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Diet Quality of Large Herbivores Across Continents

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Declaration

I hereby declare that this thesis entitled: Diet quality of large herbivores across continents, is my own work and all the sources have been quoted and acknowledged by means of complete references.

In Prague date

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Abstract

The objective of this diploma thesis was to compare diet quality of large herbivores across European and African continents. The particular aims were: 1) to determine concentrations of macronutrients (N, P, K, Ca, Mg) and fibre fractions (NDF, ADF, ADL) in faeces of selected 17 species of large herbivores, 2) to compare concentrations of these macronutrients and fibre fractions in faeces within the ruminants adopting different foraging strategies and non-ruminants separately in European and African localities, 3) to test the functional link between concentrations of nitrogen and phosphorus in animal faeces and in soil on pasture. In total, 281 faecal samples were collected from 17 animal species, i.e.: *Loxodonta africana*, *Equus asinus*, *E. caballus*, *E. zebra quagga; Bos taurus*, *Syncerus caffer*, *Alcelaphus buselaphus*, *Damaliscus pygargus pygargus*, *Hippotragus equinus*, *Kobus ellipsiprymnus*, *Antidorcas marsupialis*, *Ovis aries*, *Bison bonasus*, *Alces alces*, *Taurotragus oryx*, *Taurotragus derbianus*, *Capra aegagrus hircus* from 10 countries (Senegal, Chad, Zambia, Republic of South Africa, Czech Republic, Bulgaria, Hungary, Iceland, Netherlands, Norway) together with soil representative samples from each locality. The samples were analysed for concentrations of N, P, K, Ca, Mg, NDF, ADF, ADL and concentrations of FN and FP were used for determination of diet quality. Herbivores from Africa had lower concentrations of FN and FP and higher ratios of N:P and Ca:P in comparison to herbivores from Europe. The highest lignin concentrations had concentrate selectors (moose, Derby and giant eland), thus reflecting the high concentration of lignin in browse. Within the non-ruminants, equids from Europe were superior in diet quality to African species, with highest fibre concentrations in elephants and lowest concentration of K together with highest concentrations of Mg in faeces of zebra. Finally, the relationship between plants available N, P in soil and FN, FP in investigated herbivorous animals was not proved, proposing more investigation concern to soil-plant-herbivores relationships.

Key words: diet, nutrients, faeces, large herbivores, foraging strategies, nitrogen, phosphorus

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List of the abbreviations of chemical substances

ADF (acid-detergent fibre)

ADL (acid-detergent lignin)

Al (aluminium)

As (arsenic)

B (boron)

C (carbon)

Ca (calcium)

Cd (cadmium)

Cl (chlorine)

Co (cobalt)

CO_2 (carbon dioxide)

C_{org} (organic carbon)

Cr (chromium)

Cu (copper)

DM (dry matter)

F (fluorine)

Fe (iron)

FN, FP (faecal nitrogen/ phosphorus)

H (hydrogen)

HCl (hydrochloric acid)

H_2O (water)

$H_2PO_4^-$, HPO_4^{2-} (dihydrogen phosphate ion, monohydrogen phosphate)

H_2S (hydrogen sulfide)

H_3BO_3 (boric acid)

I (iodine)

K (potassium)

Li (lithium)

Mg (magnesium)

Mn (manganese)

Mo (molybdenum)

MoO_4^{2-} (molybdate)

N (nitrogen)

Na (sodium)

NDF (neutral-detergent fibre)

NH_3, NH_4 (ammonia, ammonium)

Ni (nickel)

NO, N_2O (nitrogen oxide, nitrous oxide)

O (oxygen)

P (phosphorus)

Pb (lead)

S (sulphur)

Se (selenium)

Si (silicon)

Sn (tin)

SO_2 (sulphur dioxide)

SO_4^{2-} (sulfate)

V (vanadium)

Zn (zinc)

List of the abbreviations of sites, animals and foraging strategy

BG (Republic of Bulgaria)
Chad (Republic of Chad)
CZ (Czech Republic)
HU (Hungary)
IS (Iceland)
NL (Netherlands)
NO (Kingdom of Norway)
RSA (Republic of South Africa)
SN (Republic of Senegal)
ZM (Republic of Zambia)
CS (Concentrate selectors)
GR (Grass and roughage eaters)
IM (intermediate eaters)
Ass (*Equus asinus*)
Bontebok *(Damaliscus pygargus*)
Buffalo (*Syncerus caffer*)
Cattle (*Bos taurus*)
Derby (*Taurotragus derbianus*)
Eland (*Taurotragus oryx*)
Elephant (*Loxodonta Africana*)
Goat (*Capra aegagrus hircus*)
Hartebeest (*Alcelaphus buselaphus*)
Horse (*Equus cabalus*)
Moose (*Alces alces*)
Roan (*Hippotragus equinus*)
Sheep (*Ovis aries*)
Springbok (*Antidorcas marsupialis*)
Waterbuck (*Kobus ellipsiprimus*)
Wisent (*Bos bonasus*)
Zebra (*Equus burchelli; zebra zebr*

1 Introduction and Literature review

The diet quality of free ranging herbivores is hardly to be assessed from nutrient levels of forage because wild herbivores are able to select the most nutrient rich plants or plant parts by adopting different foraging strategies, making the detection and sampling of proper forage species highly demanding. Thus, the information about diet quality and nutritional requirements of wild herbivores is scarce, in contrary to the most information of large herbivores diet quality comes from managed feeding experiments. For that reason more investigation is needed to be done in this area for better comprehension to wild herbivores requirement and subsequent habitat management and conservation.

1.1 Essential elements for organism functioning

Minerals are inorganic substances occurring in all body tissues and fluids and are crucial for the maintenance of the physicochemical processes essential to life. Animals and plants are principally composed from three major building bioessential elements: carbon (C), oxygen (O) and hydrogen (H). However, a number of other nutrients are needed for metabolism function, development and successful reproduction (Whitehead, 2000). In these thesis, I will use the term of nutrients equally to minerals and elements for both plant and animals just to simplify it, even though animals do not obtained the nitrogen in mineral form. The nutrients which are vital for organism functioning are called essential elements and their numbers vary according to literatures, however recently the essential animal nutrients have been increased from 22 to 28 due to modern investigation techniques in this area (Suttle, 2010). From all essential nutrients, the N and P have overriding importance in all living systems, therefore they are being described in separate chapter (chapter 1.1.1.)

The essential nutrients can be further divided according to their amount needed in living organism as macronutrients, micronutrients (trace) elements and ultra-trace elements (Soetan et al., 2010). Macronutrients in both plant and animal organism usually attain concentration above 100 ppm and micronutrients less than 100 ppm (Hillel et al., 2005; Paterson and Engle, 2005; Soetan, 2010). The essential nutrients are: Carbon (C), hydrogen (H), oxygen (O), nitrogen (N), phosphorus (P), calcium (Ca), potassium (K), sodium (Na), magnesium (Mg), sulphur (S), chlorine (Cl), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), molybdenum (Mo), boron (B), nickel (Ni), selenium (Se) and

silicon (Si), are the same for both plants and animals (Whitehead, 2000). Furthermore, animals require iodine (I), fluorine (F) chromium (Cr) and in minute amounts tin (Sn), vanadium (V) and arsenic (As) (Soetan et al., 2010; Suttle, 2010). On the other hand, plants usually require much less amounts of Na and Se (Hillel et al., 2005). For the detailed division of essential elements into the categories see chapter 1.3 and 1.4.2.

1.1.1 Nitrogen and phosphorus

The N and P are the major nutrients limiting the primary productivity together with growth of photosynthetic biota in terrestrial as well aquatic ecosystems (Ngai and Jefferies, 2004; Hillel et al. 2005; Elser et al., 2007; Vitousek et al., 2007). The indispensable role of N and P in terrestrial ecosystem is due to its large requirements by plants and animals for successful growth and reproduction and the finite ability of soils supplying them in sufficient available forms (mainly P). The N, P are core of many essential biochemical molecules essential to metabolism function in both plants and animals organism. Organic N is participating on structure of proteins as amino acid (e.g., glutamine, glycine, lysine etc.), amino sugar (e.g., glucosamine, galactosamine), nucleosides (adenine, guanine, thymine, uracil, cytosine), peptides, phospholipids (e.g., phosphatidylserine), vitamins (e.g., niacin), creatine, cyanide, allantoin, alkyl amines and urea, whereas phosphorus is primary component of energy molecules ATP (adenosine triphosphate), ADP, AMP, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), phospholipids, coenzyme phosphopyridine nucleotides (NADP+), phytin (P storage molecules mainly in seed- inositol hexaphosphate) and component of some intermediate product (e.g. glycolysis, glycolysis) (Hillel et al., 2005). N and P have very strong connexion and influence on each other, i.e. they can have stimulation as well as constraining effects on each other, particularly the excess of nitrogen can strongly support the uptake of phosphorus and other nutrients by plant, due to its boosting effect on biomass production (Elser, 2007)

Furthermore, the faecal nitrogen and phosphorus (FN, FP) are considered as major indicators of diet quality of large herbivores (chapter 1.6).

1.1.2 The N and P cycles in the environment

The N and P have different cycles in the environment since main source for obtaining N is from atmosphere, whilst P is releasing from parent rock material (apatite minerals) through environmental disturbances (e.g., erosion, weathering). The total amount of N on the Earth is assumed to be 1.68*1017 t (Tlustoš et al., 2007), albeit only 2% are in available forms for plants and microbes (Hillel et al., 2005). The N inputs to soils can be through: collection of N from atmosphere via N_2 fixation of special organisms and bacteria, which can be symbiotic/ or non-symbiotic with plants; wet/dry deposition from atmosphere, decomposition of organic matter, organic (urea, faeces) or inorganic fertilizers and slow releasing of N from rocks and other minerals. Subsequently, there are 5 major processes on N transformation in soils: mineralization (ammonification), assimilation, nitrification, denitrification (Hillel et al., 2005). All these processes are influenced by many factors, e.g. C:N ratio and C amount and forms in soil, soil moisture, temperature, aeration together with oxidation-reduction potential. In contrary, N loses from soil can be through: volatilization during denitrification (gaseous NO, N2O, N2) and mineralization (due the semi-finished product gaseous NH3), immobilization- incorporation inorganic N into biomass and then plant harvesting, leaching N into water sources with possible eutrophication effect and erosion.

On the other hand in the P cycle the soil processes together with plants and microbes activities play the primary role, while atmospheric phase have only peripheral importance.

Total soil P ranges from 0.01 to 0.30% with majority of soil P being presented in mineral forms where releasing of P is positively correlated with lower pH with optimum 6.5 (Hillel et al., 2005). Therefore the major form of P in soils is inorganic P bound in rock minerals, followed P in soil solution (mainly as HPO_4^{2-} or H_2PO^{4-}, in smaller scale in organic forms) and organic P (e.g. phosphate ester, phospholipids, nucleic acid, phytate). There are various minerals containing P, such as primary minerals (e.g. apatite group) or secondary clay minerals (being responsible creating insoluble compounds P with Al, Fe and Ca). Phosphorus losses can arise with erosion, run off (to surface water), leaching and harvest and in contrary, the inputs are through animal (mainly) and plant residues decomposition, animals' waste product (faeces) and fertilizers.

1.2 Soils

The soil was formed during the pedogenesis process by weathering of parent rock material and activities of edaphic microorganisms together with climatic influences and soil factors in the long period. It is the upper layer of Earth's crust essential for all living terrestrial organism, which is source of all essential elements as well as provide habitat to edaphic organisms, fungi and primary producers organisms. The soil consists of three phases: gaseous soil phase (soil air), liquid phase (soil solution) and solid phase (mineral an organic). The gaseous phase consists of the air filling the soil pores and the concentration is in relation with liquid phase. The content of N and O2 is almost the same like in atmospheric air, while CO_2 concentration being 8-10 times higher. The soil solution consists of dissolved ions of chemical compounds, gass (especially CO2) and some organic substances (particularly in rhizosphere) and provides the basic important source of available plant nutrients. At last, the solid phase of soil can be divided on mineral part (92-98%), which consists of primary aluminosilicate (feldspar, mica) and secondary aluminosilicate (illite, montmorillonite, kaolinite, allophane), and organic part (2-8%) formed by edaphic organism and inanimate organic materials including nonhumified organic substances (primary organic matter) and humic part (humic acid, fulvic acis and humic substances) (Hillel et al., 2005). The soil composition, productivity and fertility (nutrient availability) are closely interconnected. However, the presence of sufficient amount of nutrients in soil does not guarantee the direct availability of these nutrients to plants because there are other factors which are influencing plant nutrients uptake such as: pH, soil moisture content, soil physical condition as well as the presence of toxic elements and salts. Moreover, the soil is a continuum with constant changes of matrix, where abiotic and biotic factors play an important role.

1.2.1 Natural resources of nutrients

There are two ways how can nutrients enter to ecosystem, i.e. either from atmosphere or by weathering from parent rock material (Chadwick et al., 1999). Atmospherically derived nutrients such as C and N have an important gaseous phase (CO_2, N_2) and enter to ecosystem via plant processes such as photosynthesis and N_2 fixation, or through wet deposition (dissolved in precipitation, e.g. (SO_2)) and/ or dry deposition of elements particles. On the other hand, the rock derived nutrients which can be represented by P, Ca, Mg, K are important components of minerals and enter to ecosystem via of chemical weathering of parent rock material (complete or partial dissolution of rock minerals). On the basis of this idea the central conceptual model of soils formation and development was built by Walker and Syers (1976). From this model summarized by Vitousek (2010), the terrestrial ecosystem begin their existence with fixed amount of P meaning that even small loss of P cannot be easily replenished. Thereafter very old soil, i.e. majority of tropical soils, can become P depleted resulting in final "terminal steady state" of P depletion and biological limitation (Walker and Syers, 1976). In contrast, N appear to be mostly deficient in the young soils and outset of ecosystems formations because it enter to ecosystem either via rapid biological fixation by symbiotic N- fixer organism/ or non-symbiotic free living N- fixer prokaryotic bacteria, or via slower physically- chemical atmospheric dry and/or wet deposition. Additionally, nowadays due to raised global pollution by anthropogenic activities, there is constantly increasing trend of obtaining N by acid rain contained nitric acid (HNO3), formed in atmosphere by reaction of gasiform nitrogen, oxygen and water, which is not negligible as well.

