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Research article

Maize green forage as a partial replacement for lettuce in the diet of West Indian manatee *Trichechus manatus*

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Abstract
In zoos, West Indian manatees *Trichechus manatus* are fed mostly with different varieties of lettuce. It is a relatively expensive product and highly sensitive to improper storage conditions. In the wild, manatees feed on aquatic vegetation, whose content of dry matter, fibre and ash is usually higher than in zoo diets. However, manatees willingly ingest lettuce and reproduce on such a diet, so it is commonly used in zoos. The large amount of lettuce being fed and its high price make zoos look for alternative feeds. Maize green forage was proposed as a lettuce replacement and was introduced as a part of the diet at Wrocław Zoo (Poland) for the first time in 2019. Based on scientific literature and data from the zoo, the nutritional values of plants consumed by West Indian manatees in the natural environment and zoos were compared. The costs related to the feeding of butterhead lettuce and maize green forage were also calculated. The introduction of maize green forage increased the concentration of dry matter and fibre in the diet, and reduced concentration of the crude protein, which may have a positive influence on the functioning of the digestive system. Furthermore, the addition of maize green forage into the diet reduced the cost of feeding by 17% (€60,000) within 3 years.

Introduction

Keeping manatees in zoos requires a large financial expenditure and effort, which results mainly from the specificity of their nutrition. West Indian manatees *Trichechus manatus* are food opportunists that consume more than 60 species of saltwater and freshwater plants in the wild (Hartman 1979). These plants are of low nutritional value—high in fibre but low in protein and energy. Manatees, like terrestrial grazers, consume large amounts of low-nutrient food. In the wild, a West Indian manatee spends 6–8 hours a day foraging 5–10% of its total body weight (EAZA 2018; Hartman 1979). Due to problematic and expensive cultivation, transport and storage, institutions that maintain and breed manatees do not use aquatic vegetation in manatee nutrition, instead using different varieties of lettuce *Lactuca sativa* as the basis of the daily ration (Best 1981). So far, manatee requirements for nutrients, vitamins and minerals have not been established nor have the nutrient requirements of other species (e.g., livestock) been used as a model in manatee nutrition.

In zoos, romaine lettuce *L. sativa* var. *longifolia* is the most commonly used dietary ingredient, but it contains less dry matter (DM), ash and crude fibre than the aquatic vegetation consumed by this species in the wild (Siegal-Willott et al. 2010). Despite significant differences in the nutrient content, it is widely used. Manatees willingly ingest it, quickly gain weight and reproduce (Harshaw 2012). However, as animals with the ability to effectively ferment fibre in the hindgut, they probably require a higher concentration of fibre in the daily ration to obtain sufficient energy from hindgut fermentation and maintain proper digestive function. In Wrocław Zoo (Poland), unlike in other zoos in Europe and globally, the basis of manatee diets is butterhead lettuce *L. sativa* var. *capitata* due to its much greater availability in Poland and its lower price than the romaine variety. However, butterhead lettuce is still expensive and like other varieties of lettuce spoils quickly

if not stored in the right conditions. The sensitivity of lettuce to improper storage environments and the action of putrefactive microorganisms is a real threat to manatee health, exposing institutions that breed or rehabilitate manatees to huge financial (throwing away spoiled feed, veterinary treatment of animals) and reputational (negligence on the part of the facility) losses.

A substitute for butterhead lettuce proposed in this paper is maize green forage (MGF). Maize *Zea mays* is a crop used globally in livestock nutrition and widely grown in Europe, so its local availability is virtually unlimited. In addition, the fibre and DM content of MGF is higher than that of lettuce varieties used in manatee feed, and its elongated stems can better mimic seagrasses and prolong feed intake. The impact on feed costs is also relevant; it has a higher DM concentration so can be fed in smaller quantities, and production and transport costs are lower than for butterhead lettuce. It was hypothesised that MGF would be a better substitute for aquatic vegetation consumed by manatees in the natural environment than (butterhead) lettuce hitherto fed in zoos. Partial replacement of lettuce with MGF at the Wrocław Zoo was expected to significantly reduce the feeding costs of West Indian manatees. Therefore, the aim of this study was to evaluate the possibility of using MGF leaves and stalks to feed manatees as a substitute for the lettuce commonly used in the nutrition of these animals. On the basis of the literature and data obtained at Wrocław Zoo, a comparison was made of: 1) the chemical composition of manatees' natural food with feed available in captivity, as well as the chemical composition of MGF fed to manatees in the Wrocław Zoo; and 2) the costs of daily rations with different proportions of lettuce and MGF.

