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APPARENT METABOLISABILITY OF DIETS CONTAINING CONCENTRATE, *ZOSTI NOLTII OR TARAXACUM OFFICINALIS IN ANAS PENELOPE*

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ABSTRACT

Four metabolisability trials on captive Wigeons were conducted comparing a complete pelleted diet with others where Taraxacum officinalis and Zostera noltii were added. The daily dry matter (DM) intake of Wigeons varied from 54.4 to 65.5 g/day and the amount of nutrients received from the four diets was similar. The nitrogen (N) content of droppings statistically diminished when Wigeons were fed diets containing Zostera noltii. Correlation among cell wall components (CWC) of droppings and that of intakes was always highly significant and positive. The DM metabolisability of the four diets was 42-51 %. The crude protein (CP) metabolisability varied significantly from 21 % for the diet with Taraxacum to 39 % for that with the Zostera collected in June. The metabolizabilities of CWC also differed significantly among diets. The apparent metabolizabilities of ash with the Zostera diets were significantly higher when compared to those of two other diets.

Keywords: Wigeons, Bird captivity, Metabolisability, *Zostera noltii*, *Taraxacum officinalis*.

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Contribution/ Originality

This study is one of very few studies, which have investigated the metabolic responses of captive Wigeons (*Anas penelope*) to four different diets and compared the results of nutritional aspects to those of their wild counterparts.

1. INTRODUCTION

The Eurasian Wigeon, *Anas penelope*, the smallest grazing Anseriform, is a migratory herbivorous bird, flying from the northern regions in early autumn to winter to warmer southern coastal areas and wetlands until early spring in order to feed on nutrient rich plants high in protein and low in fibre [1, 2]. Ring recoveries suggest that the reproductive area of Wigeons wintering in northern Italy are found across different Palearctic regions. Migrations towards wintering sites begins during the months of September and October while spring back-migration occurs during March and April. If the reproductive sites do not ensure ideal conditions for moulting, Wigeons may make an earlier juvenile or post-reproductive and pre-moult migration to safer and more adequate southern feeding grounds [3]. Many environmental investigations deal with the role of this widespread dabbling duck, migratory and herbivore, on the ecology of wet grassland or marshes.

Early research carried out on Wigeon dietary habits dates back to the turn of the century [4]. It was reported that this dabbling duck fed primarily on phanerogame species on coastal mudflats [5] Its diet consists mainly of leaves, shoots, and seeds of *Enteromorpha spp.* and *Zostera spp.*, and of a varying, but a significant amount of molluscs, chironomid larvae, and arthropods [6, 7]. The worldwide classified *Zostera spp.* are 16 [8]. *Zostera noltii*

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(Hornemann) and *Z. Marina* (L.) are widespread along the tidal zones of the European Atlantic [9] and Mediterranean coasts [10, 11] where it flourishes in perennial meadows [12, 13]. Rhizomes secure it to the sandy or muddy substrates forming beds. *Zostera* take root in April and maximum development stems and leaf development is reached over a short period of time. In autumn the leaves reach a length of 30-40 cm, when the incidence of generative shoots is higher [14]. Short stems grow out from extensive, white branching rhizomes. More or less mature inflorescences and infructescences can be found from July to October; mature seeds are released in autumn and the natural decay of the plant is triggered with the onset of winter [15].

The ribbon-like leaves and shoots of this plant are eaten in large quantities (about 80 %), while rhizomes and stems make up about 5 % of the diet, and seeds about 10 % [16]. The submerged or floating vegetation is intensively grazed most of the day and night, preferably during ebbtide. Other authors also reported regular inland feeding, mainly on saltmarshes (*Puccinellia and Salicornia spp.*) but also on flooded inland pastures (*Glyceria*, *Festuca*, *Poa pratensis*, *Agrostis stolonifera*, *Lolium perenne*, *Dactylis glomerata* [17] *Ranunculus repens*, and *Trifolium repens* [18-20]. These plants differ in nutrient content and vegetal structure and consequently in palatability and digestibility. The *Taraxacum spp.* is consumed by the Wigeons together with other inland plants to integrate the *Zostera* diet when necessary, as during reproduction, moulting, and before migration.

A Wigeon, weighing about 700 g, is subject to the digestive constraints of small vertebrate herbivore [21]. Like other *Anatidae* it has a simple gut and its caeca are not as well developed as other avian grazers, so it relies on ingesting very large quantities of food, which passes through the gut rapidly and is only in part digested [22]. The two caeca, positioned backwards along the terminal portion of the ileum, are blind sacs consisting of a narrowly constricted open end and a dilated thinner-walled blind portion. The caeca retain the liquid and soluble components of the intestinal contents for long periods. Caecal length and activity in herbivorous birds are influenced by captivity [23, 24] fibre content of the diet [16, 25, 26] and ingestion rates [27].

Studies on apparent digestibility or metabolisability of feedstuffs by Wigeon are few. E.g., according to Mayhew [5] the average digestive efficiency of dry matter (DM) by Wigeons is 28.8 %; [2, 28, 29] considering that some grouse and waterfowl digest 15-35 % of the cell wall components (CWC).