According to Lambers (2010), the soils can be divided into two main categories: soil of ancient landscapes (OCBILs) and soil of young landscapes (YODFELs). OCBILs represent the old, climatically buffered, infertile landscapes with nutrient impoverished soils (mainly P), because they have not been glaciated or disturbed by other major natural catastrophic events (e.g. volcanic eruption) in recent time. Furthermore, their climate is more or less stable (buffering by oceans) with high biological diversity. On the other hand, YODFELs represent the young, frequently disturbed, fertile landscapes (e.g. Europe), where prevail N limitation to P limitation because of relative young age of ecosystems (need of N assimilation). These soil were rejuvenated by glacial recession, volcanic eruption initiating formation of new ecosystems and soils enriched

of rock derived minerals supplementation with lack of atmosphere derived nutrients (CO2, N2). On this two different soil types diverse flora with various ecophysiological plants traits have been developed.

1.3 Plant essential elements

The essential element in plants are define by three criteria, they are following: the lack of elements make impossible to complete plant life cycle, a lack of the element gives rise to the specific deficiency symptoms and finally, the element play specific role in plant nutrition and metabolism (Whitehead, 2000; Hillel et al., 2005) . The plant macronutrients are: Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S) and the micronutrients are: Iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), molybdenum (Mo), boron (B), chlorine (Cl) and nickel (Ni). In the third class are useful elements: Sodium (Na), silicon (Si) and aluminium (Al), which are presented in plants, but their abundance and importance vary with plant species (Hillel et al., 2005). The plant uptake the nutrients mainly from soil solution in form of cations, anoints or oxides by growing roots, especially then by root cap covered with roots hair. However, C, H, O and mainly absorbed from water and together with some other gasses nutrients (i.e. CO2, SO2, H2S) are directly absorbed from the air by leaf stomata. The different forms of plant accessible nutrients and their functions are shown in Table 1. Generally, the monovalent ions are absorbed more rapidly in contrary to polyvalent ions from soil solution. The majority of cations occurring in plant tissue are in the inorganic form (K, Ca, Mg), whereas anions are predominantly in the organic form. The organic ions are synthesized in plant tissue, while inorganic ions are absorbed from the soil solution.

Table 1. Forms of absorption and functions of essential nutrients in plants (Hillel et al., 2005)

Nutrient	Forms taken up by plants	Functions
Carbon	CO_2	Basic molecular component of carbohydrates, proteins, lipids, nucleic acid.
Hydrogen	H_2O	Central role in metabolism, importance in ionic balance as main reducing agent, key role in energy relation of cells.
Oxygen	CO_2 , O_2	Basic molecular component in all organic compounds.
Nitrogen	NH_4^+, NO_3^-	Important compounds ranging to nucleic acid to proteins.
Phosphorus	$H_2PO_4^-$, HPO_4^{2-}	Key role in energy transfer and protein metabolism.
Potassium	K^+,	Osmotic and ionic regulation, cofactor or activator of many enzymes.
Calcium	Ca^{2+}	Participation in cell division, maintenance of membrane integrity.
Magnesium	Mg^{2+}	Component of chlorophyll, cofactor for enzymatic reactions.
Sulphur	SO_4^{2-}	Similarity with phosphorus- participation of energetic reaction, part of some amino acid.
Iron	Fe^{2+}, Fe^{3+}	An essential component of heme and nonheme Fe emzymes and carries (cytochromes, ferredoxins), component of chlorophyll.
Zinc	Zn^{2+}	Essential component of some dehydrogenases, proteinases and peptidases, e.g. glutamic and malic dehydrogenases.
Manganese	Mn^{2+}	Involved in the O_2 evolving system of photosynthesis, component of arginase and phosphotransferase enzymes.
Copper	Cu^{2+}	Constituent of important oxidase-enzymes, e.g. cytochrome and ascorbic acid oxidase, lactase, importance in photosynthesis, protein and carbohydrate metabolism.
Boron	H_3BO_3	Activator of some dehydrogenase enzymes, essential for cell division and development, synthesis of cell walls components.
Molybdenum	MoO_4^{2-}	Component of nitrate reductase and N_2 fixation enzymes.
Chlorine	Cl^-	Essential for photosynthesis (splitting water) and enzymes activation, osmoregulation functions of plants growing in saline soils.

1.3.1 Strategies in plant nutrients uptake

Since of the natural deficient of nutrients in soils and presence of other factor limiting the plant grow, plants have developed several strategies how to acquire hard accessible nutrients and thus meet the metabolism requirements. Terrestrial plants (apart from epiphytes, parasitic and carnivorous plants) obtain the most essential nutrients from soil by two pathways: via direct absorption by roots or indirectly via symbiotic mycorrhizal fungi. In total, between 86% and 94% of plants are mycorrhizal on a global scale and majority of them can uptake P by both pathways with preference of mycorrhizal uptake (Brundrett, 2009). However, many non-mycorrhizal plant species, living in habitats with nutrients impoverished soils, adopted several strategies such as is in the carnivores, parasites and cluster-rooted species, or they lack specialised root structures which is typical for plant occurring in wet and arid habitats. As the plant available P is characterized by its scarcity in soil and determine the species richness in the same time, plants have several strategies how to gain it. Firstly, the roots have ability to secrete exudates, such as organic acids, citric acid, propanedioic acid, fumaric acid etc., thus acidifying the adjacent surrounding making alkaline compound more soluble. Secondly, they can secrete alkaline or acidic phosphatase in order to lower or raise soil pH, and thus increase P availability. Thirdly, the capability of formation proteoid roots (cluster roots) is enhancing for roots nutrient uptake due to enlarged absorption surface. And finally, arbuscular mycorrhiza or ectomycorrhizal association is also very effective for widening the area from which can be nutrients collected, due to extension of roots by fungal mycelium by hypha in the soil, which should be better in competition with soil microbial organisms for example for P acquiring. The special N acquiring strategy is mainly through symbiosis of some plant with N- fixing bacteria (e.g. legumes with rhizobia bacteria and some trees (e.g. alder, sea buckthorn) with actinomycete) which provide N in form of ammonia to plant, and get back energy in form of saccharide products (Hillel et al., 2005; Lambers et al. 2010).

1.3.2 Fluctuation of nutrients in plants

The nutritional quality of plants reflect the plant growth patterns, with the highest peak occurring usually during the beginning of vegetation period, i.e. spring/ wet season, followed by gradual declining reaching the minimum in winter/ dry season (Barnes et al., 1990). Thus, the mineral concentration varies within and among plants species, sites and seasons, where major role play the mineral status of the soil in which it grows (Stapelberg et al., 2008). However, there are a range of other factor participating on plant nutrient fluctuation, such as the stage of plant maturity, genetic predisposition, environment and abiotic (weather) factors (Stapelberg et al., 2008, Ohlson and Staaland, 2001). Even, the different plant parts, organs and tissues differ from each other in mineral concentration. For example, most N and P is located in seeds (Mattson, 1980), majority of ash minerals (e.g. Ca, Mg, S) are found in leaves and abundance of potassium is found in inflorescence and juvenile plant parts/ organs. The young plants and/or plant parts are richer on mineral nutrients in comparison to old plants/ parts due to dilute effect. Finally, all these factors are influencing herbivores foraging strategies. Thus, the plant species diversity is essential to mineral diversity and well-balanced diet for herbivores (Ohlson and Staaland, 2001). Furthermore, Ohlson and Staaland (2001) proved that for some animals (e.g. moose) the aquatic plants are an important source of minerals since they have generally higher concentration of most of minerals than terrestrial plants, except of N, B and Mn.

1.3.3 The plants protection strategies against large herbivores

Plants are equipped with variety of defensive mechanism, in order to protect themselves against their consumers, i.e. herbivorous animals (Freeland et al., 1974). The protection of plants against herbivores can be divided on 1) physical avoidance, i.e. location, visibility, mechanical barriers (spines, trichomes, tough anad waxy cuticules), 2) chemical avoidance, i.e. via chemical substances (plant secondary compounds), which differ among plant species (Danell, 2006). Generally, browse plants have higher levels of cell contents, lignin, secondary compounds and N than grass species (Gordon, 2002). Furthermore it was suggested that stronger chemical defence have particularly plants with low growth rates occurring in environments with low availability of resources (Vivas and Saether 1987). There is variety range of secondary compounds from simple organic compounds (e.g. nitrates, silicates) to alkaloids, terpenoids and glycosides, but the most important plant secondary compounds are tannins, especially condensed tannins. Tannins are phenolic compounds presented in all vascular plants, but especially in higher amounts occur in browse, with several functions, e. g.: they protect plants against herbivores and pathogens, protect the plant from ultraviolet radiation and desiccation and finally they are part of plants metabolism (Lavin, 2012). Tannins are able to precipitate plant proteins and gastrointestinal enzymes, resulting in reduced digestibility (Robbins et al., 1987). In respect to that, high intake of tannins can inhibit digestion of plant material, increase excretion of essential minerals from animal organism or even lead to physiological impairment, due to high toxicity. Nevertheless, the wild herbivores, especially the browsers can restrict and/or eliminate the negative effect of tannins by detoxification strategies, such as elevating gut pH and/or use of surfactants as well as by foraging strategies (Freeland et al. 1974; Robbins et al., 1987).

1.4 Metabolism of nutrients

1.4.1 The food and its components

Large herbivores are dependent on quality of grazing lands and plants, thus the plants and plant products are the major source nutrients for herbivores. Since the animals and plants require the similar type of substances for their nutrition, we can divide them into classes according to constitution, properties and function (see Figure 1).

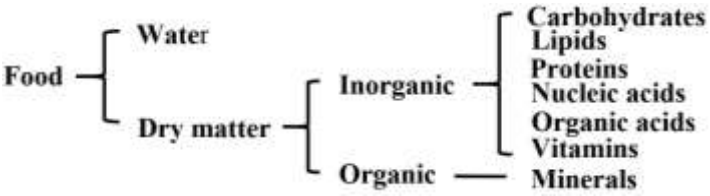

Figure 1. The main components of plants and animals food (McDonald et al., 2011).

As we can see from Figure 1, the DM (dry matter) is divided into inorganic and organic parts, however the distinction is not very sharp because many organic substances contain mineral as structural components (e.g. proteins containing S and many lipids, carbohydrates P). In plant DM the major components are carbohydrates, especially cellulose as the main component of plant cell walls, whereas the content of carbohydrates is being very low in animal body since animal cell walls are mostly composed from lipids and proteins. Similarly, the plants store the energy mainly in the form of carbohydrates (starch, fructans), contrary to animals of which main energy store is in the form of lipids (McDonald et al**.,** 2011). The proteins and nucleic acid are the major N containing compounds. The nucleic acid has the pivotal role in synthesis of proteins and carrying of genetic information. The proteins in plants are mostly present in form of enzymes while the highest concentrations in animals are found in muscles, skin, fur/ wool/ feathers/ and nails. In contrary to proteins, vitamins occur only in a minute amounts in both plants and animals, however the plants are able synthesize all vitamins needed for maintenance of metabolism functions, animals have only limited ability of synthesis (apart from vitamins of B complex, vitamin K and partially C) being much more dependent on external supply via food. Finally, the inorganic matter cover all other elements presented in plants and animals except of C, H, O (see chapter1.4.2). The fibre concentration in food can be determined in older method on CF (crude fibre) or in recent method by Van Soest on 3 fractions NDF (neutral- detergent fibre), ADF (acid-detergent fibre) and ADL (acid- detergent lignin) used as a measure of the plant

cell wall material. NDF is the residual, contacting mainly cellulose, hemicellulose and lignin, after extraction with boiling neutral solutions of sodium lauryl sulphate and ethylenediamine tetraacetic acid (EDTA). ADF represents the cellulose and lignin fractions together with Si content, obtained by refluxing the plant material with 0.5 M sulphuric acid and cetyltrimethyl-ammonium bromide. It provide us useful information about extent of digested food (digestibility). Lastly, the ADL follows the steps of ADF preparation, with additional ADF treatment with 72 % sulphuric acid dissolving the cellulose. Burning the residue we obtain the crude lignin, including cutin, Lignin is a polymer originating from three derivatives of phenylpropane (i.e., coumaryl alcohol, coniferyl alcohol and sinapyl alcohol) which closely related to carbohydrates (McDonald et al., 2011). Special attention is paid to lignin presence in animal nutrition due to its high resistance of chemical degradation, due to effect of lignin on reinforcing the plant fibres and thus making them inaccessible to animal‘s enzymes that would normally digest them. For example, the woody plants, dry plants (straw, mature hay) are rich on lignin presence resulting in poor digestion, especially to non-ruminant herbivores.

1.4.2 Essential minerals for animal nutrition

The mineral concentrations in plants play fundamental role in free ranging herbivores reflecting the physical condition, fitness as well as the quality of productivity of animals. The ingested food should provide sufficient content of essential nutrients as well as digestible energy and if the nutritional demands are not fulfilled it will be followed by weight loss, reduced animal's fertility, lowered lactation period, decreased reproductive rates as well as rising susceptibility to diseases and parasites due to weakened immune system (Stapelberg et al., 2008; Olson et al, 2010). Furthermore, the nutritional requirements of large herbivores vary during the life development and individual life stages (e.g. the growing period, pregnancy, rut). Therefore, the need to meet the nutritional requirement is the major driver of large herbivores foraging strategy as well as life history strategy in the meaning of their distribution and migration during the year (Van Soest, 1982; McNaughton, 1990).

The macronutrients for animal's nutrition are: nitrogen (N), phosphorus (P), calcium (Ca), potassium (K), sodium (Na), magnesium (Mg), sulphur (S) and chlorine (Cl). The

micronutrients include: iron (Fe), manganese (Mn), copper (Cu), Cobalt (Co), zinc (Zn), molybdenum (Mo), iodine (I), fluorine (F), nickel (Ni), selenium (Se). Finally, the ultra-trace elements include: silicon (Si), chromium (Cr), boron (B), arsenic (As), nickel (Ni), tin (Sn) and vanadium (V) (Soetan et al., 2010; Suttle, 2010). In addition, the essentiality in some other nutrients has not been proved yet, but they are considered as useful elements to animal growth and health, e.g.: cadmium (Cd), aluminium (Al), lithium (Li) and lead (Pb) (Suttle, 2010). There are four main functions which minerals perform in animal body, i.e.: structural, physiological, catalytic and regulatory. In structural functions minerals form the structural components of tissues and body organs, e.g. P, Ca, Mg and Si in teeth and bones, P and S in muscle proteins, P and Zn improve the structural stability of membrane, from which they are part of. The minerals with physiological functions occur as electrolytes in body fluids and tissues in order to maintain osmotic pressure, acid-base balance, membrane permeability and transmit the nerve impulses, e.g. Na, K, Ca, Mg, Cl in blood and the spinal fluid and gastric juice can serve as an example of this function. The catalytic minerals behave as catalyst in enzyme and endocrine systems as part of metalloenzymes, hormones and coenzymes in anabolic, catabolic or life enhancing (oxidation) and life protection (antioxidation) processes. The pivotal role of regulatory minerals is to regulate cell replication and differentiation, e.g.: signal transduction by Ca ions, influence of selenocysteine and triiodothyronine on gene transcription (Suttle, 2010). The minerals have complex and multiple functions. Many functions can be performed by the simultaneously performed by the same mineral in animals, plants as well as microbes. For examples of mineral functions see Figure 2.