Materials and methods

Animals and data collection

All studied animals were housed at Wrocław Zoo (Poland) and remained in their usual enclosures with normal husbandry throughout the study period. The research group included five West Indian manatee individuals (two adult males, two adult females and one subadult male at the start of the study in 2019). The gradual introduction of MGF into the diet and observations lasted from 2019 to 2022.

Composition of daily rations at Wrocław Zoo

Until 2019, daily feeding rations per herd at Wrocław Zoo consisted mainly of butterhead lettuce (140 kg as fed) with an addition of 5 kg of romaine lettuce and napa cabbage *Brassica rapa pekinensis*, 13 kg of a mixture of vegetables (kale, broccoli, cauliflower, carrots, celery, fennel, chicory, red pepper and beetroot; full binomial taxon names were used only for lettuce and cabbage as components very similar in appearance and for this reason sometimes confused with each other) and 0.84 kg of feeds used in medical training—0.6 kg of Boskos Grazer and Boskos Browser pellet (WES Enterprises, South Africa) and 0.24 kg of jelly (Sea mix Unimix [IT-Ichthyo Trophic, Poland], mussels, agar). Starting in 2019, the proportion of butterhead lettuce was decreased annually to gradually accustom West Indian manatees to the new component in the diet, MGF. The animals readily accepted the new diet item (Figure 1). With each successive diet change, the amount of MGF increased, the amount of butterhead lettuce decreased and the amount of all other components remained constant (romaine lettuce, napa cabbage, a mixture of vegetables and rewards used in medical training) (Table 1). It was assumed that 1 kg of MGF is equivalent to 2 kg of butterhead lettuce (considering differences in DM levels between the two feeds). Fresh MGF (whole young maize without cobs, but mainly with young leafy material and a soft stem, 1–1.5 m in height) was fed in the summer season for four months from 15 June until 15 September each year. For the rest of the year, thawed MGL—collected and frozen during the summer season and stored in zoo freezers—was fed. Several varieties of maize—but in general those with lower FAO numbers, which are an indication of the vegetation period length—were sown at different times to ensure harvest occurred before blooming.

Chemical analysis of daily rations at Wrocław Zoo

Representative samples of butterhead lettuce and MGF—the whole shoot and parts eaten by manatees (mainly leaves and soft, upper parts of the stems)—were collected and analysed. Dry matter, neutral detergent fibre (NDF), acid detergent fibre (ADF), crude protein (CP) and ash were determined according to standard AOAC methods (Górka et al. 2016).

Composition and nutrient value of natural and zoo diets

Data on roughage fed in zoos were based on breeding recommendations for West Indian manatees from the European Association of Zoos and Aquaria (EAZA), publications treating the diet of West Indian manatees in captivity and information from official zoo websites. Information on the species of vegetation consumed by West Indian manatees in the wild was taken from Hartman (1979) and other scientific publications describing manatee diets.

Table 1. Rations of diet used in West Indian manatees nutrition at Wrocław Zoo. [1]plus additional fixed part of the diet: 5 kg romaine lettuce and 5 kg napa cabbage, 13 kg of a mixture of vegetables (kale, broccoli, cauliflower, carrots, celery, fennel, chicory, red pepper and beetroot) and 0.84 kg of rewards used in medical training (0.6 kg Boskos Grazer and Boskos Browser pellet [WES Enterprises, South Africa] and 0.24 kg of jelly [Sea mix "Unimix" (IT-Ichthyo Trophic, Poland), mussels, agar]).

Ration[1]	Year	Butterhead lettuce (kg)	Maize green forage (kg)
Ration 0	until 2019	140	0
Ration I	2019-2020	120	10
Ration II	2020-2021	100	20
Ration III	2021-2022	80	30

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Figure 1. West Indian manatee at Wrocław Zoo feed on maize green forage

Comparison of natural and zoo diets

Comparison of nutrient content in vegetation consumed by manatees in the natural environment with the diet in captivity and in the Wrocław Zoo was based on calculated weighted averages and ranges of values (minimum and maximum). The weighted average for plants consumed in the natural environment was calculated by assigning weights based on the percentage share of individual plant groups in the diet of the West Indian manatee from Allen et al. (2018). These weights were 81.06% for seagrasses, 2.48% for algae and 6.72% for vascular plants (the remaining few percent were not assigned to specific groups of plants or invertebrates so were not included in these calculations). For feed eaten in zoos, nutrient value ranges were determined that included the extreme values of a given ingredient in the group of all the plants listed. The nutrient content of butterhead lettuce and MGF fed at Wrocław Zoo were separately compared to the nutrient values of vegetation eaten in the wild. Such a comparison was also carried out for the average concentration and provision of nutrients contained in individual feed rations (Table 4). Based on the diets used at Wrocław Zoo with different proportions of butterhead lettuce and MGF, the estimated dietary chemical composition was calculated (concentration and daily feed supply of DM and NDF, ADF, CP and ash expressed in % DM and kg/day/herd respectively). Calculations for concentrations used weighted averages, where the weights were the amount of specific feed (butterhead lettuce and MGF) fed in the ration. The dietary nutrient supply was calculated as the ratio of the concentration of each nutrient to the amount of feed fed.

