analysis

In a first step, a Pearson's chi-squared test was applied to test whether the nutrient content (in percent) differed between feed types. Secondly, since the amount of each feed type was normally distributed across days (Kolmogorov-Smirnov Test, $p > 0.05$), we tested whether different feed types or different sampling days would impact the amount of feed consumed, using a two-way ANOVA. We initially included the two-way interaction term of 'food type * sampling days' in the model but removed it, due to it causing the model to have convergence issues. Examining the model residuals did not reveal any violations of the model assumptions, such as a normal error distribution or homoscedasticity (Levene's Test). Lastly, we used a post-hoc Tukey HSD test for pair-wise comparison of consumed feed between food types and between sampling days. All statistical analysis were conducted using SPSS (version 28.0).

Results

Our study identified the mixture of black barley and white barley flake as the most preferred feed type (mean ± SD: 13.6 ± 3.4 kg; Figure 2). Black barley alone was the least preferred feed type (mean ± SD: 8.9 ± 2.9 kg), while white barley flake was the second most consumed feed (mean ± SD: 10.7 ± 3.5 kg), suggesting that white barley flake was particularly attractive to the gazelles. However, the Pearson chi-squared test did not reveal any significant difference between the nutrient content of different feed types (χ^2 = 4.4497, *df* = 24, *p* = 1.0; Table 1). Despite this similarity, the two-way ANOVA indicated a significant difference in the amount of feed consumed of each feed type (*F* = 5.77, *df* = 4, *p* = 0.002; Table 2, Figure 2). The pair-wise comparison disclosed gazelles consuming significant more black barley + white barley flake mixture than only black barley grain (mean ± SE = 4.63 ± 1.20, *p* = 0.006), white barley grain (mean ± SE: 5.24 ± 1.20, *p* = 0.002) or forage wheat (mean ± SE: 3.52 ± 1.20, *p* = 0.047; Supplementary material: Table S1). Moreover, the post-hoc comparison revealed a significant higher amount of feed consumed on days 3 to 7 than on day 1, 2 or 8 (Figure 3, Supplementary material: Table S2).

Discussion

Nutrient content analysis revealed no significant difference between feed types (Table 1), indicating that feed preferences did not relate to the nutritious value of the offered feed. Other factors influencing the feed choice of (mainly domestic) ruminants include flavour, aroma and texture of the feed (Arnold et al., 1980; Provenza et al., 1996, Favreau-Peigné et al., 2013), behavioural aspects such as experience (e.g., prenatal flavour exposure, *sensu* Simitzis et al., 2008) and social organisation (Beaumont 1996; Neave et al., 2018), anti-nutritional factors like toxins or secondary plant compounds (Provenza and Frederick, 1995), environmental factors like climate and temperature (Ostrowski et al., 2006; Blank and Li, 2022), or the individual's health status (Villalba and Provenza, 2007, Villalba et al., 2010a).

Anti-nutritional compounds, such as glycosides or alkaloids, are present in commercial feeds but only at low quantities (Nte et al., 2023). Moreover, behavioural aspects can be excluded since the main feed type provided to gazelles prior to trials was alfalfa, and the prevailing social organisation was a dominance hierarchy that was established well before experiments commenced. Feed sensory characteristics, such as flavour, aroma and texture represent cues that were identified to help ruminants to anticipate the post-ingestive consequences of a feed and that will affect the learning efficiency of the individual (Favreau-Peigné et al., 2013). The aroma of ruminant feed was proven to increase dry matter intake and thus daily weight gains in young ruminants (Kalam et al., 2023). Moreover, it was reported to increase milk yield in lactating females and helps keeping good body condition during pregnancy, lactation and after birth (Kalam et al., 2023). For our study this might suggest that white barley flake (as well as the combination of white barley flake and black barley) has a certain aroma (or texture) preferred by the gazelles. Given that our experiments were conducted in August, the hottest months in southern Turkey, drought and high ambient temperatures may have caused heat stress and thus influenced the feed choice of gazelles. It might be argued that the water content of white barley is higher than that of the other feed types but given the lack of significant differences in the dry matter—white barley flake had the highest percentage of dry matter (93.95%), and thus the lowest water content of investigated feed types—this interpretation is rather unlikely. Consequently, it is recommended to conduct the same feed choice experiments during a time, e.g., in December or January, when precipitation is high, temperatures are low, and heat stress in gazelles is absent (WorldClim: Hijmans et al. 2005).