Mineral element	Role	Effects of deficiency
Calcium	Bone and teeth, transmission of nerve impulses	Rickets, osteomalacia, thin eggshells, milk fever
Phosphorus	Bone and teeth, energy metabolism	Rickets, osteomalacia, depraved appetite, poor fertility
Potassium	Osmoregulation, acid–base balance, nerve and muscle excitation	Retarded growth, weakness
Sodium	Acid–base balance, osmoregulation	Dehydration, poor growth, poor egg production
Chlorine	Acid–base balance, osmoregulation, gastric secretion	Alkalosis
Sulphur	Structure of amino acids, vitamins and hormones, chondroitin	Equivalent to protein deficiency (urea-supplemented diets)
Magnesium	Bone, activator of enzymes for carbohydrate and lipid metabolism	Nervous irritability and convulsions, hypomagnesaemia
Iron	Haemoglobin, enzymes of electron transport chain	Anaemia
Copper	Haemoglobin synthesis, enzyme systems, pigments	Anaemia, poor growth, depigmentation of hair and wool, swayback
Cobalt	Component of vitamin B_{12}	Pining (emaciation, anaemia, listlessness)
Iodine	Thyroid hormones	Goitre; hairless, weak or dead young
Manganese	Enzyme activation	Retarded growth, skeletal abnormality, ataxia
Zinc	Enzyme component and activator	Parakeratosis, poor growth, depressed appetite
Selenium	Component of glutathione peroxidase, iodine metabolism, immune function	Myopathy, exudative diathesis

Figure 2. The role of selected minerals and effect of their deficiency on animal's health (Source: McDonald et al., 2011)

1.4.3 Metabolism of minerals in animals

Minerals are following convoluted pathways through the animals once are ingested. Usually they are being transported from serosal side of gut mucosa to the liver either in bound or free form via the portal blood stream or even the can get stuck in the mucosa. Subsequently, from the liver they migrate to soft tissues, bones and udder and vice versa. The digestive process of animals can enhance or constrain the amounts of absorbed minerals and even change the forms in which they are being absorbed. The absorption of minerals are cautiously regulated by divalent metal regulators, which can be either specific or shared by more minerals. The turnover rate differ according the tissues as well as depends on the individual nutritional and physiological status, however in general the highest is in intestinal mucosa and liver, intermediate is in soft tissues and slowest is in bones. The nutrients are being lost by secretion (e.g. milk,

sweat, gastric juices) and excretion in form of urine (mainly N, K) and faeces (P) (Suttle, 2010).

1.5 The digestion of nutrients in herbivores

The large herbivores have evolved two strategies how plant material could be digested and thus according to morphology of digestive tract we can divided them on ruminants and non-ruminants (monogastric animals). The non-ruminant herbivores are represented by order Perissodactyla (equids, tapirs and rhinoceros), with well-developed cecal digestive system, whereas the most species of ruminant herbivores are found in Cetartiodactyla order (*Ruminantia* suborder) being characterized by four-compartment stomach, where fibre is digested. However, in every animal the digestion begin in mouth following by pharynx, oesophagus, simple/ compound stomach, small/ large intestine and ending with anus, lined with mucous membrane. The process of digestion can be distinguished on mechanical, chemical and microbial activities. Firstly, before the own digestion the large particles have to be break down to smaller particle size in order to pass through the mucous membrane into the blood and lymph. The mechanical processes involved the mastication of food with muscular contraction of alimentary canal (i.e. peristatic movement of intestines). The chemical processes are primarily secured by enzymes (secreted by digestive juices), responsible for breaking down plant nutrients, with subsequent utilization by herbivores. There are number of complex reactions involved in digestion process of nutrients in animal's body for which the catalyst (enzymes) secure the high velocity. Microbial digestion is performed by various bacteria, protozoa and fungi, which are mainly responsible for anaerobic fermentation of cellulose, where the location of where the fermentation take place is the most important differences in ruminant and non-ruminant herbivores (chapter 1.5.1 and 1.5.2).

The own digestion start in mouth, where small amount of enzymes α-amylase, which break down the carbohydrates, especially the starch, and lysozyme complex (breaking down peptidoglycans which are the cell walls component of many species of bacteria, resulting killing and dissolving them) are presented. However some monogastric animals lack the presence of α-amylase (e.g. horses, cats, dogs) or its activity is limited (e.g. pigs). When food enter the stomach it is not immediately mixed with gastric juices, so the α-amylase are able to continue with starch hydrolysis. The stomach can be

divided on cardia (entrance site), fundus and pylorus (terminal site), where the cardia and pylorus are sphincters controlling the passage of food through the stomach. The cardia and pylorus area produce the protective alkaline mucous, in order to protect the epithelium from acid effect of the gastric juice, which have low pH (2.0) suitable for partial hydrolysis of proteins by HCL and pepsins. Overall, the gastric juice consists of water, pepsinogens (inactive forms of pepsins), inorganic salts, mucus, hydrochloric acid and the factor important for the efficient absorption of vitamin B12. The main absorption site of nutrients occur in small intestine which have three parts: the duodenum, jejunum and ileum, containing the villi which greatly extend the area available for nutrients absorption. The food is mixed there with secretions from liver, pancreas and gut wall (duodenum), which contain enzymes for hydrolysis of various food components. The duodenum secretions are alkaline due to protect its wall from HCl acid entering from the stomach and it same time act as lubricant. The liver secrete bile entering to duodenum via the bile duct, which responsible for emulsifying fats and activating the pancreas lipase and it consist of sodium and potassium salts of bile acids, phospholipids and pigments (bilirubin, biliverdin) as the end product of haem, mucin and cholesterol catabolism. The pancreas gland has two secretary functions: endocrine function by producing insulin and exocrine function by producing the number of digestive enzymes and pancreatic juices secreted via pancreatic duct into duodenum. There are number of proenzymes, e.g. trypsinogen, chymotrypsinogen, procarboxypeptidases A and B, proelastase, α-amylase, lipase, lecithinases and nucleases activating the relevant enzymes responsible for hydrolysis of particular chemical substances having the optimum pH 7-9. Whereas the small intestine is the main absorption site, the large intestine can be described as main fermentation site, especially in monogastric herbivorous animals (horses). Large intestine has an irreplaceable role in retrieval of nutrients, electrolytes and water in the digesta, which were not absorbed in small intestine. Additionally, the five parts can be distinguished: cecum, appendix, colon (ascending, transverse, descending and sigmoid), rectum and anus (McDonald et al., 2011).

1.5.1 Digestion in monogastric herbivores

The monogastric herbivores are post gastric fermenters, with well- developed cecal digestion of fibre material and short retention time of digesta in stomach (2-6 hours) (McDonald et al., 2011). The main differences from ruminants digestion is that the fermentation of fibre by microbes takes place in the large intestine, with enlarged caecum, thus the enzymatic digestion occurs before the microbial fermentation. Hence, non-ruminants lose majority of microbial protein because the fermentation occurs after the main absorption site and thus only a small amount of microbial protein and vitamins can be recycled (Van Soest, 1982). Additionally, the equids lack the α-amylase enzyme in saliva as well as the gall bladder, so they cannot store the bile, but does not seem to affect the digestion of fat in small intestine (McDonald et al., 2011).

1.5.2 Digestion in ruminant herbivores

Ruminants have evolved a special system of digestion that involves microbial fermentation of food before its exposure to their own digestive enzymes (pregastric fermenatation). The fermentation of plant material occurs in four-compartment stomach, i.e. in rumen, reticulum, omasum, and abomasum, providing the capability of regurgitation of cud and its repeated chewing resulting in smaller particle size suitable for better digestion. Also the detoxification in ruminants is better than in simple stomach animals, in which the detoxification take place in liver. The anaerobic fermentation takes place in rumen, where symbiotic protozoa, bacteria and fungi transform the plant protein, starch and carbohydrates to higher quality animal protein as well as produce vitamin B complex. Subsequently, the chymus enter to true stomach (abomasum) and intestines, were majority of nutrients are absorbed. However, despites of many benefits the nutrients fermentation by microbes before absorption can lead to energetic loss if substrates like sugars/starches are fermented rather than being digested auto-enzymatically as well as it can lead to higher degree of saturation in the body fats (Clauss et al., 2010). Furthermore, ruminant herbivores widely differ in digestive morphology among themselves according to their foraging strategy (feeding style), i.e. whether they are grass and roughage eaters (GR), concentrate selectors (CS) or intermediate feeders (IM), firstly described by Hofmann (1973). More recently, they are distinguished according to the degree to stratification i.e. the papillation pattern of

rumen wall, with little stratification in ‘moose-type’ (browsers/CS) ruminants and a high degree of stratification into gas, particle and fluid layers in ‘cattle-type’ ruminants (grazers/ GR) (Clauss et al., 2010). These differences in stratification are expected to constrain a ruminant species to a browsing or grazing (or mixed-feeding) niche (Clauss et al. 2003; Lechner et al. 2010). The differences between these two types are following: Firstly, the ‘cattle-type’ ruminants have longer retention time of digesta, since the grass ferments slower than browse, and in the same tame they have more voluminous forestomach in order to avoid the constraint of food intake, resulting in higher fibre digestibility. Secondly, the large forestomach in the ‘cattle-type’ sp. compete for space with other organs (lungs, distal colon) resulting in higher respiratory rates and moist consistency of faeces. Thirdly, the stratification of rumen content is higher in ‘cattle-type’ sp. with stronger rumen pillars used for contracting against the plant material, whereas in ‘moose-type’ sp. the stratification is not at all or much more less, additionally they lack the gas dome, have weaker rumen pillars, more viscous rumen fluid and a less distinct difference between fluid present in the dorsal and the ventral rumen (summarized by Clauss et al., 2010). Also the ‘moose-type’ sp. might developed the larger saliva glands containing defences against to plant secondary metabolites in browse plants.

1.5.3 Foraging strategies of large herbivores

Diet of large herbivores is a linkage between habitat, feeding type strategies and nutritional requirements and differences in content of minerals according to different type of forage, i.e. fruits, browse and grass. The complex parameters such as: body size and digestive tract morphology, rumino-reticular volume to body weight (determines which food type is most efficient for processing) and mouth size (i.e. smaller mouth size is being related to browser and vice versa) determine the foraging strategy of ruminant herbivores (Hanley, 1982; Danell, 2006). Moreover, the diet decisions of herbivores are trade of between foraging time (time minimizing vs. time maximizing strategy, Bergman et al., 2001) and the quality of forage (nutrients versus antinutrients). In respect to that, the diet selection is often done against to plant secondary compounds based on experience of aversive post-ingested effect. The lower nutritional quality of forage is balanced via higher volume of food intake and by higher spectrum of selected

plant species (Provenza et al., 2003). Moreover, the availability of nutrients to animals is dependent on nutrient ratios plants, particularly the Ca:P ratio of 1:1 or 2:1 is considered as favourable to absorption of these minerals (Stapelberg, 2008).

The feeding type strategies are traditionally categorized on grass and roughage feeders (grazers/GR), concentrate selectors (browsers/CS) and intermediate feeders (IM) according to Hofman (1989), however they can be broadened of hypergrazers and hyperbrowsers with >95% of C_4 grass or C_3 browse respectively (Cerling et al, 2003). From recent investigation, it was proved that both browsers and grazers evolved from intermediate feeder ruminants (Codron et al., 2010)). The grazers can be represented by cattle (*Bos taurus*), African buffalo (*Syncerus caffer*), Wildebeest (*Connochaetes taurinus*), equids (horses, zebras, asses). Browsers are, for example, giraffe (*Giraffa camelopardalis*), kudu (*Tragelaphus strepsiceros*), Derby eland (*Taurotragus derbianus*), moose (*Alces alces)* and mix-feeders can be represented by impala (*Aepyceros malapus*), springbok (*Antidorcas marsupialis*) and nyala (*Tragelaphus angasii*) (Grant et al., 2000; Codron et al., 2007; Stapelberg et al., 2008).

1.6 Methods of determination of large herbivores diet

As the diet quality of free ranging herbivores are hardly to be assessed directly from nutrient levels of forage since wild herbivores are able to select the most nutrient rich plants/ plant parts by adopting different foraging strategies, the faecal analysis become a reliable and relatively simple method how to determine the nutrient status of large herbivores. For these purposes the FN and FP are widely use as index of diet quality of large herbivores and their actual body condition. Moir (1960) and Belonje (1980) proved the FP correlation with P intake of large herbivores, and according to Grant et al. (1996) the FN reflect the weight gain of herbivores. However, for assessment of diet quality FN and FP should be used together because of their linked excretion by animals (Moir, 1966). From many surveys, various concentration of FN and FP have been set up for particular herbivorous animals indicating the threshold of dietary deficiency. For example, according to Grant et al. (2000) the FN concentrations between 13- 16 g kg^{-1} an FP concentrations in 1.9- 2.0 g kg^{-1} interval are above the threshold of dietary deficiency in majority of large herbivore species. So far, several techniques have been developed and used for dietary analysis from faeces. They are: microhistological

technique, based on faeces maceration and later identification of plant residues under microscope and comparing them with prepared reference slides, technique based on natural alkanes analysis of plant cuticular wax (Cuartas and Garcia, 1996), method of stable carbon isotope composition of faeces and animal tissues (Botha and Stock, 2005, Codron et al., 2007), use FN and FP as indicators of large herbivores diet quality (e.g. Wrench and Meissner,1997; Grant et al., 2000; Stapelberg et al., 2008; Leslie et al., 2008) NIRS- near infrared reflectance spectroscopy (e.g. Lyons and Stuth, 1992; Coates, 2000) and recently DNA faeces analysis, which can provide use the most precise information not only about the diet composition but also about the gender of animals and other population characteristics (Pegard et al. 2009; Valentini et al, 2009; Pompanon et al., 2012). Especially, the stable carbon isotope method from animal tissues (e.g. bones, teeth, hair) or excreta is very useful in Africa since it reflects the relative proportions of browse (i.e., trees, shrubs with C_3 photosynthesis pathway) to grass (monocotyledonous plant with C_4 photosynthesis pathway) being ingested by herbivores (Codron et al., 2007).