The present study was conducted on *Anas penelope* kept in captivity to measure the apparent metabolisability of four diets containing similar amounts of concentrate pellet (the habitual feed, given alone), *Zostera noltii* and *Taraxacum officinalis*. Two successive trials were performed to compare *Zostera* collected in June, which should represent the feed consumed during reproductive and moulting period, to that vegetating in late September, at the time when Wigeons arrive for wintering. *Taraxacum*, a perennial wild herb native to the northern hemisphere, was also given as it is a common species along the lagoon banks of our region and represents a widespread source of complementary forage for Wigeons. The results obtained on captive animals at an experimental facility were compared with those previously published obtained on wild animals.

2. MATERIALS AND METHODS

Four trials were carried out to test the apparent digestibility. This consisted of administering either a pelleted complete feed (P), feed alone as a control, or with *Zostera noltii* collected in June(PZJ) and in late September (PZS), in the Grado and Marano lagoons (Friuli Venezia Giulia – Italy), or *Taraxacum officinalis* (PT) leaves, harvested just before flowering (April) at the University farm, where the experiments were conducted.

The same 4 three-year-old European Wigeons (2 males and 2 females) born and reared on the farm were used. The animals were habitually housed outdoors, in an aviary and given *ad libitum* pellets of a complete feed (Table 1) and on occasion some seasonal fresh forage available on the farm.

To establish the amount of daily feed to administer during digestibility trials, preliminary feeding tests were carried out offering forage (*Taraxacum officinalis* and *Zostera noltii*) *ad libitum* to accustoming Wigeons to the feed and to appraise their palatability. It appeared that *Taraxacum* was more palatable than concentrate, while the daily intake of *Zostera* appeared to be less regular and residues were always recovered in the feeders. To maintain a comparable daily intake and digestibility condition, even with less palatable *Zostera*, as well as prevent weight loss during the experimental periods, the original idea was that of administering a maintenance diet consisting of at least an equal amount of pellet and forage on a wet basis. Given the chemical differences among forages, the experimental diets were formulated to obtain the chemical characteristics and nutritive proprieties as close as possible, with particular attention to crude protein (CP) and energy content (Table 2). The nutrient and energy contents of concentrate pellets, *Taraxacum officinalis*, and *Zostera noltii* are reported in Table 1. Thus, the scheme of the amount of daily food offered to Wigeons was outlined (Table 2).

Table-1. DM and nutrient content of single feed used in experimental diets (% on DM)

Diet	Energy kJ/kg DM	DM %	CP	CF	EE	Ash	NDF	ADF	NFE	WSC	HEMI	CELL
P	18581	91.9	18.9	15.3	3.36	7.7	36.7	18.6	54.8	33.4	18.1	12.2
PT	16890	12.8	16.7	14.7	3.8	16.4	27.1	22.0	48.3	35.9	5.1	14.1
PZJ	11968	19.7	10.0	11.5	0.4	39.3	42.3	32.0	33.9	7.9	10.4	25.4
PZS	12982	20.7	10.2	9.5	0.4	33.3	38.9	25.0	46.5	17.1	13.9	21.2

CF: crude fibre; CP: crude protein; EE: ether extract; NDF: neutral detergent fibre; ADF: acid detergent fibre; OM: organic matter; NFE: nitrogen free extracts; WSC: water - soluble carbohydrate; HEMI: hemicellulose; CELL: cellulose.

The metabolisability trials were performed in November (P), April (PT), June (PZJ), and September (PZS). During the digestibility trials, each bird was kept and individually fed with pelleted concentrate and forages daily harvested, in a single cage (60 x 50 x 50 cm), fitted with a removable tray for excreta collection.

After a preliminary adjustment period of 5-7 days, all Wigeons were individually weighed. The lengths of the experimental trials were 5 days for P, 7 days for PT, 7 days for PZJ, and 12 days for PZS. As the amount of the DM daily intake resulted more variable when forages were added to the diet, the option of extending the length of the collection period was chosen when mixed diets were administered. Wigeon live weights were recorded at the beginning and end of each experimental period to check that all animals maintained their live weight. After each trial, birds were brought back to their habitual housing.

Table-2. Ingredients of the experimental diets

	Feeds					
	Pellet	**Forage**	**Total**	**Pellet**	**Forage**	**Total**
Diet	As sampled (g)	As sampled (g)	As sampled (g)	DM (g)	DM (g)	DM (g)
P	66		66	61.4		61.4
PT	60	100	160	55.8	12.8	68.6
PZJ	60	85	145	55.8	16.7	72.5
PZS	60	85	145	55.94	17.6	73.5

Pellet: complete feed; Forage: *Taraxacum officinalis* or *Zostera noltii*.

Wigeons were fed twice daily, at 8:00 a.m. and 5:00 p.m., before and during the experimental periods. Fresh water was always available, and animals were given grit (sand) to support the feed breakdown in the gizzard. Feed consumption during the experimental period was recorded daily. Spilled food was separately weighed to obtain the effective intake. At the same time, droppings were collected, weighed and then dried, and ground.