Feed purchase costs

Component prices were provided by the zoo. All prices were given in Polish złoty (PLN) but converted into EUR (National Bank of Poland; EUR 1=PLN 4.47, as of 15 July 2020). Two prices are given for each ingredient: for February 2020 (winter season) and July 2020 (summer season). The exception is MGF, which was purchased only in the summer. Based on the quantity of the daily ration and average prices, the annual purchase cost of each component of the ration was calculated.

Freezer work and cost calculations

Two freezers (13.5 kW, Thermo King and 11.55 kW, Daikin Industries Ltd.) were used for MGF storage. The freezers were loaded with maize from 15 July to 30 August and contained about 4,000 kg of MGF each. They were replenished sequentially until full. During loading, the freezers worked with the highest power (about 80%) and consumed the most electricity. After loading, the freezers maintained a constant temperature until unloading and were operating at about 20% capacity. Unloading of the first freezer began on 16 September and about 40 kg of frozen MGF

was taken out every two days. During unloading the freezers were assumed to operate with about 25% power. Unloading of the second freezer started after the first was emptied and lasted until it was completely empty, i.e., until fresh feed was used again (at about 15 June). Freezer lifespan was not specified, so an average lifespan for this type of equipment was assumed after consultation with a refrigeration specialist. The cost of purchasing one freezer was divided by its lifespan to determine an estimated annual purchase cost. The assumed estimates of freezer capacity influenced the calculated cost of freezer use, but had little impact on the total sum of manatee feeding costs (calculated, but not presented in the final results). Power consumption was calculated using the number of days each freezer was in operation during the given periods (loading, storing, unloading). Each of these values was then multiplied by the power consumption corresponding to a given period of freezer operation. The results were added up for each freezer. The average annual electricity consumption was calculated and multiplied by the current electricity price for Wrocław Zoo to obtain the average annual cost of using one freezer.

Comprehensive analysis of feeding costs

In order to compare the total cost of feeding each ration, the annual costs of purchasing feed and purchasing and working the freezers used in each ration were added together. Based on the results of these calculations, the profitability of the rations used at Wrocław Zoo was compared.

Results

Chemical composition of natural diet

Table 2 presents data on the nutritional value of vegetation consumed by West Indian manatees in the wild. Due to the paucity of publications describing the nutritional value of plants, six species were used to create the table and assigned to the following groups: seagrasses, algae and vascular plants. Also presented is the average concentration of nutrients in the estimated diet eaten by manatees in the wild, taking into account all groups of these plants (hereafter referred to as natural vegetation).

Comparison of nutritional value of natural and captive diets

Table 3 summarises the nutritional value of feeds used as the main roughage in captivity in manatee nutrition with distinction from Wrocław Zoo. The range of individual nutrient concentrations in these feeds differs from those in natural vegetation. However, there are some similarities. Napa cabbage has NDF, ADF and NFC values (% DM) that fall within the natural vegetation range. Wheat has similar values for NDF, ADF and CP (% DM). Similarities in terms of CP content are also shown by elephant grass and barley. On the other hand, romaine lettuce (both June and December harvests) has similar values only for acid detergent lignin. None of the feeds contained in Table 3 correspond to the DM, ether extract and ash content of natural vegetation. Comparing feeds used at Wrocław Zoo to natural vegetation shows that butterhead lettuce has values corresponding to natural vegetation only for NDF, and MGF only for CP. The other nutrients of both feeds differ from those of natural vegetation, in particular ash, which is two times lower in butterhead lettuce and five times lower in maize. NDF content in MGF is two times higher than in natural vegetation.