2 Aims

The general aim of my thesis was to compare diet quality of large herbivores across European and African continents.

Particular aims were to determine:

1) To determine concentrations of macronutrients (N, P, K, Ca, Mg) and fibre fractions (NDF, ADF, ADL) in faeces of selected species of large herbivores.

2) To compare concentrations of macronutrients (N, P, K, Ca, Mg) and fibre fractions (NDF, ADF, ADL) in faeces of ruminants adopting different foraging strategies in European and African localities.

We hypothesised, that in Africa animals will have less nutritive diet than in Europe because of soil age and thus better availability of nutrients (based on Lambers et al., 2011). Contrasting hypothesis was that there will be no difference because of evolved strategies of selection for nutrients. And that CS will have higher fibre concentration than IM, GR.

3) To compare concentrations of macronutrients (N, P, K, Ca, Mg) and fibre fractions (NDF, ADF, ADL) in faeces of non-ruminants in European and African localities.

We hypothesised that that animal in Europe will have higher diet quality due to better soil fertility (based on Lambers et al., 2011).

4) To test the functional link between concentrations of nitrogen and phosphorus in animal faeces and soil on pasture.

We hypothesised, that There will not be direct relationship between faecal N, P and soil N, P because herbivores can balance potential nutrient deficiency through foraging strategies (diet selection and migration).

3 Methods

The procedure of sampling included collection of 281 faecal samples from 17 animal species (Table 1-2) and 13 representative soil samples from given localities

3.1 Model Animals

As model large herbivore species, following ones were chosen from the European continent: European bison (*Bison bonasus*), cattle (*Bos taurus*), sheep (Ovis aries), goat (*Capra aegagrus hircus*), moose (*Alces alces*), Ass (*Equus asinus*), horse (*Equus caballus*) and from the African continent: elephant (*Loxodonta africana*), zebra (*Equus quagga*), buffalo (*Syncerus caffer*), hartebeest (*Alcelaphus buselaphus*), bontebok (*Damaliscus pygargus pygargus*), roan antelope (*Hippotragus equinus*), watrebuck (*Kobus ellipsiprymnus*), springbok (*Antidorcas marsupialis*), eland (*Taurotragus oryx*), derby (*Taurotragus derbianus)*, sheep (*Ovis aries*). Foraging strategy of ruminant species assigned in Table 1-2, was determined into three categories according to following authors:

1) Grass and roughage eaters: Cattle and sheep (Hoffman, 1989), African buffalo, hartebeest, bontebok and waterbuck (Grant et al., 2000; Stapelberg et al., 2008, Wilson and Mittermeier, 2011), roan antelope (Schuette et al, 1998; Codron et al, 2007; Wilson and Mittermeier, 2011). However, the roan antelope is grazer in most places, in West-African region during the hot- dry season adopt the intermediate feeding strategy, consuming shrubs and legumes with less than 50% of grass (Codron et al., 2007; Wilson and Mittermeier, 2011).

2) Intermediate feeders: elephant (Cerling et al., 1999; Dolmia et al., 2007; Wilson and Mittermeier, 2011), goat (Hoffman, 1989; Omphile et al., 2004; Jonsson, 2010), wisent (Hoffman, 1989; Gębczyńska et al., 1991; Braukmann, 2011), springbok (Grant et al., 2000; Stapelberg et al., 2008).

3) Concentrate selectors: giant eland (derby) (Hejcmanová et al., 2010), common eland (Cerling et al., 2003; Codron et al., 2005, 2007; Wallington et al., 2007), moose (Hoffman, 1989; Wilson and Mittermeier, 2011).

3.1 Localities

The soil and faeces samples were collected in Europe and Africa together in 13 localities. The European samples were collected at 7 localities either in semi-natural fenced pastures or in free ranging conditions. Samples from Africa were collected at 6 localities from National parks or wildlife reserves.

European localities

There were two localities in Czech Republic Židlov and Pálava. **Židlov** (50.62° N, 14.86° E) is a fenced park on the former military area situated in the Czech Republic, in the district of Česká Lípa near to city Mimoň. It is the second largest game park in Czech Republic with size of 3780 ha. All animals there are free ranging within the area without additional feeding. The landscape is characteristic by sandy soil with predominance of forest (pine, spruce, beech, shrubs etc.) and interspersed by open grasslands (Appendices 1-2). Faeces of European bison were collected there. Židlov is under the management of Vojenské lesy a statky České republiky (www.vls.cz.).

Pálava (48°50'N, 16°38'E) is the National Nature Reserve located 40 km south of Brno in with total area 109.06 ha. The altitude is ranging 350–445 m a.s.l. The annual rainfall is about 571 mm and annual mean temperature is 9.6°C. There are two soil types present: rendzina and chernozem. The grazing area is species-rich dry grassland with a mosaic of vegetation: *Festucion valesiacae* (dominated by *Carex humilis* and *Aster linosyris*) on the southward slope of the hill, *Cirsio-Brachypodion pinnati* (dominated by *Bromus erectus* and *Brachypodium pinnatum*) on the lower slopes, and *Berberidion* dominated by scrubs *Crataegus monogyna, Prunus mahaleb,* and *P. spinosa,* which indicate abandoned pastures in the area (Chytrý et al., 2001; Pokorná et al., 2013). The faeces from sheep, goats and horses were collected there.

Next locality was **Kraansvlak** national park in Netherlands (GPS 52°07'40.29" N, 5°31'43.32" E). It is the coastal dune area, situated in Netherlands, west of Amsterdam as a part of the Zuid-Kennemerland national park (Figure X). It is the location of the European bison and konik horses reintroduction pilot project (McCulla, 2012). It is a fenced area with size of 226 ha, with large variety of habitats from coastal dunes to old-growth forests (Appendices 6-7). The landscape used to be dominated by open sand and grasslands, however, in the last twenty years there has been a dramatic change in the

landscape from limited shrubbery, open sand and grassland areas to an area that has lost almost all of the open sand areas, due to increased overgrowing (McCulla, 2012). Shortly before the reintroduction the area consisted of 41% shrubs and trees, while only 57% were grassland and open sand. The remaining 2% were fresh water and marshland (Braukmann, 2011). From this locality faeces samples from European bison, cattle, sheep and konik horse were collected.

South European localities were in Hungary and Bulgaria. In **Bulgaria**, the site was located nearby the town **Obzor** (42°49′N 27°53′E) on the Black Sea coast. The climate is Mediterranean with hot summers and mild winters with annual average temperature 12°C and annual average rainfall around 400-600 mm, receiving little rainfall all months. Samples were collected on Mediterranean semi-natural dry grassland used as pasture for extensive cattle, sheep and goat grazing (Appendix 8). Faeces samples from horses, cattle and goats were collected there. In **Hungary**, the samples collection was located in the **Hortobágy**, an 800 km^2 national park in eastern Hungary (N 47°39.02197', E 21°7.48080'), rich with folklore and cultural history. In Hortobágy, the average annual temperature is 10.4 °C and rainfall there averages 556 mm/ year. There is the largest semi-natural grassland in Europe, an alkaline grassy steppe grazed widely by Hungarian Grey cattle, racka sheep, water buffalo, and horses tended by herdsmen (Appendices 4-5). The plant species can be represented by a grazing tolerant graminoids and forbs e.g.: *Cynodon dactylon*, *Poa angustifolia*, *Festuca pseudovina*, *Festuca rupicola*, *Carex stenophylla*, *Galium verum*, *Euphorbia cyparissias*, *Cruciata pedemontana*, *Achillea collina* (Török et al., 2011). Faeces from cattle, sheep were collected there.

Northern European localities were in Iceland and Norway. In **Iceland**, the site was located in the open landscape in the North Iceland (65°40' N, 17°33'W) on large unfenced semi-natural pasture dominated by boreal vegetation with grass *Deschampsia cespitosa* and dwarf shrubs *Betula nana* and *Salix lanata* (Appendix 4). The climate is cold oceanic with the mean annual temperature for around 5°C, the annual rainfall in the sampling area is between 1000 and 400 mm. Faeces samples sheep were collected there. In **Norway**, the site (N 59°45.61567', E 8°47.10187') is also located in the open landscape (altitude approx.. 550 m a.s.l.), in pine forest with undergrowth vegetation formed by *Vaccinium myrtillus* and *V. vitis-idea* on the granitic rock. The average

annual temperature is 4.0 °C and the rainfall here averages 737 mm (data for Rjukan, Norway). There were collected samples of moos faeces.

African localities

There were two localities in **Senegal** Bandia and Fathala reserves. The **Bandia Reserve** is a 1500 ha fenced wildlife nature reserve, which lies 65 km south of Dakar. It was developed in order to habitat and wildlife conservation and safari-tourism in Senegal. The fenced breeding enclosure (60 ha) of western Derby eland is included inside of reserve. The wet season is lasting from July to October with an average annual precipitation of 484 mm. The average temperature in the dry season is 25°C (Al-Ogoumrabe, 2002). The vegetation type is *Acacia ataxacantha-Acacia seyal* bushland (Lawesson 1995) and the breeding enclosure for western Derby eland is characterized by several Acacia species, *Azadirachta indica*, *Boscia senegalensis*, *Combretum micranthum*, *Grew*ia *bicolor*, *Feretia apodanthera*, *Ziziphus mauritiana*, the annual grass *Brachiaria lata* and forbs *Abutilon pannosum*, *Achyrantes aspera*, with the vine *Merremia aegyptiaca* in the undergrowth (Hejcmanová et al., 2010) (Appendices 9, 10, 11). The faeces samples of African buffaloes, zebras, roan antelopes and Derby and common eland antelopes were collected there.

The **Fathala Reserve** (13°9'N, 16°27'W) is a part of the Delta du Saloum National Park in Western Senegal. It is a fenced area managed for tourism covering 2000 ha. Within the reserve a fenced enclosure (70 ha) with access restrictions to the public was established in 2006 as part of a conservation breeding programme for the critically endangered western giant eland. The climate is characterized by a warm, rainy season from June to October, and a dry season from November to May. The mean annual precipitation is 1022 mm, and the mean annual temperature is 26°C. The area is at the interface of the Sudanese and Sudano-Guinean savannas. The major vegetation types are wooded grassland and woodland dominated by plant families Caesalpiniaceae (16.3%), Combretaceae (16.3%) and Mimosaceae (12.2%). The fenced enclosure was dominated by *Acacia macrostachya* with several species of the *Combretum* genus, *Piliostigma thonningii*, *Prosopis africana*, *Pterocarpus erinaceus*, *Terminalia laxiflora* and *T. macroptera*, and with scarce undergrowth formed mostly by *Andropogon gayanus* and *Schizachyrium sanguineum*, and almost no forbs (Hejcmanová et al.,

2013). The faeces samples of African buffaloes, zebras and roan antelopes were collected there (Appendices 12-13).

Other localities were in Chad, Republic of South Africa and Zambia.

In **Chad** it was the Zakouma national park (10°50'15.0"N 19°39'19.9"E), which is 3000km^2 large area located in southeast Chad. The climate is of the Soudano-Sahelian type characterized by a rainy season from May to October and a dry season the remainder of the year. The dry season can be divided into two periods: a cool dry season from November to January and a hot dry season from February to April. The hydrographic network is concentrated in the eastern half of the park. The plant savanna species composition is changing according to a north–south gradient, with *Acacia* sp. dominating in the north of the park, *Combretaceae* in the centre and *Caesalpiniaceae* in the south (Dolmia et al., 2007). The faeces samples of African buffaloes, African elephants, zebras, hartebeests, waterbuck antelopes and sheep were collected there.

In **Republic of South Africa** were two localities the Augrabies and Bontebok national parks. **Augrabies National Park** (28°35'59.9"S 20°19'59.9"E) is located 120 km west of Upington in the Northern Cape Province and covers the area of 820 km^2 around the Augrabies Falls. It is a semi desert rocky areas, with infertile soil where plant species such as *Aloe dichotoma, Aloe claviflora, Acacia erioloba, Acacia karroo, Boscia albitrunca, Ficus cordata, Pappea capensis, Sisyndite spartea, Stipagrostis hochstetteriana, Rhigozum trichototum* are prevailing. The faecal samples from zebras, common eland and springbok antelopes were collected. **Bontebok National Park** (34°03'56.4"S 20°28'07.4"E) is located south of Swellendam, in the foothills of the Langeberg Mountains and it is the smallest species specific national park in South Africa, covering an area of 28 km^2. The park was established in order to preserve bontebok antelopes and it is a part of Cape Floristic Region. The area is characterized by fertile soil and fynbos vegetation type, were plant species such as restioids, ericoids, proteoids are found. The faeces samples from zebras and bontebok antelopes were collected there.

In **Zambia** it was the **Mosi-Oa- Tunya National Park** (-17°52'15.2"S 25°48'37.7"E) where the faeces samples of African elephants were collected. It is the smallest National park in Zambia situated in south- western boundary of Livingstone city covering 66km^2. It is divided in two sections- a wildlife park, where faecal samples

were collected, and Victoria Falls area. The wildlife park consists of riverine forest, miombo woodland and grassland (Appendix 14).

3.2 Data collection

3.2.1 Faeces samples

From European localities 112 faecal samples were taken from 7 animal species and from African localities 169 faecal samples were taken from 11 animal species. At least 2 faecal -samples were taken from one animal species (Table 1-2). Faeces were collected specifically in fresh stadium and dung with beetles' activity were excluded. Faeces samples were subsequently dried and sent to accredited national laboratory Ekolab Žamberk (http://www.ekolab.zamberk.cz) for analyses of concentrations of macro-elements (N, P, K, Ca, Mg), residual ash content (ash–P,K,Ca,Mg), neutral- (NDF) and acid-detergent fibre (ADF) and acid-detergent lignin (ADL) were determined as well. NDF represents cellulose, hemi-cellulose and lignin together, ADF represents cellulose and lignin. The N concentration was determined using an automated analyser TruSpec (LECO Corporation USA) by combustion with oxygen in an oven at 950 °C. Combustion products were mixed with oxygen and the mixture passed through an infrared CO2 detector and through a circuit for aliquot ratio where carbon is measured as CO2. Gases in the aliquot circuit were transferred into helium as a carrying gas, conducted through hot copper and converted to N. Faeces samples were burnt in a microwave oven at temperature of 550 °C and weighed in order to determine ash content. After that were samples mineralized using aqua regia and P, K, Ca and Mg concentrations were then determined in the solution using ICP-OES (Varian VistaPro, Mulgrave, Vic., Australia). NDF, ADF and ADL contents were determined by standard methods of AOAC (1984).