The water content of feeds and dropping samples was determined by oven-drying (55° C). Proximate Analysis; Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF), and Acid Detergent Lignin (ADL) were determined on samples to calculate by difference Hemicellulose and Cellulose [30, 31] Water-soluble carbohydrate contents (WSC) on DM was calculated according to the following: WSC = 100 % - % Ash - % Ether Extract (EE) - % NDF -

% CP; Nitrogen-free extract (NFE) was calculated according to the following: 100 % - % Ash - % EE - % Crude Fibre (CF) - % CP. An adiabatic calorimeter (IKA Werke C7000) was used to measure the gross energy content of feeds and dropping samples.

The apparent metabolisability -uncorrected for endogenous losses- of different nutrients was calculated using the following formula: metabolisability (%) = [(1-Intake (g)/Excreta (g)] x 100. Data were statistically analysed by ANOVA (SPSS Statistics 17.0), setting the significance levels at $P < 0.05$; the Pearson test to detect correlation among variables was also applied.

3. RESULTS

We sampled and analysed *Zostera noltii* in our coastal lagoon at different times, obtaining a set of different data on DM, nutrients, and energy contents. The vegetative state primarily determines the proportion and the chemical composition of rhizomes, shoots and leaves. A fraction of the leaves prevailed in the *Zostera* collected in June, while in September Wigeons received a higher quota of rhizomes and shouts. It seems that Wigeons prefer the green shoots and young leaves. The higher incidence of young shoots in autumn could support the differences of ADF, and consequently of hemicelluloses, cellulose, and of soluble carbohydrates (NFE and WSC) contents observed between *Zostera* handpicked in June and in September (Table 1). The comparison of the chemical composition of *Zostera noltii* with *Taraxacum* illustrates that the former contains less protein, fat, carbohydrates, and gross energy, but more ash, hemicelluloses, and celluloses (Tables 1 and 2). Table 3 shows the effective feed intake measured across the four trials. The DM intake of concentrate and of forages examined separately was not significantly different among diets. When forages were added to the ration, the total daily DM intake significantly increased with respect to P diet and varied from 61.0 to 65.5 g/d. When the birds were given *Taraxacum* and *Zostera* collected in September DM intake was significantly greater when compared to those measured with P diet (Table 3). The intake of PZJ was intermediate and not statistically different from the other three diets. The same trend was observed for organic matter (OM) intake.

Table-3. Concentrate, forage, and nutrients intake (DM, g/day)

Contents	P	PT	PZJ	PZS	F
Energy KJ	1009	1168	1089	1167	3.37
DM	54.4^{b}	64.0^{a}	61.0^{ab}	65.5^{a}	4.52
CF	7.4^{a}	9.9^{b}	9.2^{b}	9.9^{b}	13.28
CP	11.6	11.1	10.5	11.2	1.25
EE	1.8^{a}	2.4^{b}	1.8^{b}	1.8^{b}	24.36
ASH	4.6^{c}	5.9^{b}	6.5^{b}	7.3^{a}	13.26
NDF	19.6^{b}	19.9^{b}	$21.4a^{b}$	26.7^{a}	16.03
ADF	9.3^{c}	11.9^{b}	13.3^{a}	13.1^{a}	11.71
OM	49.8^{a}	58.1^{b}	53.4^{b}	58.2^{b}	4.64
NFE	28.1^{c}	34.8^{ab}	31.8^{bc}	36.1^{a}	8.69
WSC	16.8^{c}	24.8^{a}	20.8^{b}	18.4^{bc}	10.83
HEMI	10.4^{b}	9.0^{c}	8.6^{c}	11.5^{a}	13.64
CELL	5.7^{c}	8.3^{b}	10.2^{a}	8.1^{b}	43.07

$^{a-b}$Means within a row with different superscripts differ significantly for $P < 0.05$

CF: crude fibre; CP: crude protein; EE: ether extract; NDF: neutral detergent fibre; ADF: acid detergent fibre; OM: organic matter; NFE: nitrogen free extracts; WSC: water - soluble carbohydrate; HEMI: hemicellulose; CELL: cellulose.

The ingestion of CP does not vary among diets, while a statistically higher intake of soluble carbohydrates was observed when Wigeons were fed with forages. With *Zostera* collected in September, the ash intake of Wigeons significantly increased. The daily individual average amount of excretions is reported in Table 4. The amount of daily excretion was also variable within the same feeding treatment, as the number of droppings collected *per diem* ranged between 50 and 85. The NDF constitutes the major component of DM Wigeon dropping in all diets. The chemical composition of droppings sampled differed among treatments in CP and in some CWC components (Table 4). A higher amount of DM was excreted when the Wigeons were fed with *Zostera*, corresponding to a significantly higher content of NDF, which indicates a poorer nutritional quality of the fibre found in this forage.

The CP content of DM was always higher in droppings than in the rations consumed by Wigeons, as well as, rather appropriately occurred for ash and for the components of the cell wall. The nitrogen (N) content of droppings of diet containing *Zostera noltii*, poorer in CP than other feeds (Tables 1 and 4), compared to those from P diet statistically diminished. The addition of *Taraxacum officinalis* also caused a reduction of N content of dropping, lower but still significant. It is highly probable that these results do not solely depend on the N content of the diets, but also on the different chemical characteristics and on the fermentability of forages CWC. In fact, correlation between CWC, NDF, and ADF of droppings and intake is always significantly and positively correlated (r = 0.97, r = 0.92, and r = 0.88, respectively). The correlations between intake and droppings calculated for other constituents, as CP, ash, and EE are also statistically correlated, but to a lesser degree.