Estimated chemical composition of the diet rations with different proportions of butterhead lettuce and MGF at Wrocław Zoo is presented in Table 4. The concentration of DM in all rations is lower than for natural vegetation. The concentration of NDF (% DM) increases in subsequent rations, and is within the range of natural vegetation only in ration 0. In rations II and III, NDF exceeds the natural vegetation range by a few percent. The ADF

Table 2. Nutritional value of vegetation eaten by West Indian manatees in the wild. DM, dry matter; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; CP, crude protein; EE, ether extract; CF, crude fibre; NFC, non-fibrous carbohydrates; DE, digestible energy for horses; N/A, not analysed; [1]seagrass (weight 81.06); [2]algae (weight 2.48); [3]vascular plant (weight 6.72); [4]Siegal-Willot et al. 2010; [5]Heuzé et al. 2015; [6]Sokoloff et al. 1949; [7]weighted average of all plants were calculated using the weights assigned to each group of plants based on the information on the percentage share of individual plant groups in the diet of the West Indian manatee contained in the work of Allen (2016); [8]calculated without values for *Eichhornia crassipes* and *Rhizophora mangle*

	%	%	% dry matter (DM)								kcal/kg DM
Plant	Ingested part	DM	NDF	ADF	ADL	CP	EE	CF	Ash	NFC	DE
Thalassia testudinum[1,4]	Rhizomes	19-20	18-23	11-18	0.9-1.4	8-12	0.4-0.5	N/A	61-67	12-19	623-886
Thalassia testudinum[1,4]	Leaves	16-28	21-39	14-26	1.7-2.0	9-13	0.4-0.8	N/A	53-59	9-25	787-1321
Halodule wrightii[1,4]	Rhizomes	21-27	19-32	10-19	1.0-2.6	10-17	0.5-1.2	N/A	48-50	19-21	557-1248
Halodule wrightii[1,4]	Leaves	23-25	23-32	17-19	1.4-2.3	8-18	0.6-0.8	N/A	34-41	18-34	956-1615
Syringodium filiforme[1,4]	Rhizomes	26-30	16-30	12-27	0.9-2.4	6-8	0.5-0.9	N/A	52-70	17-24	1220-1721
Syringodium filiforme[1,4]	Leaves	17-19	20-28	14-18	1.5-2.6	11-16	0.8-1.4	N/A	40-51	17-22	1270-1460
Chara sp.[2,4]	Leaves	17	9	5	1.2	10	0.9	N/A	56	25	1254
Eichhornia crassipes[3,5]	Whole plant with roots	6	57	33	N/A	18	2.0	23	21	N/A	N/A
Rhizophora mangle[3,6]	Leaves	24	N/A	N/A	N/A	14	2.9	14	7	N/A	N/A
Average[7]		20-24	22-32	14-22	1.2-2.28	9-14	0.7-1.1	N/A	46-53	15-248	904-1375[8]

Table 3. Nutritional value of feeds used as the main roughage in feeding of the West Indian manatee in captivity and at Wrocław Zoo. DM, dry matter; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; CP, crude protein; EE, ether extract; CF, crude fibre; Ash; NFC, non-fibrous carbohydrates; DE, digestible energy for horses; n.a., not analysed; [1]Heuzé et al. 2020; [2]Yang 2011; [3]Strzetelski et al. 2014; [4]Siegal-Willot et al. 2010; [5]Song et al. 2020; [6]Hopkins 2003; [7]Morales et al. 2010; [8]Francis et al. 2018; [9]EAZA 2018; [10]University of Agriculture in Kraków - average of analyses carried out in years 2018-2020; [11]parts of the plant most eagerly eaten by West Indian manatees in the Wrocław Zoo (mainly leaves and soft stem parts).

Feed	Type	%	% dry matter (DM)								kcal/ kg DM
		DM	NDF	ADF	ADL	CP	EE	CF	Ash	NFC	DE
Used in captivity											
Elephant grass[1]	aerial part, fresh	17.9	71.5	42.5	5.7	9.7	2.0	36.1	13.8	N/A	N/A
Banana plant[2]	leaves, fresh	19.1	69.4	40.0	N/A	11.4	4.0	N/A	N/A	N/A	N/A
Alfalfa[3]	whole plant, fresh	18.9	51.3	34.3	N/A	17.8	3.7	31.5	12.1	N/A	N/A
Romaine lettuce[4]	leaves, fresh, June harvest	5.0	13.0	12.0	1.9	24.0	2.2	N/A	11.0	41.0	2758
Romaine lettuce[4]	leaves, fresh, December harvest	8.0	15.0	13.0	2.1	25.0	3.6	N/A	18.0	51.0	3212
Napa cabbage[5]	mainly leaves, fresh	6.3	28.8	17.7	4.6	20.2	2.0	13.8	29.2	24.7	N/A
Italian rye-grass[6]	whole plant, fresh	17.3	45.3	27.3	4.3	20.0	N/A	N/A	N/A	N/A	N/A
Barley[7]	whole plant, fresh, hydroponic	16.1	N/A	N/A	N/A	13.5	2.6	16.3	4.8	N/A	N/A
Wheat[8]	whole plant, fresh, hydroponic	15.6	29.7	19.9	N/A	15.7	5.8	N/A	2.2	N/A	2870
Wheat[9]	whole plant, fresh, hydroponic	13.0	N/A	N/A	N/A	21.5	3.8	N/A	1.5	N/A	N/A
Used at Wrocław Zoo											
Butterhead lettuce[10]	whole plant, fresh	4.0	27.8	25.7	N/A	26.9	N/A	N/A	26.0	N/A	N/A
Maize[10]	whole plant, fresh	17.5	54.6	30.5	N/A	9.4	N/A	N/A	5.0	N/A	N/A
Maize[10]	eaten[11], fresh	15.5	54.1	28.5	N/A	11.1	N/A	N/A	5.1	N/A	N/A