3.2.2 Soil samples

Soil samples were collected in areas where investigated animals were foraging. Samples had been taken from the upper 0 - 10 cm soil layer. From each locality 2 to 5 samples were collected. Subsequently, the soil samples were air-dried, grounded in a mortar, and sieved to 2 mm after removal of living roots. Samples were analysed in accredited Czech national laboratory Ekolab Žamberk (http://www.ekolab.zamberk.cz) for concentrations of plant available P, K, Mg, Ca in (mg kg^{-1}), total N (STN) and organic C (C_{org}) (g kg^{-1}). Determination of plant available concentrations of P, K, Mg, Ca was done by using Mehlich III extraction (Mehlich, 1984). The determination of total N was performed by using a TruSpec f. Leco instrument, where the soil samples were combusted at 950°. Organic C (C_{org}) concentrations were performed spectrophotometrically after oxidation in $K_2Cr_2O_7$ solution in H_2SO_4, at 135°. Soil pH (H_2O) was measured in suspension of 10g dry soil mixed with 50ml of distilled H_2O.

3.3 Data analyses

The diet quality of investigated animal species were determined using descriptive statistic and main effect ANOVA with Tukey post-hoc test (aim 1). The macronutrients and fibre fraction (N, P, ADL) concentrations were used as dependent variables and animal species and locality were used as categorical predictor.

For comparison of concentrations of macronutrients (N, P, K, Ca, Mg) and fibre fractions (NDF, ADF, ADL) in faeces of ruminants with different foraging strategies in European and African localities, the factorial ANOVA with Tukey post-hoc test were used. As dependent variables the macronutrients and fibre fraction concentrations were used and foraging strategy and continents were used as categorical predictor (aim 2).

For comparison of concentrations of macronutrients (N, P, K, Ca, Mg) and fibre fractions in faeces of non-ruminants in European and African localities the one- way ANOVA were used together with Tukey post-hoc test (aim 3). The concentrations of macronutrients and fibre fractions were used as dependent variable and animal species as categorical predictor.

For testing of functional link between soil N, P and diet quality (N, P in faeces) simple linear regression was applied. Mean values of N, P concentrations in soil were

calculated for these analyses. All analyses were processed in STATISTICA 13.0 program (StatSoft, Tulsa, USA).

To analyse mutual relationships among concentrations of N, P, K, Ca, Mg in faeces and to test the effects of localities and species for ruminants and non- ruminants separately, constrained redundancy analyses (RDA) in the CANOCO 5 program (Ter Braak and Šmilauer, 2012) were used and followed by Monte Carlo permutation tests (999 permutations). Data were log-transformed, centred and standardised in the course of the analyses. The results were visualized in the form of an ordination diagram constructed by the Canoco program (Ter Braak and Šmilauer, 2012).

4 Results

4.1 Diet quality of investigated animal species

In total, we have collected **281** faecal samples from **17** animal species (i.e. elephant (*Loxodonta africana*), Ass (*Equus asinus*), horse (*Equus caballus*), zebra (*equus zebra quagga*), cattle (*Bos taurus*), buffalo (*Syncerus caffer*), hartebeest (*Alecelaphus buselaphus*), bontebok (*Damaliscus pygargus pygargus*), roan antelope (*Hippotragus equinus*), watrebuck (*Kobus ellipsiprymnus*), springbok (*Antidorcas marsupialis*), sheep (*Ovis aries*), wisent (*Bison bonasus*), moose (*Alces alces*), eland (*Taurotragus oryx*), Derby eland (*Taurotragus derbianus)*, goat (*Capra aegagrus hircus*) from **2** continents and **10** countries (Africa: Senegal, Chad, Zambia, Republic of South Africa and Europe: Czech Republic, Bulgaria, Hungary, Iceland, Netherlands, Norway).

Detailed results of faecal analysis of macronutrients are given in **t**he **Table 2** and concentration of ash and fibre fractions (NDF, ADF, ADL) in **Table 3**. The minimum number of species samples are 3 (moose, bontebok, springbok), maximum is 58 (Derby eland). For the graphically displayed faecal concentrations of macronutrients N, P and lignin of all investigated animals species see Figure 1**.** The highest FN concentration of all investigated animals had sheep together with goat, wisent, derby antelope and cattle ($P < 0.001$) in contrary the zebra had the lowest FN concentration (see Figure 1-a). The highest concentration of FP (Figure 1-b) had also sheep and goat together with cattle and ass from Europe, in contrary the lowest concentration had animal from Africa (e.g. bontebok, springbok) with exception of moose from Europe ($P < 0.001$). Finally, the lignin concentration were highest in concentrate selector, i.e. derby and eland antelope together with moose ($P < 0.001$), followed by elephant and roan antelope. The lowest lignin concentration had the springbok as the intermediate feeder (see Figure 1-c).

Table 2. Faeces concentrations of N, P, K, Ca, Mg, N:P, Ca:P and (mean ± SE) of investigated animals and their foraging strategy, site of occurrence with number of faecal samples.

Animal	Foraging strategy	Site	Number of samples	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})	N:P	Ca:P
Elephant	-	CHAD	9	15.41±1.01	2.87±0.32	15.36±1.30	14.53±0.97	2.60±0.26	5.62±0.32	5.43±0.56
Elephant	-	ZM	2	13.18± 1.0	2.00±0.10	8.6±0.6	22.65±1.45	2.45±0.15	6.63±0.83	7.55±3.60
Zebra	-	SN	20	13.41±0.52	3.44±0.45	8.84±0.49	12.03±2.06	3.93±0.23	4.88±0.50	3.28±0.29
Zebra	-	RSA	6	14.08±0.70	2.3±0.55	5.73±0.69	16.93±8.08	3.92±1.32	8.14±1.83	5.97±2.54
Horse	-	NL	10	18.11±0.31	2.88±0.21	8.23±0.55	6.38±0.56	2.27±0.14	6.61±0.50	2.24±0.14
Horse	-	CZ	8	17.86±1.80	6.64±0.46	16.60±1.32	10.36±1.01	2.83±0.19	2.68±0.15	1.57±0.13
Horse	-	BG	4	18.96±0.96	5.75±0.21	12.33±0.87	13.15±0.73	4.10±0.25	3.32±0.23	2.29±.0.9
Horse	-	HU	5	16.19±0.54	3.76±0.28	10.96±1.19	4.30±0.68	2.10±0.16	4.39±0.33	1.19±0.22
Ass	-	CZ	5	17.12±0.46	5.64±0.16	12.06±0.98	9.76±0.20	2.76±0.10	3.05±0.13	1.73±0.03
Ass	-	HU	2	16.7±0.37	4.05±0.35	9.90±0.90	5.15±2.15	2.05±0.05	4.15±0.27	1.33±.0.65
Sheep	GR	NL	5	29.01±0.39	3.10±0.14	3.32±0.40	27.50±1.32	5.92±0.61	9.43±0.41	8.89±0.34
Sheep	GR	CZ	12	25.01±1.29	8.87±0.69	11.99±0.89	33.07±3.18	6.09±0.56	2.98±0.26	3.68±0.17
Sheep	GR	IS	3	32.75±0.53	6.00±0.12	16.20±0.74	22.77±3.27	7.03±1.31	5.46±0.16	3.81±0.60
Sheep	GR	HU	4	22.24±0.54	7.20±0.65	8.83±0.54	15.70±2.86	4.10±0.40	3.15±0.22	2.15±0.28
Sheep	GR	CHAD	1	28.23±0.00	5.10±0.00	10.80. ±0.00	25.90. ±0.00	7.80. ±0.00	5.54±0.00	5.08±0.00
Cattle	GR	CZ	12	24.32±1.44	7.65±0.63	7.27±0.75	30.17±1.96	5.12±0.28	3.27±0.14	4.14±0.32
Cattle	GR	NL	5	22.80±0.66	3.72±0.25	7.80±0.59	19.28±1.28	4.44±0.30	6.21±0.32	5.28±0.49
Cattle	GR	BG	4	21.96±1.37	5.58±0.34	5.90±0.23	24.48±1.80	6.30±0.66	4.02±0.49	4.49±0.61
Cattle	GR	HU	7	20.15±0.93	3.79±0.63	5.53±0.98	9.74±0.96	3.29±0.31	6.19±1.02	3.00±0.66
Buffalo	GR	CHAD	7	16.55±0.96	3.50±0.32	10.33±0.53	12.73±1.25	3.14±0.21	4.85±0.34	3.74±0.39
Buffalo	GR	SN	16	16.12±0.72	3.79±0.39	7.89±0.5	15.56±1.31	5.69±0.39	4.98±0.57	4.46±0.38
Hartebeest	GR	CHAD	3	20.02±0.70	4.77±0.27	9.30±0.55	11.47±0.47	4.67±0.34	4.22±0.23	2.41±0.11
Bontebok	GR	RSA	3	16.87±1.10	2.20±0.26	5.57±0.38	5.67±0.69	2.33±0.52	7.78±0.47	2.58±0.07
Roan	GR	SN	20	18.63±0.59	3.30±0.22	5.47±0.43	20.30±2.69	4.90±0.42	6.04±0.35	5.96±058
Waterbuck	GR	CHAD	5	18.38±1.45	3.80±0.55	11.32±0.92	17.28±2.56	4.00±0.57	5.08±0.59	4.60±0.47

Wisent	GR	NL	5	23.95±0.72	3.82±0.49	9.48±0.92	17.58±0.87	3.88±0.13	6.60±0.66	4.88±0.59
Wisent	GR	CZ	5	22.41±1.53	4.60±0.39	5.08±0.50	19.84±2.41	4.52±0.59	4.90±0.13	4.29±0.31
Moose	CS	NO	3	18.95± 3.99	1.83±0.39	3.30±0.55	7.37±2.04	1.47±0.58	10.42±0.78	3.97±.0.49
Eland	CS	RSA	4	19.14±1.25	2.05±0.18	4.35±0.23	30.25±4.22	2.85±0.65	9.49±0.72	14.71±1.39
Eland	CS	SN	11	25.67±1.67	3.63±0.24	8.84±0.86	24.72±2.12	4.46±0.32	7.18±0.38	7.22±0.87
Derby	CS	SN	58	20.94±0.44	3.84±0.15	7.00±0.28	31.80±1.82	4.71±0.21	5.83±0.21	8.65±0.54
Springbok	IM	RSA	4	14.33±0.29	2.13±0.09	2.83±0.29	22.18±0.79	4.13±0.6	6.76±0.14	10.45±0.24
Goat	IM	CZ	10	25.04±0.65	7.50±0.17	8.48±1.14	38.80±2.80	5.29±0.26	3.35±0.11	5.21±0.41
Goat	IM	BG	4	21.72±1.90	4.48±0.23	7.35±0.65	22.35±1.98	6.08±0.62	4.83±0.22	4.97±0.24

Table 3. Faeces concentrations of Ash, NDF, ADF, Lignin in g kg-1 and (mean ± SE) of investigated animals with their foraging strategy, site of occurrence and number of faecal samples.

Animal	Foraging strategy	Site	Number of samples	Ash (g kg^{-1})	NDF (g kg^{-1})	ADF (g kg^{-1})	Lignin (g kg^{-1})
Elephant	-	CHAD	9	15.41±1.01	2.87±0.32	15.36±1.30	14.53±0.97
Elephant	-	ZM	2	13.18± 1.0	2.00±0.10	8.6±0.6	22.65±1.45
Zebra	-	SN	20	13.41±0.52	3.44±0.45	8.84±0.49	12.03±2.06
Zebra	-	RSA	6	14.08±0.70	2.3±0.55	5.73±0.69	16.93±8.08
Horse	-	NL	10	18.11±0.31	2.88±0.21	8.23±0.55	6.38±0.56
Horse	-	CZ	8	17.86±1.80	6.64±0.46	16.60±1.32	10.36±1.01
Horse	-	BG	4	18.96±0.96	5.75±0.21	12.33±0.87	13.15±0.73
Horse	-	HU	5	16.19±0.54	3.76±0.28	10.96±1.19	4.30±0.68
Ass	-	CZ	5	17.12±0.46	5.64±0.16	12.06±0.98	9.76±0.20
Ass	-	HU	2	16.7±0.37	4.05±0.35	9.90±0.90	5.15±2.15
Sheep	GR	NL	5	29.01±0.39	3.10±0.14	3.32±0.40	27.50±1.32
Sheep	GR	CZ	12	25.01±1.29	8.87±0.69	11.99±0.89	33.07±3.18
Sheep	GR	IS	3	32.75±0.53	6.00±0.12	16.20±0.74	22.77±3.27
Sheep	GR	HU	4	22.24±0.54	7.20±0.65	8.83±0.54	15.70±2.86
Sheep	GR	CHAD	1	28.23±0.00	5.10±0.00	10.80. ±0.00	25.90. ±0.00
Cattle	GR	CZ	12	24.32±1.44	7.65±0.63	7.27±0.75	30.17±1.96
Cattle	GR	NL	5	22.80±0.66	3.72±0.25	7.80±0.59	19.28±1.28
Cattle	GR	BG	4	21.96±1.37	5.58±0.34	5.90±0.23	24.48±1.80
Cattle	GR	HU	7	20.15±0.93	3.79±0.63	5.53±0.98	9.74±0.96
Buffalo	GR	CHAD	7	16.55±0.96	3.50±0.32	10.33±0.53	12.73±1.25
Buffalo	GR	SN	16	16.12±0.72	3.79±0.39	7.89±0.5	15.56±1.31
Hartebeest	GR	CHAD	3	20.02±0.70	4.77±0.27	9.30±0.55	11.47±0.47
Bontebok	GR	RSA	3	16.87±1.10	2.20±0.26	5.57±0.38	5.67±0.69
Roan	GR	SN	20	18.63±0.59	3.30±0.22	5.47±0.43	20.30±2.69
Waterbuck	GR	CHAD	5	18.38±1.45	3.80±0.55	11.32±0.92	17.28±2.56