Table-4. Average daily excretion and chemical composition of droppings

Contents	**P**	**PT**	**PZJ**	**PZS**	**F**
g/bird	235.6 ± 48.8	311.4 ± 39.1	240.5 ± 49.0	301.8 ± 21.7	
g DM/bird	26.7^{c}	32.0^{b}	29.5^{bc}	37.9^{a}	10.22
Energy kJ	468.1^{d}	555.7^{b}	510.6^{b}	643.0^{a}	9.72
Chemical composition					
DM (%)	11.5^{ab}	10.4^{b}	12.4^{a}	12.6^{a}	4.01
CF % on DM	23.3^{b}	25.4^{a}	25.0^{a}	22.7^{b}	6.08
CP % on DM	29.2^{a}	27.2^{b}	21.8^{c}	21.3^{c}	176.58
EE % on DM	0.7^{b}	1.2^{a}	0.8^{b}	0.6^{bc}	29.89
ASH % on DM	14.1	14.7	14.1	13.2	2.6
NDF % on DM	49.4^{bc}	45.5^{c}	55.4^{b}	66.8^{a}	10.65
ADF % on DM	24.6^{c}	37.4^{a}	35.3^{a}	28.5^{b}	55.5
OM % on DM	85.9^{ab}	85.3^{b}	85.9^{ab}	86.8^{a}	2.6
NFE % on DM	32.7^{c}	31.6^{c}	38.3^{b}	42.3^{a}	51.36
WSC % on DM	6.7^{b}	11.6^{a}	7.9^{ab}	10.9^{a}	3.2
HEMI % on DM	24.8^{ab}	14.2^{c}	20.1^{b}	25.7^{a}	8.4
CELL % on DM	19.2^{b}	22.5^{b}	30.8^{a}	18.1^{b}	13.41
Energy kJ/kg DM	17981	16132	15266	17115	2.34

$^{a-b}$Means within a row with different superscripts differ significantly for $P < 0.05$

CF: crude fibre; CP: crude protein; EE: ether extract; NDF: neutral detergent fibre; ADF: acid detergent fibre; OM: organic matter; NFE: nitrogen free extracts; WSC: water - soluble carbohydrate; HEMI: hemicellulose; CELL: cellulose.

The overall effect following the addition of different forages to the ration on the amount and composition of dropping is better defined by energy excretion, which was significantly greater in PZS (643 kJ/d), even if the energy concentration of *excreta* did not statistically differ among treatments.

The average DM metabolisabilities measured with four diets varies from 42.2 % for PZS to 51.4 % PZJ, the former value being significantly lower than the others (Table 5). Apart from the least result, from the outcome of DM metabolisabilities some distinct associative effects between forages and pellet can be excluded.

A similar trend of assimilation efficiencies were found for OM, even if the digestive and metabolic utilization of ash statistically differed among diets.

The apparent metabolisability of CP ranged from 21.4 % for diets containing *Taraxacum* to 38.8 % with PZJ. The protein of the diet containing *Zostera* harvested in June appears to have been more efficiently metabolised, more so than that of PZS, while the presence of *Taraxacum* statistically reduced the protein metabolisability of the diet.

As expected, the metabolisability of NDF differed significantly across diets, being the highest in the control diet P. The average value of ADF metabolisability was significantly lower in PT than in P and PZJ. The metabolisability of hemicelluloses was statistically higher in PT and lower in PZS and PZJ. The cellulose content of diets containing forages was less digestible than that of P diet. The values of WSC metabolisability was lower for the PZS diet.

Table-5. Apparent metabolisability of diet contents (%)

Contents	P	PT	PZJ	PZS	F
Energy	53.6^{a}	52.4^{a}	52.9^{a}	43.7^{b}	6.45
DM	50.9^{a}	50.1^{a}	51.4^{a}	42.2^{b}	4.87
CF	16.2	17.6	19.9	13.6	0.55
CP	32.6^{ab}	21.4^{bc}	38.8^{a}	28.3^{b}	7.31
EE	90.0^{a}	84.3^{c}	86.8^{b}	87.7^{b}	5.7
ASH	18.0^{b}	20.0^{b}	36.0^{a}	31.7^{ab}	3.54
NDF	33.2^{a}	27.1^{ab}	23.3^{b}	21.0^{b}	5.44
ADF	29.1^{a}	11.5^{b}	21.2^{ab}	18.0^{b}	4.07
OM	53.9^{a}	53.1^{a}	53.2^{a}	43.5^{b}	7.86
NFE	69.9^{a}	71.1^{a}	65.4^{b}	54.5^{c}	29.13
WSC	89.0	85.1	88.5	78.3	2.81
HEMI	37.0^{ab}	50.0^{a}	30.8^{b}	15.5^{c}	9.43
CELL	10.7	13.2	12.1	15.6	0.29

$^{a-b}$Means within a row with different superscripts differ significantly for $P < 0.05$

CF: crude fibre; CP: crude protein; EE: ether extract; NDF: neutral detergent fibre; ADF: acid detergent fibre; OM: organic matter; NFE: nitrogen free extracts; WSC: water - soluble carbohydrate; HEMI: hemicellulose; CELL: cellulose.