and CP values are outside the ranges of natural vegetation, while the ash for each ration is lower than for natural vegetation.

Diet costs

Table 5 presents the cost of feeds used in diets with different proportions of butterhead lettuce and MGF, including the costs of an additional fixed part of the diet, and costs of purchasing a freezer and its maintenance. Despite the increasing number of freezers used, the total cost of feeding shows a decreasing trend as the proportion of maize in the diet increases (ration 0>ration I>ration II>ration III). As a result, almost EUR 60,000 was saved over three years.

Table 4. Estimated chemical composition of the rations with different proportions of butterhead lettuce and maize green forage fed to West Indian manatee in the Wrocław Zoo. DM, dry matter; NDF, neutral detergent fibre; ADF, acid detergent fibre; CP, crude protein; [1]The basis of the diet is 140 kg of butterhead lettuce; [2]The basis of the diet is 120 kg of butterhead lettuce and 10 kg of maize green forage; [3]The basis of the diet is 100 kg of butterhead lettuce and 20 kg of maize green forage; [4]The basis of the diet is 80 kg of butterhead lettuce and 30 kg of maize green forage.

	%	% dry matter (DM)			
Ration	DM	NDF	ADF	CP	Ash
Ration 0[1]	4.0	27.8	25.7	26.9	26.0
Ration I[2]	4.9	29.8	25.9	25.7	24.4
Ration II[3]	5.9	32.2	26.2	24.3	22.5
Ration III[4]	7.1	35.0	26.5	22.6	20.3
kg/day/herd					
Ration	DM	NDF	ADF	CP	Ash
Ration 0[1]	5.6	1.6	1.4	1.5	1.5
Ration I[2]	6.4	2.2	1.7	1.5	1.3
Ration II[3]	7.1	2.8	1.9	1.4	1.2
Ration III[4]	7.9	3.4	2.1	1.4	1.1

Discussion

Data available in the literature allowed the preparation of a list of nutritional values for natural vegetation based on six plant species from three different groups: seagrasses (n=3), algae (n=1) and vascular plants (n=2). These species represent less than 10% of all plant species consumed by West Indian manatees in the wild (Hartman 1979). Therefore, comparison of the nutrient content of the natural diet with those fed in zoos may not be accurate. However, a significant part of the data used in this work is cited in scientific publications, where the nutritional values of this narrow group of plant species are treated as a reliable source of information on nutrients in the natural diet of manatees (Allen et al. 2018; Rodrigues et al. 2021). On this basis, it was assumed that the data may be a valuable reference when assessing the similarity of the zoo diet to the natural diet.

The nutrient content of West Indian manatee feed in zoos hardly corresponds to that of vegetation consumed by this species in the wild, at least compared with data found in the literature. Large differences were also observed between the chemical composition of butterhead lettuce and MGF. For example, MGF has a higher DM concentration, although neither attain that of natural vegetation. The introduction of MGF into the manatee diet and the reduction of butterhead lettuce increases the average dietary DM concentration; however, even with the complete removal of butterhead lettuce in favour of MGF, this value will still not be within the natural vegetation range. This shows how hard it is to replicate the food available in the wild for wild animals kept in zoos. For terrestrial animals, including farm animals, the DM level of feed determines, among other things, the palatability of the food or its daily intake (e.g., in dairy cows, Lahr et al. 1983). In zoos, it is assumed that manatee feed should be soft, mimicking natural vegetation, and therefore not dried or desiccated (above 50–60% DM). It is also suspected that hay may cause manatees to develop colic (Harshaw 2012). On the other hand, EAZA (2018) dietary recommendations for manatees indicate that dried timothy or alfalfa can be added to the diet. However, it is not known what effect, if any, dietary DM levels have on manatee feed intake.