Studies on domestic ruminants revealed that their well-being depends on environmental and behavioural factors, but most important on the physical and mental health of individuals (Barrell, 2019; Mattiello et al., 2019). Hereby it was emphasized that the gastro-intestinal health plays a substantial role. Gastro-intestinal health is closely related to the amount of concentrated

feed or grains in the diet (Cooper et al., 1995), which makes it an important supplement to pure roughage, especially during the hot summer months when gazelles suffer from heat stress (Yavuz and Biricik, 2009; Dikmen et al., 2012). We thus carefully hypothesise that the feed preferences observed in this study (predominantly white barley flake) might be the best suited feed to balance the gastro-intestinal health of the gazelles. Gastro-intestinal health was also flagged of major importance in a study conducted on captive Arabian sand gazelle at BCEAW in Sharjah, UAE (Whitehouse-Tedd et al., 2016). The study recommended to supply local browse (indigenous and non-indigenous forbs, shrubs and trees), which comprised 30% of the sand gazelles' diet and which contributed 11% to the metabolizable energy intake. Unfortunately, the study did not include alfalfa or grains, but it emphasised the high starch content of pelleted diets (22–29%), suggesting that a reduction in the proportional provision of pellets (and grains which have even a higher starch content than pellets; this study) would improve the diet suitability and thus the gastro-intestinal health of the gazelles.

Moreover, low starch, i.e., low carbohydrate content in the feed of ruminants is also important to avoid the formation of ruminal acidosis. Studies on domestic livestock (i.e., dairy cattle; Nordlund et al., 1995), as well as on wild ruminants in captivity such as blackbuck (*Antilope cervicapra*), barbary sheep (*Ammotragus lervia*) and addax antelope have shown that easily digestible, high-carbohydrate feeds combined with fibre deficiency damage the rumen epithelium due to a decrease in rumen pH (Schilcher et al., 2013). Furthermore, several studies (e.g., Zenker et al., 2009; Schilcher et al., 2010) reported that laminitis, an inflammatory condition of the hooves, can occur due to ruminal acidosis if ruminants were fed with high carbohydrate diets. To consider the fibre needs of gazelles and to avoid diseases like acidosis and laminitis, alfalfa was given *ad libitum* to the gazelles at Kızılkuyu Gazelle Breeding Station. In addition, gazelles were able to forage on natural vegetation at the breeding station, increasing the amount of fibre consumed and thus reducing the risk of acidosis. This feeding regime is in

line with that proposed by Whitehouse-Tedd et al. (2016) at BCEAW, attempting to be as close as possible to the natural diet of gazelles and thus corresponding to recommendation given by IUCN/SSC (2014) according to which the feeding regime should mimic that consumed in the wild.

Our second analysis revealed significant differences in the amount of feed consumed between days (Figure 3). Initially (day 1 and 2), gazelles were carefully exploring the new feed (except the black barley / white barley flake mixture which was preferred from the first day onwards), followed by a few days (day 3 to 7) of increased consumption, and a decrease (including the mixture) on the last day. This feeding behaviour points towards a satisfaction or saturation of a specific need after some days, i.e., that amount of grain feed needed to maintain and balance the gastro-intestinal health. Gazelles seem to recognise the black barley + white barley flake mixture, and the level of consumption remained high throughout the test period. This either suggests that this mixture is beneficial to the gazelles or that optimal foraging theory may apply. Food supplies the animal with energy, while the processes of searching for and capturing that food demands both, energy and time. To enhance its fitness, a gazelle may therefore employ a foraging strategy that maximizes its benefits (high energy feed) while at the same time minimizing its costs (competition with other gazelles), thereby optimizing the net energy obtained.