The EE metabolisability was significantly lower only when Wigeons were given *Taraxacum*. Both *Zostera* diets showed the highest ash metabolisability, so that the OM metabolisability resulted only partially limited by the high ash content of *Zostera*.

4. DISCUSSION

Very little information is available on the chemical composition of *Zostera noltii*, however the composition of *Zostera* collected and offered Wigeons in June and September are consistent with those reported by Mathers and Montgomery [32]. The vegetal part of foodstuff collected always contained leaves, shoots, and rhizomes, but in different proportions. Shoot and rhizome fractions prevailed in September. According to [32, 33] Wigeon prefer to dabble for green shoots or short leaves and [34] observed that the above ground parts predominated in the diet. The contents of DM, NDF, and CP were similar while June *Zostera* contained more ADF and cellulose, but less WSC, and hemicellulose. Fox [35] found that the rhizomes of autumnal *Zostera* contain significantly less DM, CP, fibre or ADF, and ash, but more WSC than shoots. Mathers, et al. [36] chemically differentiated shoots and rhizomes, observing that rhizomes comprise 56 % of DM of the whole plant and are lower in fibre and higher in WSC than shoots. The high content of the ash of *Zostera noltii*, as that of other saltmarsh species, was not indicated or commented in the literature, but does not seem to affect food selection and intake [32].

The gross energy content of September *Zostera* given to Wigeons was 13 kJ/g DM, higher than June plants (12 kJ/g DM), values comparable with those were reported by Mathers, et al. [36]. Inger, et al. [37] observed that the energetic value of *Zostera* shoots varied widely between sites: at Strangford it was 13 kJ/g [32] compared with 20 kJ/g for southern England [38] 19 kJ/g for the Wadden Sea [34] and 18.6 kJ/g for Lindisfarne in north-eastern England [39].

In our experiments, when forages were available, the DM intake significantly increased from 13 % (PZJ) to 20 % (PT) (Table3).

The observed levels of DM intakes we observed are comparable to those (65.2 g/d ash free dry weight) measured by Madsen [34] in Wigeons eating *Zostera*, as the sole feed source. A higher intake of *Zostera* (120 g/day DM) [40] of grass (91.6 g/day DM) [5] and of *Salicornia* (110.7 g/day DM) [41] was found. Whereas in another experiment the same forage determined a lower intake of 53.1 g/d DM [41]. All these investigations were conducted on wintering Wigeons. The gross energy intake measured during the trials was 1009 kJ/d with P and increased to an average of 1141 kJ/d (1470 kJ/kg$^{0.75}$), when forages were also administered. Woollhead [6] estimated that the energy consumption of a wild Wigeon was 2055 kJ/d, that is 2630 kJ/kg$^{0.75}$/d, which is consistent, but almost about double our values, measured at about maintenance level.

There is evidence that, even if captive, the rationed Wigeons have maintained an aptitude to consume forages to attain a higher intake of utilizable DM. Miller [25] found that not only did gut length increase in mallards on a high fibre diet, but that the food consumption of these birds also increased. The diet quality regulates the intake: if digestive efficiency decreases in terms of bulk, the animal may try to eat more resulting in gut volume enlargement, and retention time is increased to cope with extra food to process each day [24]. The modification in gut size and feed consumption elicited by diet quality is often ephemeral, having its allometric downsides resulting in weight modifications and limitations to flight capability with a higher risk of predation [42]. In fact, the energetic cost of flight increases proportionally to Body Mass$^{1.56}$ [43]. Captivity can have a major effect on gut size, morphology, and digestive physiology of *Anatidae* [23, 44]. In a select number of dead captive animals the length of the paired caeca measured 12.0 and 12.5 cm while that of wild Wigeons shot in the lagoons (n = 71) was 19.1±2.8 and 19.3±2.4 cm (Fabro and Susmel, unpublished data). The dimensions of the caeca of wild Wigeons we measured are consistent with those reported by [5, 45].

The chemical composition of dropping differed statistically among diets and was related to the intake and discloses the complexity of the digestive process. DM excretions are not directly comparable with other results of other experiences, as it depends on DM intake, on the quality of forages, or on the NDF digestive outcomes. Similar to our figures, Madsen [34] along with an intake of 64.8 g DM/d of *Zostera,* measured a defecation of 35 g DM/d, but [18] collected 65.2 g DM/d droppings from Wigeon ingesting only 91.6 g DM/d of grass. The high CP content of droppings not only represents the digestibility of protein source, but also endogenous faecal excretion composed of different components, including the remains of intestinal bacteria and the metabolic excretion of uric and other N chemical compounds (Table 3). Average CP excretion was statistically lower in the PZJ diet than in other diets. Considering the PZJ and PZS excretions, it seems clear that CP emissions go hand in hand with the DM and NDF ones. If less protein were enzymatically digested and assimilated, less N would also be excreted in urine. The droppings of Wigeons given the *Zostera* diets contain a higher percentage of NDF and hemicelluloses. For all diets the correlation between NDF and hemicellulose excretions are statistically positive ($r = 0.81$), indicating that the hemicellulose digestion is progressively limited when the intake increases ($r = 0.78$) while for cellulose there is no significantly correlation ($r = 0.30$). When intake and excretions of these two components of the cell wall are correlated, cellulose fermentation is found to be very limited and higher intake linearly results in higher excretion ($r = 0.91$), whilst the excretion of hemicellulose increases to a lower extent ($r = 0.74$).