One of the most important aspects of dietary chemical composition in the context of feeding herbivores in zoos is fibre content, which should be a major source of energy and guarantees proper functioning of the digestive system (Clauss and Dierenfeld 2008). The addition of MGF to the diet significantly increases NDF concentration; it is slightly too high in the diet with the greatest MGF content (ration III). There is an increased supply of DM (41%) and fibre fraction (NDF up to 110% and ADF 50%) and slightly reduced CP and ash compared to the baseline diet (ration 0). Studies on cattle indicate that NDF is negatively correlated with DM intake and ADF is negatively correlated with energy in the feed. Thus, increasing fibre concentration of feed reduces its digestibility (Beauchemin 1996). Similar relationships are observed in horses (Sharpe 2019). Notably, NDF concentration in the rations fed to these livestock species is twice as high as the highest NDF concentration in the current study (ration III). However, the effect of increased fibre intake on the digestive tract of West Indian manatees is unknown (Harshaw 2012). Furthermore, most facilities keeping manatees base their diets on romaine lettuce, which has lower concentrations of individual fibre fractions than natural vegetation and analyses for manatees in scientific publications were conducted on the basis of these diets (Harshaw 2012; Larkin 2007; Siegal-Willott et al. 2010). By contrast, the Singapore Zoo relies heavily on banana leaves as a main part of the diet, which have even higher concentrations of NDF and ADF than MGF (F. Cabana, personal communication). Therefore, it is difficult to unequivocally state the appropriate level of dietary fibre for these animals. However, at Wrocław Zoo, faecal consistency changed from water-dissolving, uncompacted faeces to better quality, compact and solid faeces after the introduction of MGF into the diet (Figure 2). In addition, no increased incidence of gastrointestinal disorders was observed and manatees continue to successfully reproduce and maintain their body condition. Due to anatomical adaptations for fermentation in the final sections of the digestive tract, long retention time and twice higher efficiency in fibre digestion by manatees than by horses or elephants (Harshaw 2012; Marsh et al. 2011; Reynolds and Rommel 1996), it can be assumed that manatees cope well with digesting higher

Table 5. Annual costs (€) of different rations at Wrocław Zoo[1]. [1]all costs based on 2020 prices (€); [2]5 kg of romaine lettuce and 5 kg napa cabbage, 13 kg of a mixture of vegetables (kale, broccoli, cauliflower, carrots, celery, fennel, chicory, red pepper and beetroot) and 0.84 kg of prizes used in medical training - 0.6 kg of Boskos Grazer and Boskos Browser pellet (WES Enterprises, South Africa) and 0.24 kg of jelly (Sea mix "Unimix" [IT-Ichthyo Trophic, Poland], mussels, agar); [3]The basis of the feed diet is 140 kg of butterhead lettuce; [4]The basis of the feed diet is 120 kg of butterhead lettuce, 10 kg of maize green forage per day and 1 freezer; [5]The basis of the feed diet is 100 kg of butterhead lettuce, 20 kg of maize green forage per day and 2 freezers; [6]The basis of the feed diet is 80 kg of butterhead lettuce, 30 kg of maize green forage per day and 3 freezers.

Ration	Maize green forage	Butterhead lettuce	Additional fixed part of the diet[2]	Purchase and use of a freezer	Sum	Costs difference (%)
Ration 0[3]	0	98,428	16,291	0	114,717	0
Ration I[4]	1,633	84,366	16,291	2,537	104,827	-9
Ration II[5]	3,266	70,305	16,291	5,073	94,935	-17
Ration III[6]	4,899	56,244	16,291	7,610	85,044	-26

amounts of fibre. Nevertheless, detailed studies are necessary to confirm this. It would also be helpful to analyse the DM of manatee faeces in the wild and in zoos, on diets with different levels of fibre. Unfortunately, it is difficult to collect faeces because manatees defecate directly into the water.

Observations in zoos indicate that manatees live and reproduce on diets with low (lettuce), moderate (lettuce with MGF added) or high (lettuce with banana leaves) fibre concentrations. Probably, possible energy deficiencies resulting from reduced nutrient digestibility due to too high fibre intake are compensated by the already high proportion of other components with a high concentration of easily digestible energy in the diet, such as vegetables, pellets and invertebrates. Confirmation of this hypothesis is provided by the similar body condition of manatees both during the period when maize was not used (2019) and when it accounted for up to 20% of the diet (2021–2022). Studies on many species of domesticated and wild animals (e.g., hindgut-fermenting elephants) show that body condition is directly correlated with body fat levels and thus the energy status of animals (Morfeld et al. 2014). Therefore, it can be concluded that such a high supply of MGF is unlikely to have a negative effect on the energy status of the manatees.