Wisent	GR	NL	5	23.95±0.72	3.82±0.49	9.48±0.92	17.58±0.87
Wisent	GR	CZ	5	22.41±1.53	4.60±0.39	5.08±0.50	19.84±2.41
Moose	CS	NO	3	18.95± 3.99	1.83±0.39	3.30±0.55	7.37±2.04
Eland	CS	RSA	4	19.14±1.25	2.05±0.18	4.35±0.23	30.25±4.22
Eland	CS	SN	11	25.67±1.67	3.63±0.24	8.84±0.86	24.72±2.12
Derby	CS	SN	58	20.94±0.44	3.84±0.15	7.00±0.28	31.80±1.82
Springbok	IM	RSA	4	14.33±0.29	2.13±0.09	2.83±0.29	22.18±0.79
Goat	IM	CZ	10	25.04±0.65	7.50±0.17	8.48±1.14	38.80±2.80
Goat	IM	BG	4	21.72±1.90	4.48±0.23	7.35±0.65	22.35±1.98

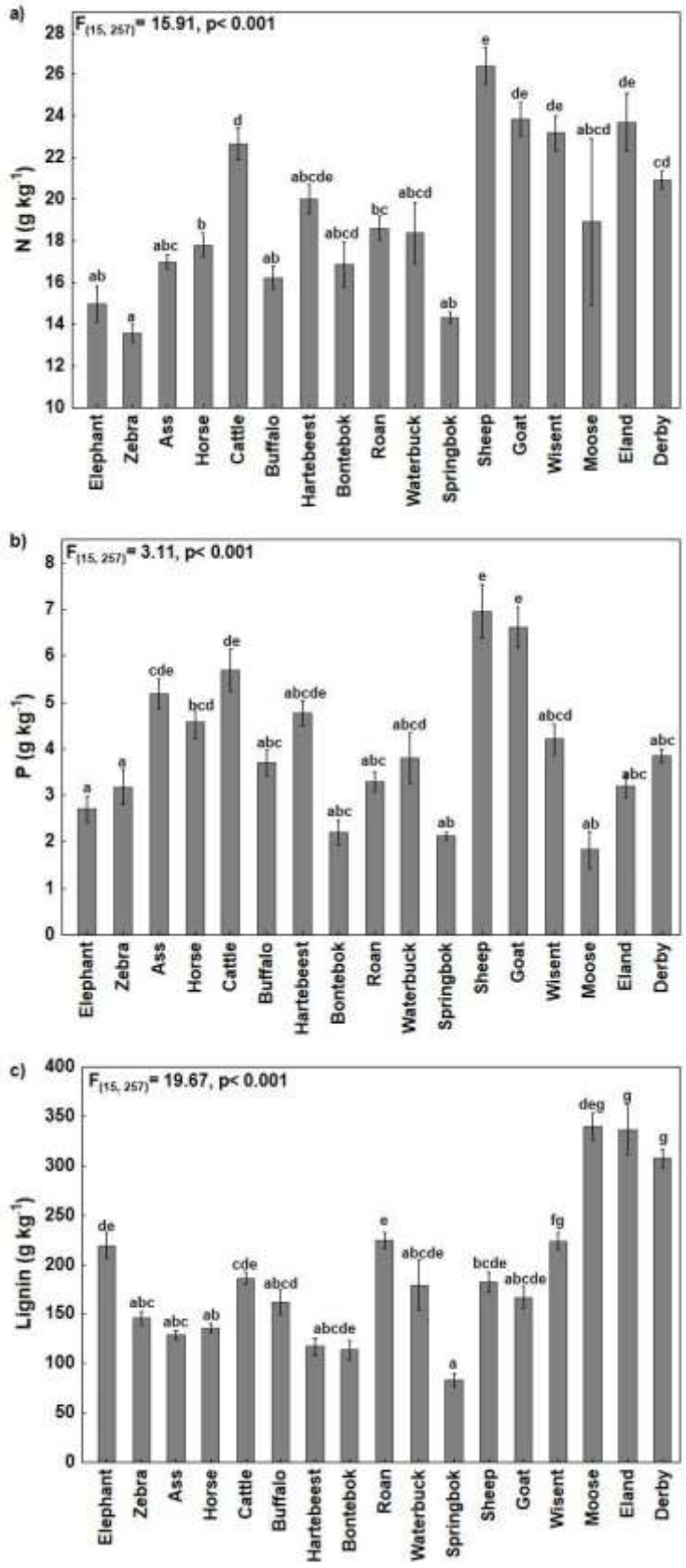

Figure 3. Faecal concentrations of N (a), P (b) and Lignin (c) in g kg^{-1} of all investigated animal species

4.2 Diet quality of ruminants in Europe and Africa

In general concentrations of faecal macronutrients (further only nutrients) are higher in favour to ruminants from Europe in comparison to ruminants from Africa (see Figure 2, 3, 4). In contrary, the ratio of N:P, Ca:P were higher for African ruminants, except of N:P of moose from Europe. The higher concentration of all macronutrients had GR and IM in comparison to CS, with exception of Ca concentrations. The fibre concentration were more balanced among continents, with the highest lignin concentrations in CS in compare to GR and IM. However, the concentration of all fibre fractions were higher in European intermediate feeders (wisent, goat) than in African springbok (Figure 4- h, i, j). The relationships of distribution of nutrients in ruminant faeces samples are shown in Figure 5. RDA (redundancy analysis) analysis reveal that the first ordination axis explained 24%, the first two axis together 37% and four axis together 45% variability of faeces chemical composition. Permutation tests, were statistically significant. It can be seen that the concentration of macronutrients as well as fibre fraction more rely on particular localities were ruminant species occur than on continents. All results given above were statistically significant ($P < 0.001$).

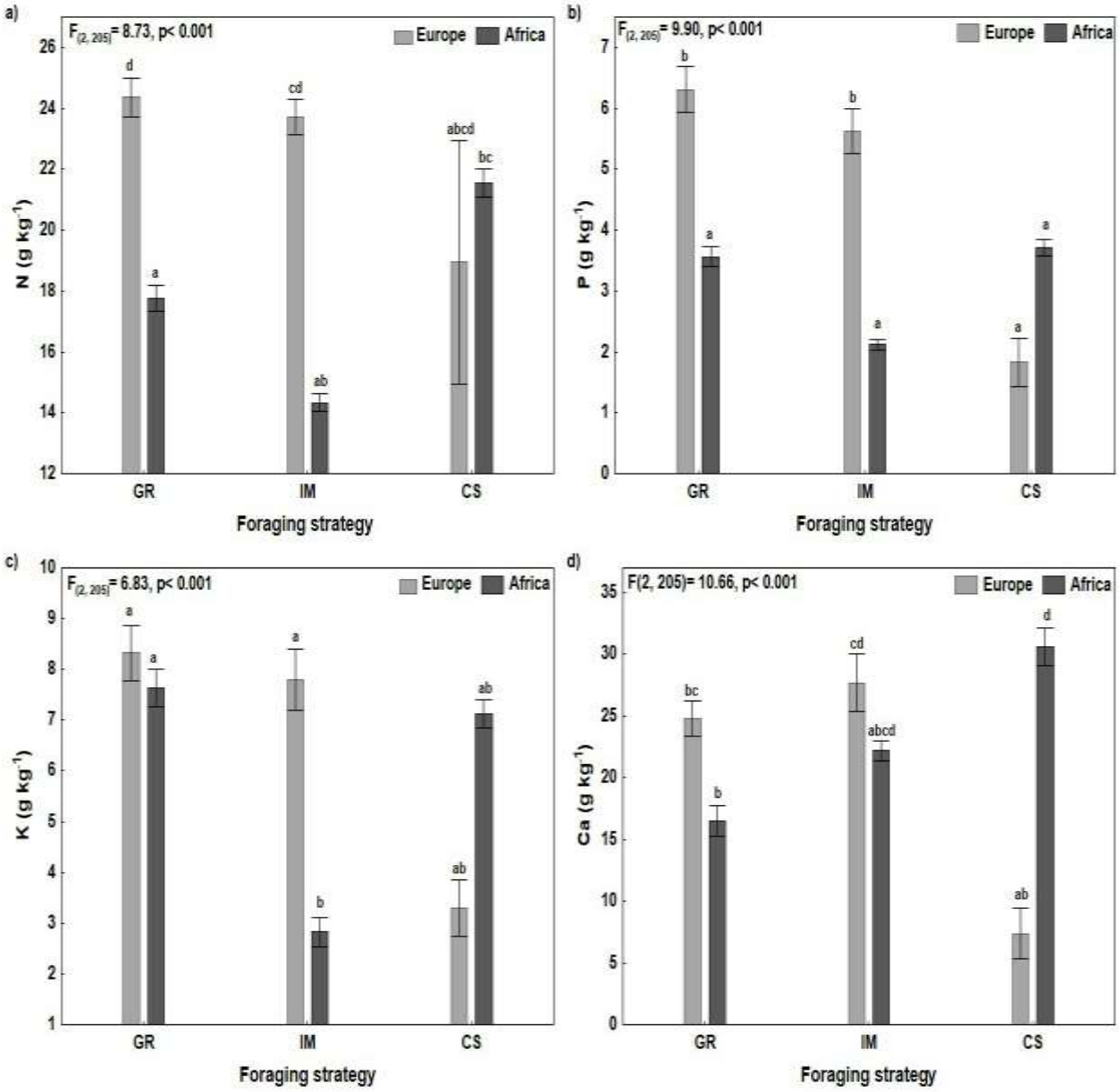

Figure 4. Faecal concentration of N (a), P (b), K (c), Ca (d) macronutrients of selected ruminant animal species, divided according their foraging strategies in Africa and Europe.

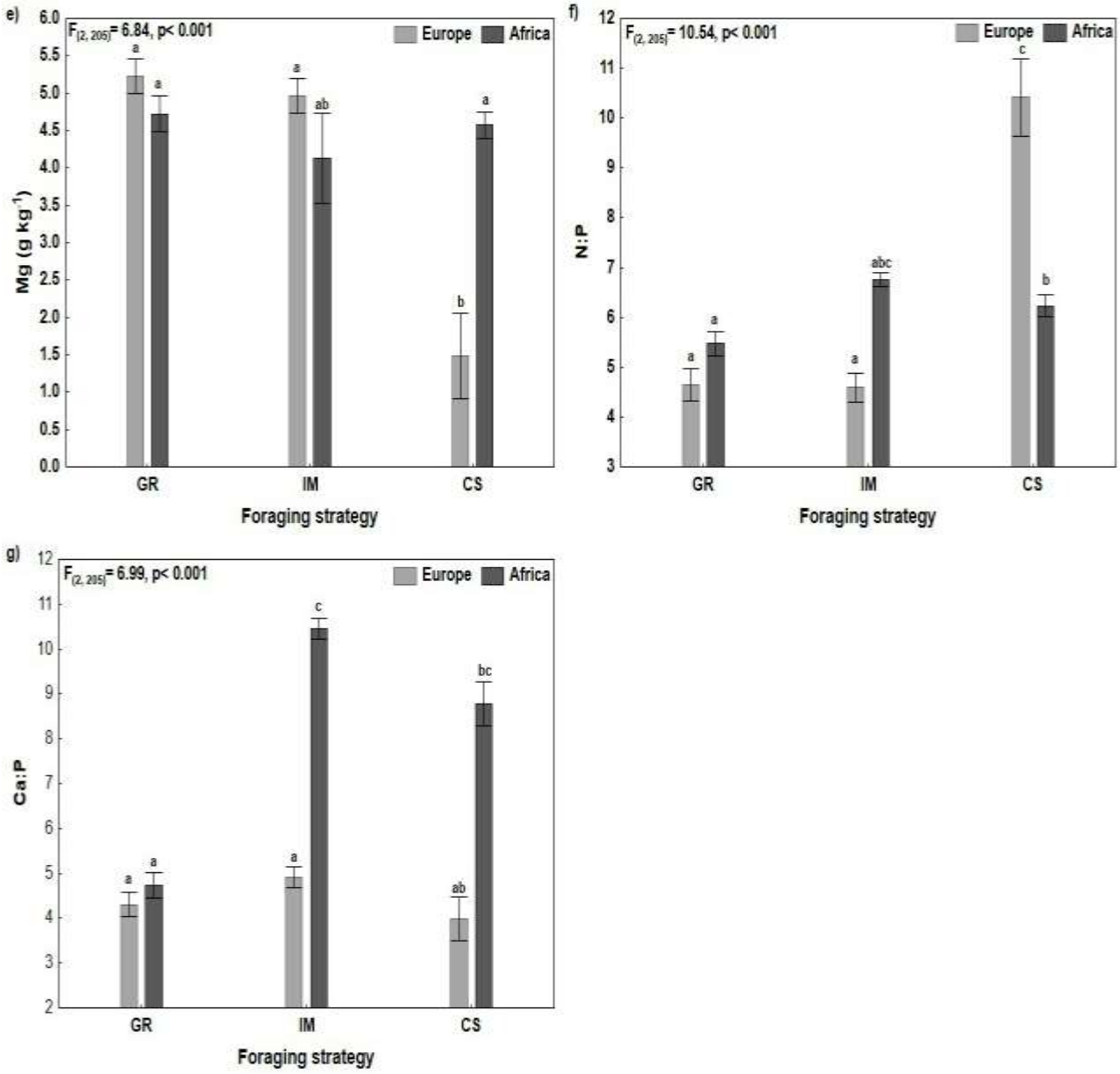

Figure 5. Faecal concentration of Mg (e) in and ratio of NP (f), Ca:P (g) of selected ruminant animal species, divided according their foraging strategies in Africa and Europe.