Methodologically, it may be argued that the results obtained with the criteria of partial substitution also have some limits, as the level of substitution of a test ingredient in a diet may *per se* change the metabolisability values. Farrell [46] authoritatively asserts that there is little experimental evidence to support this, concluding that the effects of substitution, even at high levels, appear to be additive rather than associative. Our results suggest that the DM metabolisability of *Taraxacum* and *Zostera* collected in June appears to be similar to that of P diet, close to 50 %, and significantly greater than that of PZS. A comparable value was found by Madsen [34] who on Wigeons grazing on *Zostera* meadows measured a DM digestive efficiency of 46 %. These values are higher than the average value of 28.8 % measured by Mayhew [18] on wild Wigeons eating grass.

The metabolisabilities of NDF and ADF diminished at various extents when pellet was associated to forage (Table 5), displaying in this case a dissimilarly negative associative effect. A number of papers settle that *Anatidae* can digest fibre to a variable extent [33]. [2, 28, 29] indicate that some grouse and waterfowl species digest 15-35 % of CWC, which is in the range of the metabolisability of NDF, measured in our experiments in Wigeons. The rare findings on *Anas penel*ope, report values of 30.8 % and 5.0 % of NDF and ADF digestibility of autumnal grass, respectively [33]. These values are similar to those measured on PT diet. Jamroz, et al. [47] measured the metabolisability of NDF, ADF and hemicellulose of cereals obtaining values of 18.1±8.6 %, 2.8±10.0 % and 27.8±12.4 % respectively, which are quite different from the metabolisability of P diet. The metabolisability of the hemicellulose of P diet is similar to that of seeds and tubers (35.0 %) measured by Bruinzeel, et al. [48].

Waterfowls can partly solubilise and digest hemicellulose through acid hydrolysis in the proventriculus and in the gizzard followed by rapid fermentation in the lower small intestine and caeca [49, 50]. The role of caecal microorganisms in fermenting CWC has been studied in different bird species. What is lacking is specific research on Wigeons with less developed caeca. Grazing geese [51-53] are considered to poorly digest [18] or not digest cellulose at all, as cellulolytic bacteria failed to show up in the intestinal tract [54]. Instead, Durant [33] reported that some ADF digestibility was measured in Wigeons. During the experimental trials, our captive Wigeons were able to metabolise from 15.5 % to 50.0 % of hemicellulose and from 10.7 % to 15.6 % of cellulose. The question raised by the data is whether cellulose can be prevented from entering the caeca.

Little information is available on protein metabolisability on wild and, even less, on captive Anseriformes. Buchsbaum, et al. [2] depicts the CP apparent digestibility in different herbivore waterfowls in a range of values between 61 % and 80 %, but CP digestibility is always higher than metabolisability. The addition of less than 20 % of forages to the concentrate quota was sufficient to significantly modify the CP metabolisability among diets. Our results, varying from 21.4 % for a *Taraxacum* diet to 38.8 % for a PZJ one, are more comparable to the value of 40 % for rye grass CP reported by Van Eerden [55]. This author suggests that the digestibility of foliage CP is not affected by body mass, as that of CWC, but could also indicate that Wigeons are less efficient than other herbivores at digesting protein. In our study the CP metabolisability coefficients are not correlated with protein intake (r = - 0.19), but the coefficient is negative. The correlation coefficient between CP intake and excretion is fairly positive (r = 0.60) and consequently the figure between CP metabolisability and content of droppings is higher and negative (r = - 0.90), or highly dependant on CP excretion. In brief, high CP excretion might be attributable to intake, which over exceded the maintenance requirement while the imputable fraction coming from the weight loss (see below), can be estimated to be no more than 10 % of total CP excretion (0.20 g N/kg$^{0.75}$).

In fact, the Wigeons retained on average 0.67g N/kg$^{0.75}$/d - more than enough to cover the maintenance requirements or at least to prevent the depletion body protein in the short term –, that [56] equalled to 0.49 g N/kg$^{0.75}$/d. The smallest daily N retention was 0.46 g N/kg$^{0.75}$ (PT) and the highest 0.84 g N/kg$^{0.75}$(PZJ).

The metabolisability of lipids by Wigeons is high, suggesting they may efficiently absorb the soluble compounds of a large molecular mass.

The ash content of *Zostera* is elevated and its apparent metabolisability resulted higher when compared to those of the other two diets. This result should not be considered positive because Wigeons, since their salt glands are not fully developed to reduce levels of salt in the blood, respond mostly by drinking fresh water [57]. Secondly, the excretion of salt is likely to have a high energy cost due to the process of the active transport of Na^+ and K^+ ions [58].