Another nutrient that is usually balanced in rations for animals is protein. The concentration of CP (% DM) in butterhead lettuce is much higher than in natural vegetation. The addition of maize to the diet lowers this value, but MGF would have to make up the majority of the diet to bring the CP value into the natural vegetation range. Too high CP content is suspected to contribute to obesity and other nutritional disorders in manatees (Harshaw 2012). Excess CP intake can also increase nitrogen excretion in urine and may cause a reduction in water quality.

The ash value reflects mineral content and inorganic impurities in the feed. Its high content in natural vegetation may be related to the incrustation of calcium carbonate by organisms in marl (Best 1981). Lower levels of ash in MGF and butterhead lettuce may affect microbial fermentation and functioning of the digestive tract in manatees (Siegal-Willott et al. 2010). On the other hand, considering the way manatees forage (collecting vegetation in underwater sandy meadows), ingested natural vegetation may be high in ash or, alternatively, the high level of ash may result from incomplete cleaning of sand from natural vegetation prior to ash analysis. A similar phenomenon was observed in the Feed Laboratory during ash analysis in butterhead lettuce, which contains huge amounts of sand between the leaves. Thus, unwashed lettuce may have up to twice as much ash as lettuce thoroughly rinsed before chemical composition analysis. In a zoo, it is impossible to wash lettuce thoroughly to remove sand due to the large quantities of lettuce that are fed every day; each head would have to be washed by hand, leaf by leaf. According to the specific foraging characteristics of manatees, they should be well adapted to food with a high ash concentration (e.g., possessing a specific gastrointestinal structure), which is also observed in zoos through the low frequency of colic, but manatees are not adapted to the highly abrasive properties of sand (Reep and Bonde 2021). Manatee teeth are replaced throughout life and the rate of replacement is likely to depend on the mechanical stress associated with chewing fibrous plant food—the more shedding associated with grinding fibre, the faster the rate of tooth replacement (Domning and Hayek 1984). However, sand is much more abrasive than plant tissue. Even in combination with soft lettuce leaves (which have a low fibre content), tooth wear can occur much faster than tooth replacement. There are silica bodies (phytoliths) in maize leaves (as in most monocotyledonous plants) (Piperno 2009), which have abrasive properties. However, their effect on dental abrasion may be smaller and more balanced. Phytoliths are smaller (2–250 µm) than sand particles (0.05–2 mm) (e.g., Müller et al. 2014); moreover, they are evenly distributed and possibly act more like fine sandpaper, causing uniform teeth abrasion. However, these are conjectures and speculations—without further research, it is impossible to clearly determine how zoo diets affect tooth wear in manatees and the clinical relevance. In summary, partially replacing butterhead lettuce with MGF may have reduced the supply of sand and increased the stimulation of tooth replacement by introducing more fibre into the diet.

None of the analysed roughages reflect the nutritional value of natural vegetation. However, hydroponic wheat and napa cabbage can be distinguished among these feeds, in which the concentrations of NDF and ADF (% DM) are within the range corresponding to natural vegetation. Unfortunately, hydroponic cultivation of cereals is not widely practised in Poland (Gembicka 2020), which limits access to feed and probably increases its price. Napa cabbage, like butterhead lettuce, is susceptible to diseases and difficult in store (Wojdyła et al. 2018). Nevertheless, given its chemical composition and economic considerations, it may be a better option than butterhead lettuce. Its nutritional value is closer to natural vegetation and its price per kg is 2.5 times lower than for butterhead lettuce (EUR 0.68 versus EUR 1.93 per kg). Replacing butterhead lettuce with napa cabbage would slightly improve the quality of manatee nutrition. It is impossible to select a perfect replacement for natural vegetation. A better solution would be to introduce a mixture of a greater number of roughages and to select their proportions to achieve values within the natural vegetation range. In addition to nutritional values, a number of other factors should be considered when designing such a diet: availability of each component, storage methods,

Figure 2. Faeces of a West Indian manatee at Wrocław Zoo feeding on a diet with maize green forage (Ration III)

duration of freshness in storage conditions, feeding methods and, due to the large quantities of food that manatees consume, the cost of all components.