Conclusion

Given the above, we recommend that the preferred feed type, i.e., the combination of black and white barley flake, should be offered as a supplementary feed to the gazelles, ideally once a month on three to five days. However, this should be alternatively to pelleted feed and not offered at the same time to prevent gazelles from consuming too much starch. To supply

gazelles with more natural browse in captivity to enhance their health and well-being should be considered in future studies.

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Ethical approval

This study was carried out after obtaining ethical approval from the Ministry of Agriculture and Forestry, General Directorate of Nature Conservation and National Parks (approval number: E-21264211-288.04-12703472). Ethical approval was also obtained from the Local Ethics Committee of Harran University (approval number: 298725) and from Liverpool John Moores University, UK (approval number: TW_AU/2024-19).

Conflict of interest

The authors declare no conflicts of interest.

Authors' contributions

AU: field work and data collection, AO: statistical analysis, ÇK: data maintenance and analysis, AU, AO and TW: manuscript wrting.

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Figure legends

Figure 1. A group of *Gazella subgutturosa* x *G. marica* hybrids at Kızılkuyu Gazelle Breeding Station in Şanlıurfa Province, Turkey.

Figure 2. Total amount of each feed type in kilogram (and percentage proportion) consumed by goitered gazelles during the feed choice experiment in Kızılkuyu Gazelle Breeding Station in Şanlıurfa Province, Turkey.

Figure 3. Daily amount of each feed type (in kilogram) consumed by goitered gazelle during the feed choice experiment in Kızılkuyu Gazelle Breeding Station in Şanlıurfa Province, Turkey.

Figures

Figure 1

507 Figure 2

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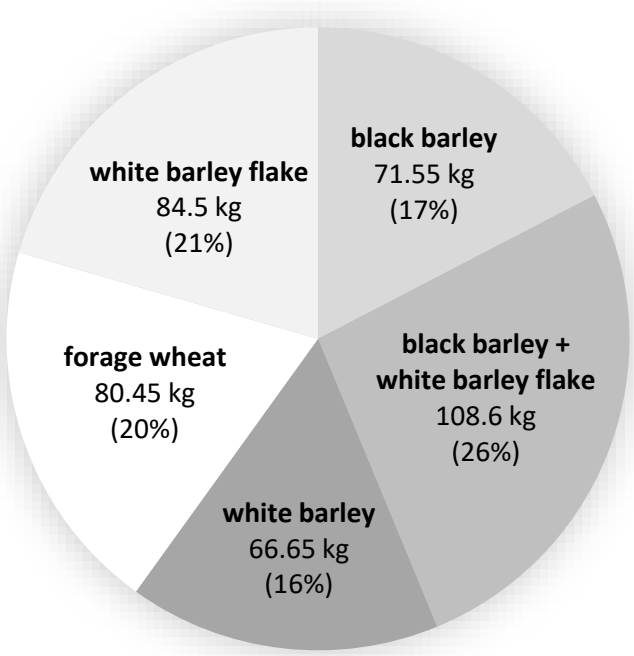