The net benefit of a feed can be measured in terms of the changes in the body weight over time [59]. During the experimental period, an average weight loss of 7 g/d was measured. As the results of the trials demonstrate, the diets cover the N and energy requirements, and weight loss is most likely attributable to the disturbance due to the change of rearing conditions during experimental periods. A higher weight loss of 18.2 and 36.6 g/day were measured during digestibility trials [41] due to inadequate energy intake. Wigeons are considered quite sensitive to human presence and activities [18, 60] and their behaviour was constantly observed as being timid or fearful, even if born in captivity. The inevitable tending, handling and feeding Wigeons in metabolisability cages generate a state of anxiety in the animals. Mayhew [5] noted that Wigeons might be under a great deal of stress when handled or when in contact with researchers, even if they have been imprinted by them.

Independently from the cause, weight loss contributes to the energy excretion of endogenous origin and could affect energy or protein metabolisability. If we accept the indication given for Wigeons by Durant, et al. [41] for an equivalent 22.6 kJ/g of weight loss-quoted as half from fat and the other half from muscular tissues, our animals would rate a daily energy loss of 170 kJ/d. Otherwise [61] proposed to subtract 34.4 kJ from metabolisable energy (ME) for each gram of N lost to account for the energy required in the excretion of urinary nitrogen. For a loss of 3.5 g/d of muscular mass, which is about 170 mg/d of N while the amount of ME is negligible at approximately 6 kJ. Then, we should conclude that the energy from weight loss is almost entirely converted into heat and a correction to N equilibrium for the scope of this type of research appears not to be necessary [62].

We measured the energy assimilated by the difference between the energy contents of feeds and droppings, just to estimate the level of nutrition achieved over the course of the trials. In fact, we were not able to find in the literature data on intake of metabolisable DM or OM of Wigeons receiving comparable mixed diets, but only occasional information on energy intake from forages.

ME is the conventional measure of the energy available to birds from their diet. In avian energetics, ME is used to convert daily energy budgets into the weight of food required to supply energy needed by individuals or populations [62, 63]. The energy values measured of feeds and droppings collected from test birds, fed at maintenance, using a bomb calorimeter is recognised as a proper measure of energy metabolisability. ME can be expressed as either apparent or true metabolisable energy. The true ME value, in most cases measured on starved animals, is adjusted by the energy of non-food origin lost through faeces and urine. This component of excretion is rather independent of energy consumption, in fact as energy intake increases, the true ME value progressively approaches that of the apparent ME. According to Miller and Reinecke [61] in the nearness of maintenance, the energy requirement weighs up at 2.5 times the basal metabolic rate (BMR) in *Anas plutyrhynchos* (1 kg of body weight), the difference between the two values is small, about 3 %.

In our experiments, the differences between gross energy in foods on the one hand and droppings on the other amount to 540 kJ/d with pellets and an average of 571 kJ/d in birds fed mixed diets. No other experiences have been found where the metabolisability was measured on captive animals kept in metabolic cages.

In feeding ecology researches, few balanced measures of food energy available to birds were instead obtained in semi-natural conditions on wild birds, using tracers, mobile aviaries, roost, and grazing patch enclosures. Wintering on grass pastures Wigeons grazed for 17.5 h/d, Mayhew [18] measured a ME intake of 630 kJ/d. Madsen [34] observed that wintering Wigeons spent 12.7 h/d feeding on *Zostera*, and evaluated a ME intake of 592 kJ/d, which he assumed to be the average daily energy expenditure (DEE) value for Wigeons (700 g of body weight). Durant, et al. [41] using mobile aviaries to keep *Anas penelope* grazing on *Salicornia* marsh, quantified a daily ME intake of 182 and 345 kJ/d, in two successive experiments.

In ecological and behavioural studies, the energy intake is quoted as DEE (otherwise indicated as Field Metabolic Rate), an all - inclusive field maintenance requirement, which conceptually excludes productive and growth exigencies [64]. DEE is measured, or more often estimated as a multiple of one category of the metabolic parameters.

The minimum maintenance metabolic rate (resting, post - absorptive, non growing, non-reproductive at thermo neutral environmental temperature), or BMR, measured under experimental conditions, represents a well-defined baseline energetic (heat) parameter concerning the animal [65-67]. Allometric equations have been widely used to predict avian BMR from metabolic weight – BMW = $kg^{0.75}$ - [68-72]. The BMR was calculated using the average body weight of our Wigeons (722 g) with the non-passerines equation of Aschoff and Pohl [73] correspond to 242.3 kJ/d. Kendeigh, et al. [63] for a Wigeon weighing 723 g gives a BMR of 241.8 kJ/d (2.799 W). Instead, McNab [74] provides a non-passerines equation, containing six variables matching factors which accounts for 97.7 % of the variation in avian BMR measures to adjust the estimate of BMR to specific environmental conditions. When applied to our situation, using the suggested corrections, the BMR of Wigeons averaged 259.9 kJ/d. Standard Metabolic Rate (SMR; basal, not thermo neutrality) results slightly higher. For example, with a non-passerines equation the value adapted to our data is 258.3 kJ/d [18, 75]. Resting Metabolic Rate (RMR; minimal activity, not post-absorptive), which accounts for the conditions under which data are obtained from test animals, is more variable and higher. An example could be that of using a general equation valid for dabbling ducks, the requirement corresponding to the weight of our experimental Wigeons should be 354.9 kJ/d [76]. The choice of the metabolic parameter among the numerous possible options for an assessment of DEE depends upon the conditions of the study and influence the multiplication factor. The factor value used to derive the DEE is differently designated, between 2.5 and 3 [54, 77].