Analysing the issue of feeding manatees in terms of economics, introducing MGF while reducing butterhead lettuce is very cost-effective. The costs associated with feeding diets based on MGF and butterhead lettuce are lower than the costs of feeding a diet based on butterhead lettuce alone, despite the need to purchase and maintain a freezer. The cost of feeding with ration 0 is about EUR 115,000 per year, without taking into account losses due to spoilage or costs of cold storage (storage is not included because feed for other animals is stored together with butterhead lettuce for manatees at Wrocław Zoo). In comparison, the annual cost of feeding with ration III, i.e., with the largest addition of maize, is almost EUR 30,000 less, even taking into account the purchase and use of additional equipment. The susceptibility of butterhead lettuce to diseases and associated spoilage generates additional costs. Lettuce diseases such as bacterial soft rot or grey mould infect lettuce on the farm. Their manifestation is associated with poor agro-technical conditions (inappropriate humidity, airiness and insolation; Janas and Grzesik 2017). Butterhead lettuce is also exposed to inappropriate conditions during its distribution to and storage at the zoo. Each lettuce delivered to the department must be carefully inspected and discarded if spoiled. Optimal storage conditions for butterhead lettuce can only be achieved by using a special cold chamber in which the atmosphere is strictly controlled (CO2=2.5%, O2=2.5%). Consultation with a refrigeration specialist determined that the cost of manufacturing and installing such a cold chamber (size 6×3 m) could exceed EUR 35,000 and retrofitting existing equipment could reach EUR 22,000. Keeping butterhead lettuce in optimum conditions can extend its lifespan up to 75 days (Salunkhe et al. 1972). This exceeds the zoo's requirements (storage for a few days) but provides a constant supply of fresh feed. Therefore, the correct way of feeding lettuce (requiring additional specialised cold storage) further increases the already high cost of this product.

Despite the use of freezers to store maize outside the summer period, its use seems to be more sustainable and the possibility of freezing it is an advantage. MGF is purchased from a local farmer only a few kilometres away from the zoo. As transport is limited to the summer season when MGF is available, use of fuel and other resources is minimised. Butterhead lettuce in Poland is usually grown in spring and summer, and for the rest of the year is imported from Spain or Italy, which increases the amount of fuel and resources needed. Depending on the season, whole shoots of MGF are fed fresh or thawed and both are readily consumed by manatees. Additionally, when placed in a metal grate and submerged, MGF mimics natural vegetation to some extent and allows consumption at different depths. The maize stalks are long and have protruding leaves—if manatees manage to pull the entire plant out of the feeding trellis, tearing off a piece of the plant requires effort due to the need to properly manipulate the flippers and grasping lips (Figure 1). Feeding butterhead lettuce is easier and requires less effort from the manatees as it is thrown into the water and floats on the surface. Therefore, feeding MGF can provide a valuable form of dietary enrichment and encourage the expression of natural behaviour in manatees. Manatees have a natural preference for submerged vegetation, followed by floating vegetation and then emergent vegetation (Hartman 1979).

Water quality due to differences in faecal consistency (with firmer faeces on MGF), may be another advantage that was not assessed in the present study. Water parameters were not tested before and after the introduction of maize into the diet. Therefore, it is difficult to determine the impact on microbiological aspects of filtration. However, there was a mechanical effect on the filters—lettuce as a low fibre and easily digestible component caused formation of faeces that looked like 'fog' in the water after defecation. Therefore, visibility of the animals to zoo visitors was limited but the filters were not affected as the faecal particles were tiny. However, in the case of maize, the faeces contained more indigestible particles, mostly corn stalk fragments. At the very beginning of the diet change, thick, long, woody pieces of maize stalks that were not eaten by the manatees were drawn into the filtration system. There, they accumulated and formed blockages at 90-degree pipe connections in the filtration system. This problem has been solved by harvesting maize forage earlier (dependent on the variety of maize and its growing conditions, climate etc., to be determined experimentally in each zoo). Once these leftovers were in the filter, a solids separator was used, where large particles of feed or faeces are separated and thrown into a container (as also routinely used in hippopotamus pools).

Given the changing chemical composition of MGF during the growing season (e.g., changes in DM, fibre and protein), it is advisable to sporadically analyse the chemical composition of forage during subsequent weeks of growth, in order to optimise the timing of harvesting and better match the chemical composition of natural vegetation.

Taking all of the above into consideration, neither lettuce nor maize are ideal substitutes for natural vegetation. However, the addition of maize to the diet may reduce the cost of feeding, increase fibre intake and be more sustainable. The manatees at Wrocław Zoo fed a diet based on a mixture of butterhead lettuce and MGF are in good condition, and in 2018–2020 the females gave birth to four juveniles. It is assumed that subtracting another portion of butterhead lettuce from the diet would continue to provide sufficient nutrient supply and bring further benefits to the manatees and the zoo.

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