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513 Figure 3

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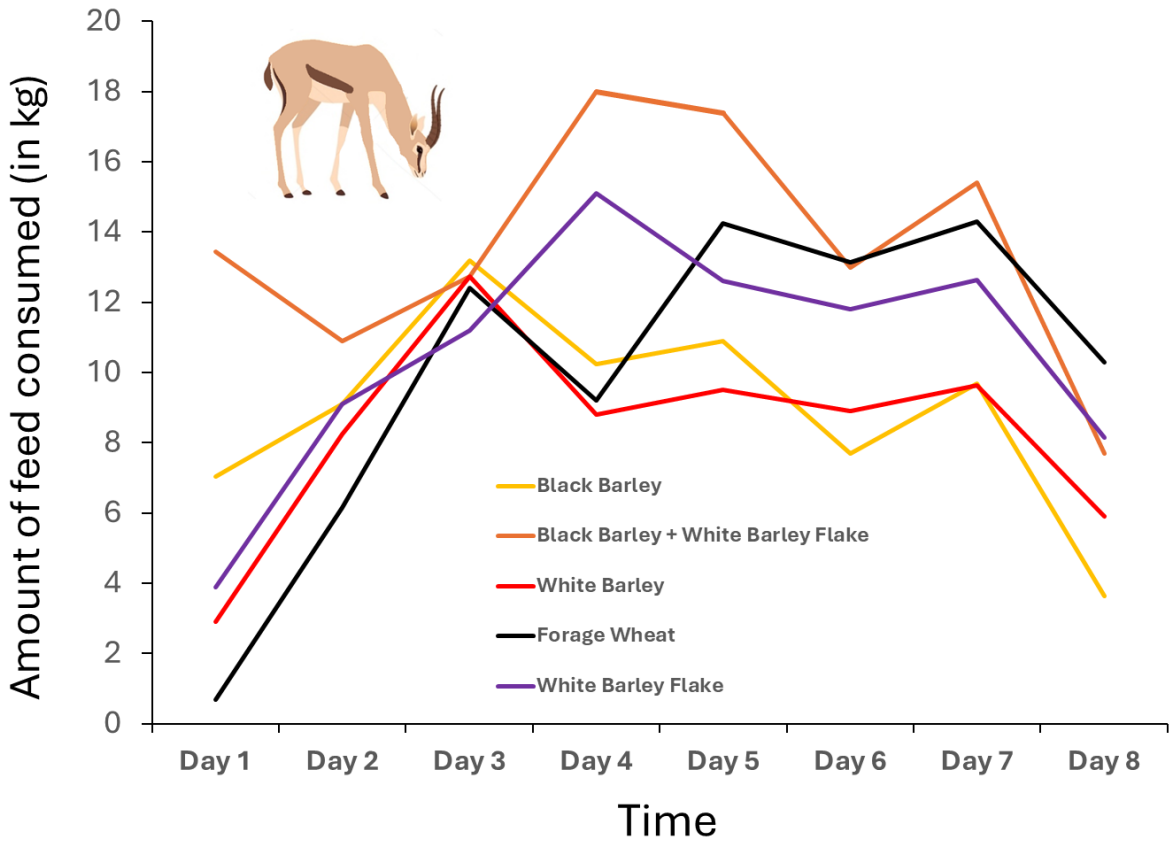

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Table 1. Nutritional value of five feed types tested during feed choice experiments (percentage proportions for dry matter, crude starch, crude ash, crude fat, crude protein and neutral detergent fibre [NDF]).

feed type	dry matter (%)	crude starch (% range)	crude ash (% range)	crude fat (%)	crude protein (%)	NDF (%)	Volumetric weights (g) in 1000 ml
black barley	92.56	45.22-46.74	2.97-3.26	2.48	10.38	20.73	740
black barley + white barley flake	93.27	43.48-45.00	2.86-2.95	2.19	10.34	20.57	660.5
white barley	93.09	45.43-46.09	2.68-2.92	2.49	10.88	20.62	723
forage wheat	93.05	50.43-52.61	1.99-2.19	1.82	11.84	14.18	783.5
white barley flake	93.95	46.30-46.74	2.95-3.19	2.09	11.04	20.41	538

Table 2. Two-way ANOVA test results of between-subjects effects (sampling days and feed type).

source	sum of squares	df	mean square	F	*p*
corrected Model	405.72[a]	11	36.88	6.43	<.001
intercept	4238.45	1	4238.45	738.47	<.001
sampling days	273.16	7	39.02	6.8	**<.001**
feed type	132.55	4	33.14	5.77	**0.002**
error	160.71	28	5.74		
total	4804.87	40			
corrected Total	566.42	39			

[a.] R squared = 0.716 (adjusted R squared = 0.605)