With reference to *Anas penelope*, in two of the above reported studies, DEE was also calculated. Madsen [34] evaluated the DEE, as suggested by [73, 78] which resulted in 617 kJ/day, close to the measured value. Mayhew [5] calculated a DEE of 631 kJ/d [75, 78]. Notwithstanding the difference of experimental conditions, these values are very similar. In fact, although *Anas penelope* has different and varying patterns of activity during the day and night, energy expenditures for behaviour and for diurnal and nocturnal habits are almost the same [79]. In a wintering area of *Anas penelope* in the Wadden Sea, to maintain the balance of vegetal biomass grazed by waterfowls, Jacobs, et al. [40] quantified the number of Wigeons feeding on seagrass bed and the consumption of *Zostera noltii*. They estimate the ME content of *Zostera* and the average DEE of Wigeons through their SMR [75] choosing a multiplication factor of 3. The calculated SMR was 226 kJ/d for Wigeons (700 g of body weight), which is a DM intake of 54.1 g/d.

Theoretically, in adult animals at maintenance, daily ME intake should correspond to DEE. The ME intake we have measured shows a close correspondence to DEE values determined in the studies mentioned above. The fact that Wigeons were kept in metabolic cages should have reduced the energy demand for maintenance. Robbins [56] and Kirkwood [80] devised that the maintenance requirement of adult captive birds in energy balance and kept in a comfortable thermal environment, come close to twice the BMR. If we consider that the average BMR of our Wigeons ought to be 260 kJ/d, as calculated by the McNab [74] equation, the ratio with a ME intake of 564 kJ/d equals 2.2, but the factor approaches 1.6 if compared with the BMR value proposed by Miller and McaEadie [76].

In any case, the level of nutrition which were applied in our trials were satisfactory, but unlike protein, did not appreciably exceed the maintenance requirements.

5. CONCLUSION

This set of experiments dealt with two related objectives. The immediate one was to evaluate the metabolisable energy and nutrients concentration using the standard procedure of total collection on captive Wigeons. The second purpose, essentially speculative, was to compare the results of the nutritional response of captive birds to those of their wild counterparts. Methodological, ecological and behavioural aspects, which characterize the experimental settings, represent unavoidable differential elements. So scientific knowledge has to help in defining which part of nutritional explanation represents unmodifiable specific physiological characteristic and which parts of the feeding response depend upon the flexible and adaptable process, which is given in the biochemical and functional digestive and metabolic configuration. In other words, what is functional and what is behavioural? Captive Wigeons have less developed caeca compared to other herbivorous ducks, but we could assume that the basic functionality is maintained. Wild grazing Wigeons rely on ingesting highly variable and relatively large quantities of fresh food, higher than captive birds, but in both situations *ingesta* pass through the gut rapidly and is inefficiently digested. The digestive system of herbivorous *Anas penelope* can be regarded as an adaptation to differences in energy requirements and environmental conditions. The same way of thinking could apply to other aspects, like faecal and urinary excretion or BMR. These aspects are different from feeding behaviour, preferences, or real energy expenditure of waterfowl in natural conditions. The differences among diets were determined by the addition of two types of forages, which represented less than the 20 % of total DM intake. The differences of DM intake among the four diets denote a preference or palatability for forages rather than a response to a need for a greater intake of energy or DM. Statistically significative changes in droppings excretion and in dietary nutrients metabolisability were observed. Most of the differences in chemical composition between *Zostera noltii* leaves harvested in June and September significantly affected metabolisability of CP, OM, NFE, and WSC. Not all the differences in metabolisability observed between *Taraxacum* and *Zostera* are attributable to chemical contents, but rather to the quality of the fibre. The *in vivo* trial seem to have screened the differences in the quality of forages more effectively than the chemical analysis. From the results it appears that, cellulose was almost partly digested leading to the consideration that caeca of captive Wigeons have and keep the ability to ferment cellulose. The CP metabolisability coefficients were low and failed to correlate with protein intake, while this occurred significantly with the correlation coefficient between CP metabolisability and CP in droppings. More likely, the protein positively interacts in the digestion of other nutrients, particularly CWC. In fact, CP, ash, hemicellulose and cellulose intake was statistically correlated with crude protein metabolisability. The ash content of *Zostera* also requires further investigation. In principle, the inorganic matter may be considered detrimental but this does not match the fact that this phanerogam represents a common feed for Wigeons. This experiment suggests that with captive birds it is possible to obtain some useful information on the nutrition of Wigeons, which can be extended to feeding in the wild. Although the consequences arising from fear and stress that Wigeons still exhibit in relation to man remain difficult to elude.

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