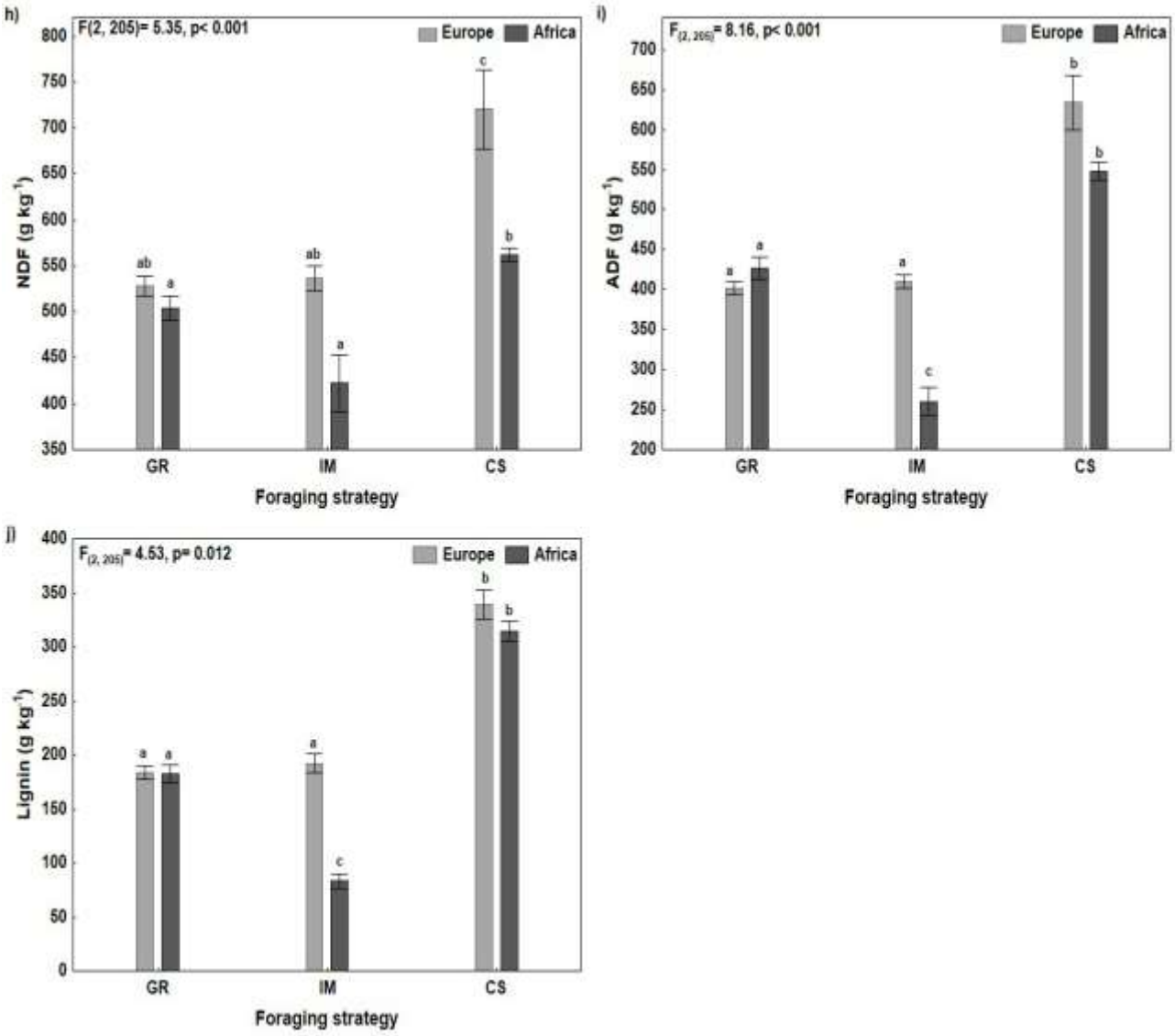

Figure 6. Faecal concentration of NDF (h), ADF (i) and Lignin (j) fibre fractions of selected ruminant animal species, divided according their foraging strategies in Africa and Europe.

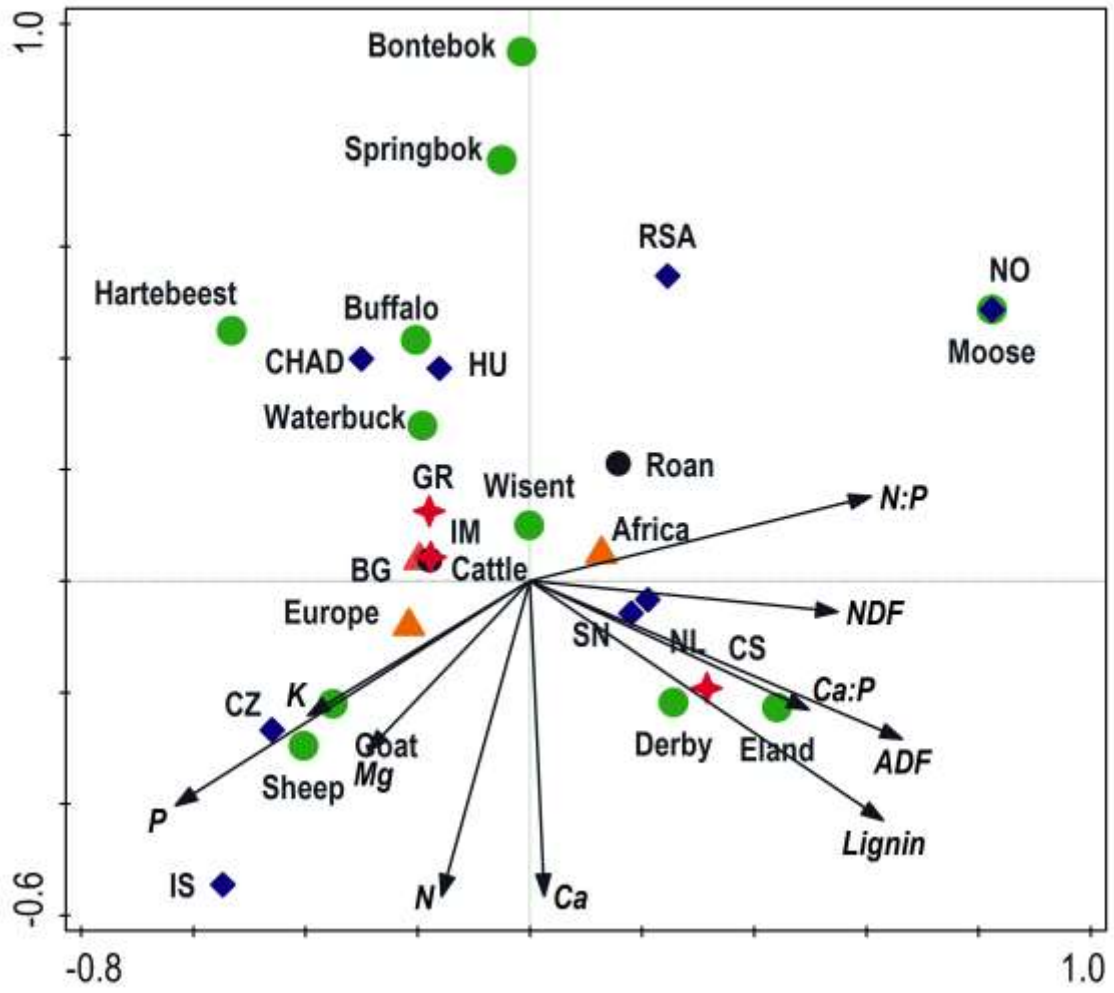

Figure 7. Diagram of RDA results of mutual relationships of concentration of macronutrients (N, P, Ca, Mg, K), NDF, ADF, lignin and N:P, Ca:P ratios in faeces of ruminants in selected localities in Europe and Africa

4.3 Diet quality of non-ruminants in Europe and Africa

Non-ruminants from Europe (i.e. horses and asses) had higher faecal concentrations of N, P and lower concentration of Ca in comparison with elephant and zebra from Africa (Figure 6). Zebras significantly differ in lowest concentrations of K and highest concentration of Mg ($P< 0.001$) see Figures 6 (c), 7 (a). The significantly highest concentrations of lignin and ADF had elephant (Figure 8- i, j).

The relationships of distribution of nutrients in non-ruminant faeces samples are shown in Figure 9. RDA analysis reveal that the first ordination axis explained 20.5%, the first two axis together 33% and four axis together 44% variability of faeces chemical composition.

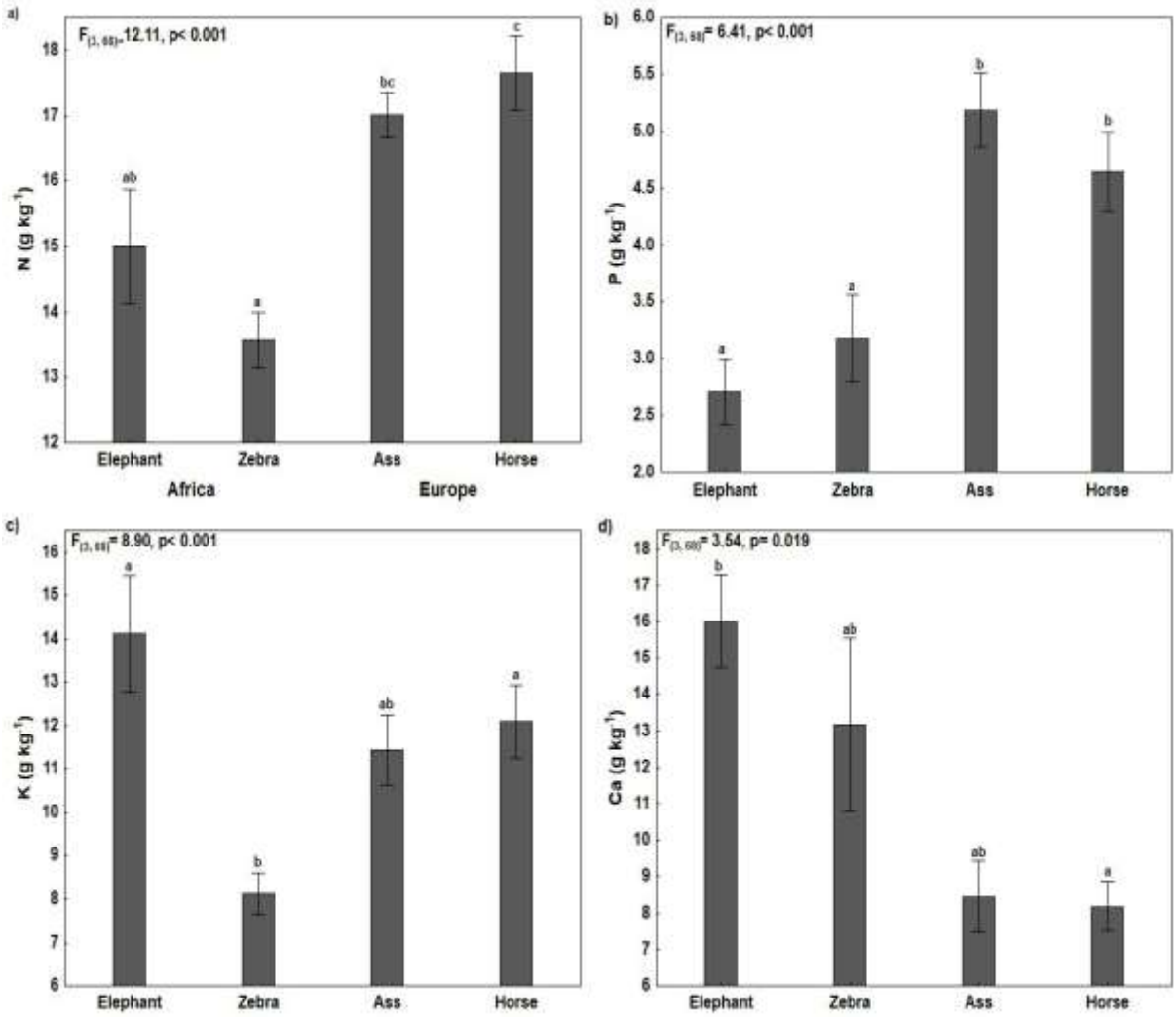

Figure 8. Faecal concentration of N (a), P (b), K (c), Ca (d) macronutrients of selected non-ruminant animal species, divided according their foraging strategies in Africa and Europe.

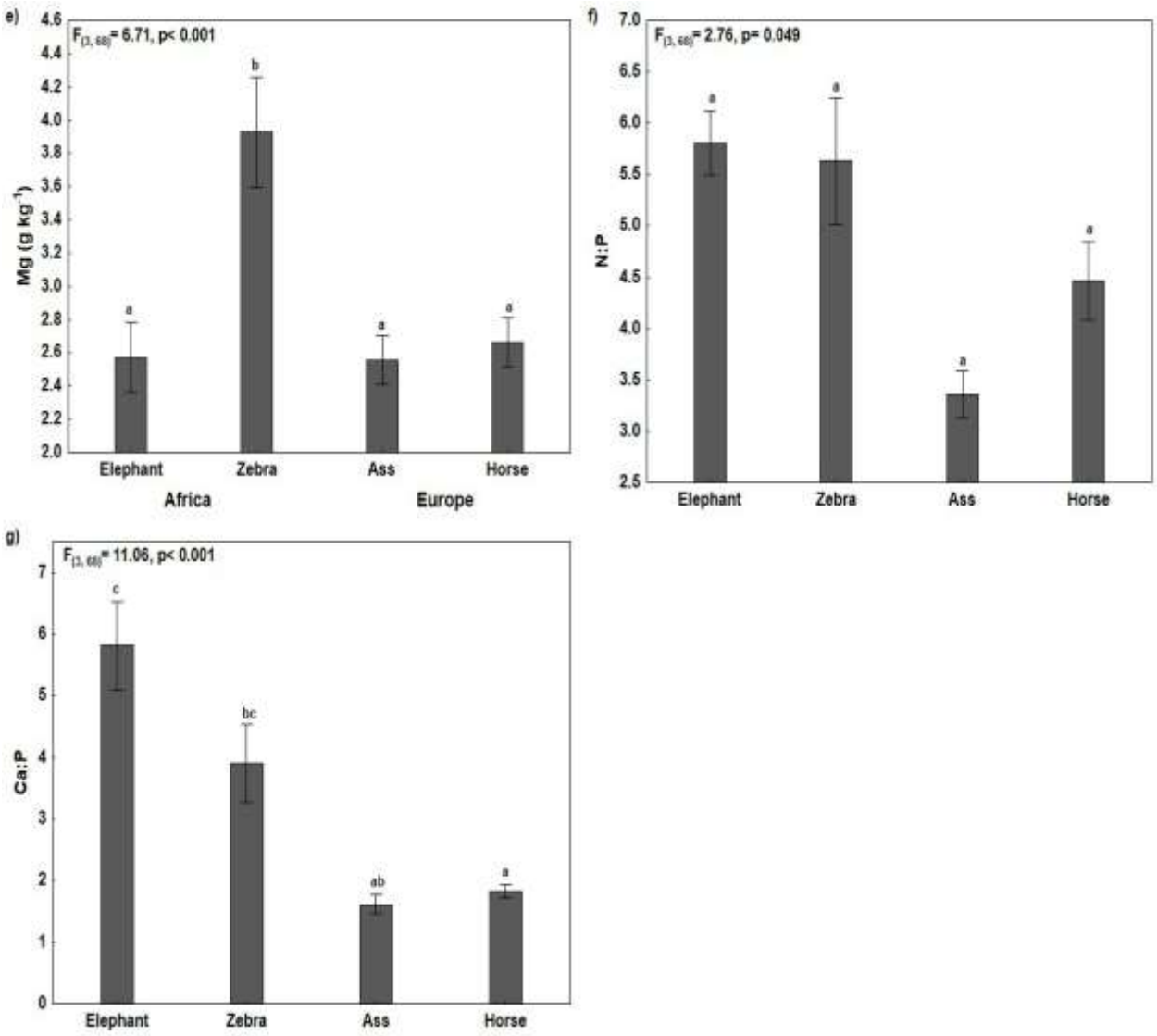

Figure 9. Faecal concentration of Mg (e) and ratio of NP (f), Ca:P (g) of selected non-ruminant animal species, divided according their foraging strategies in Africa and Europe.

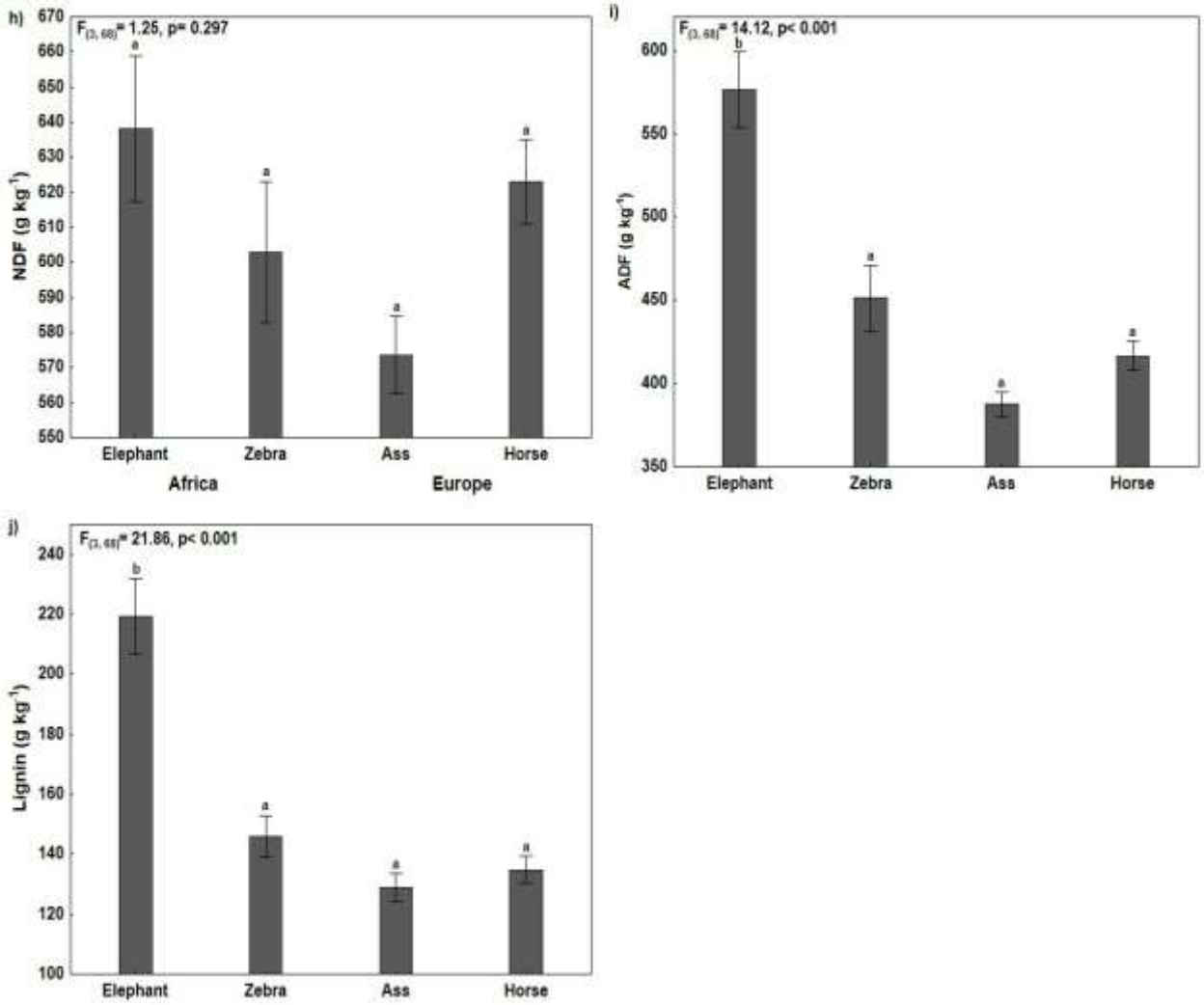

Figure 10. Faecal concentration of NDF (h), ADF (i) and Lignin (j) fibre fractions of selected non-ruminant animal species, divided according their foraging strategies in Africa and Europe.

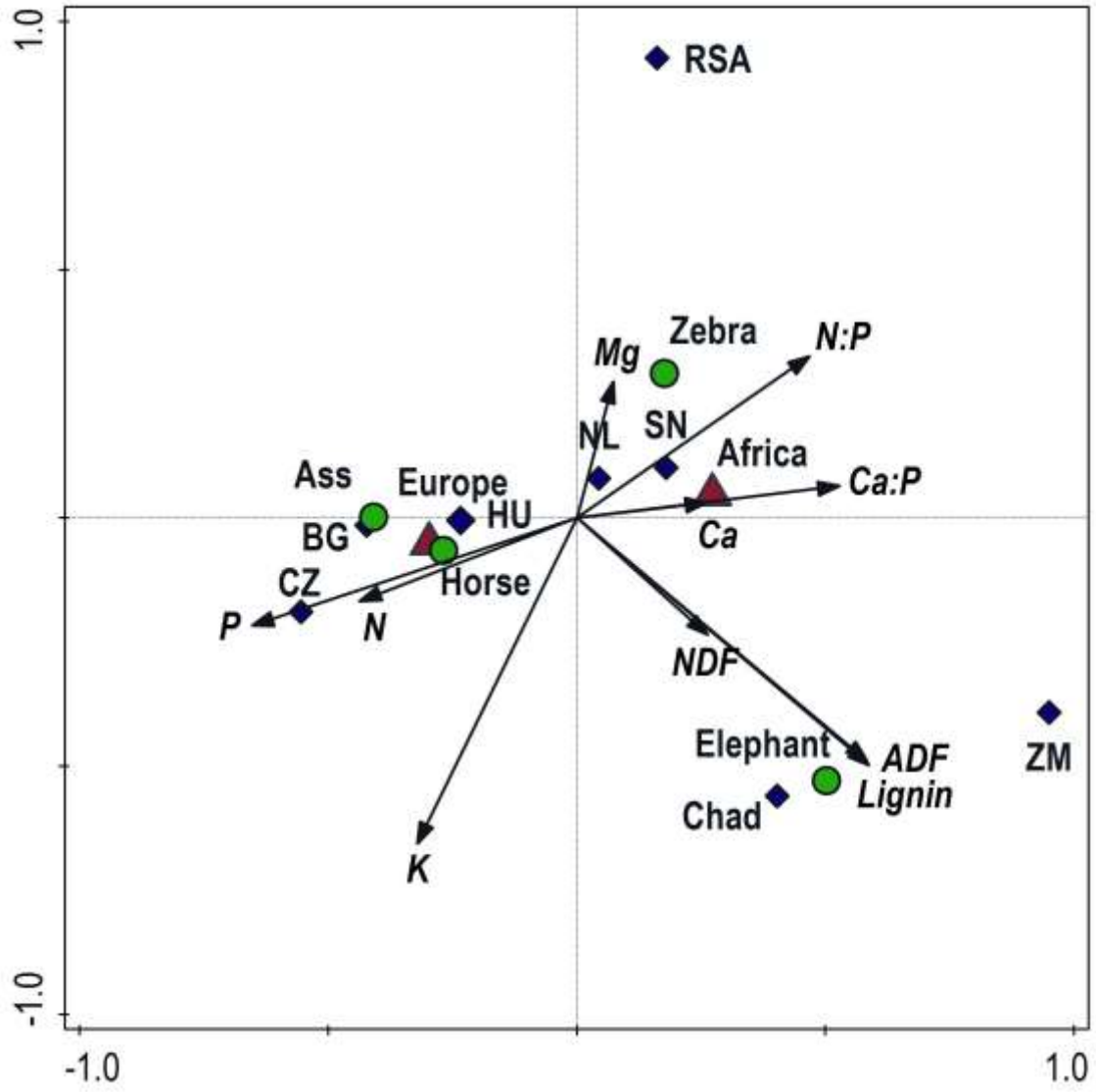

Figure 11. Diagram of RDA analysis results of mutual relationships of concentration of macronutrients (N, P, Ca, Mg, K), NDF, ADF, lignin and N:P, Ca:P ratios in faeces of non-ruminants in selected localities in Europe and Africa.

4.4 Functional relationship between concentration of plant available N, P in soil and in faeces of investigated animals

There was no significant relationship (P> 0.05) between concentrations of N, P in soils and faeces of investigated animal species (Figure 10)

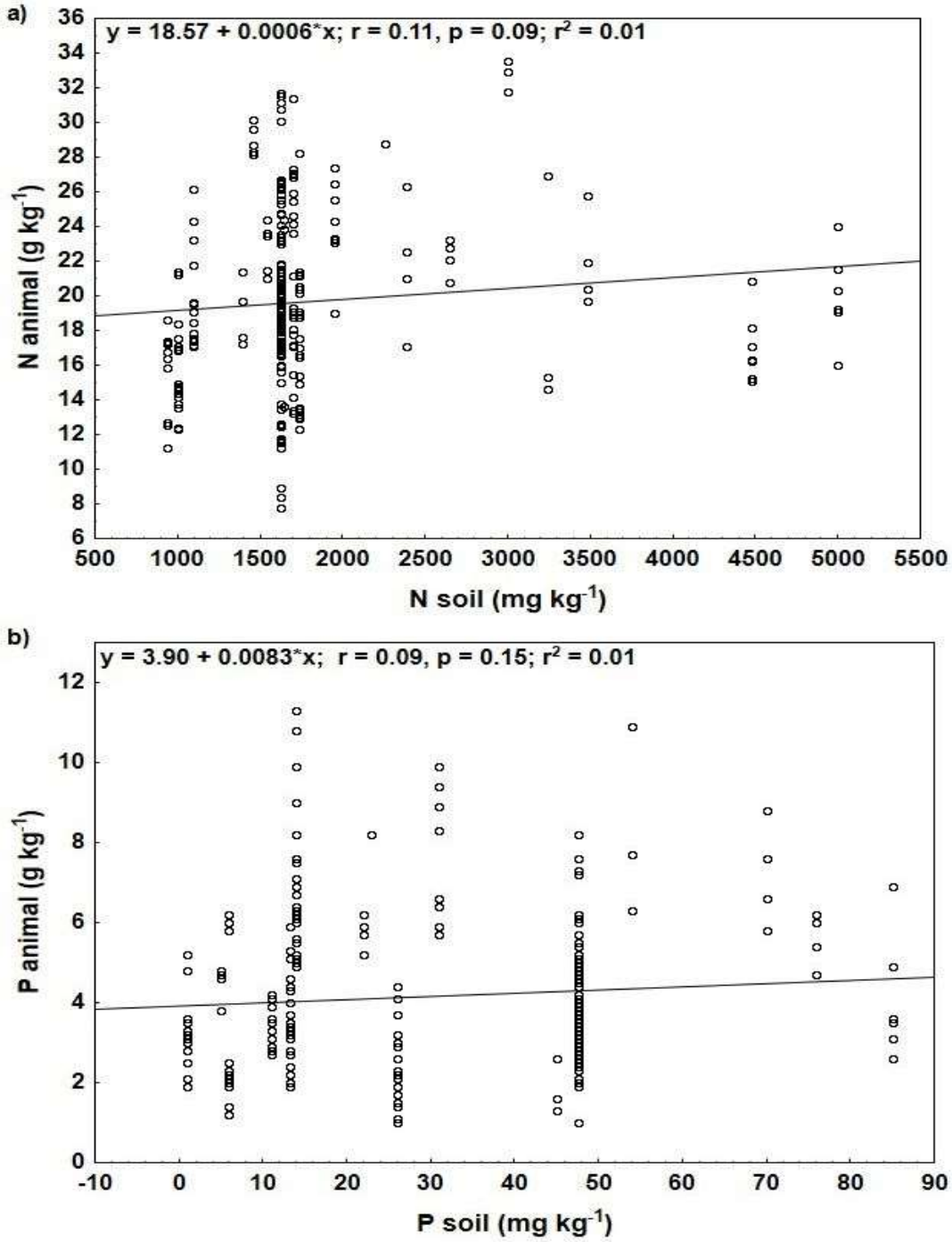

Figure 12. Simple linear regression of soil N, P and faecal N, P of investigated animal species.

5 Discussion

From all investigated animals the highest concentrations of FN and FP have the herbivores from Europe, namely for FN concentrations sheep, goat and wisent with addition of asses and cattle in FP concentrations. Although, he Derby eland had also diet rich on N but it can be due to possible feed supplementation in the Bandia reserve. Therefore, we can affirm that our findings correspond with hypothesis of different soil age and thus different availability of nutrients in old and young ecosystems, i.e. in African and European continents according to Lambers (2010). Thus, the poorer diet quality of African herbivores reflect the nutritional status of soils in Africa, which are characterized by nutrients deficiency, especially for P. These findings are also in agreement with Grant et al. (2000), who stated that P was found as major limiting nutrient in South African pastures and also that FP reflect the P status in soils. Although, the moose did not fit in this scheme, but it can be the result of insufficient amount of data (3), their large variability in N concentrations as well as strict wildlife condition, without additional feeding or mineral supplements, which could occur in rest of investigated animals.

The highest lignin concentration from all investigated species was achieved by CS (moose, Derby and common eland), thus following the foraging strategies of ruminants, from which is known that CS select the diet high on lignin and fibre concentrations.

Within the ruminants we found that GR and IM had the tendency of higher concentration of most macronutrients (except of Ca) in compare to CS, which is in agreement with many other studies, reviewed by Codron et al. (2006) that despite of higher protein/ fibre ratios of browse compare to grass, the CS have poorer quality of diet than GR, because DM digestibility of browse is lower due to high concentration of lignin and its indigestibility. Furthermore, from review by Steuer et al., 2012 there is an evidence that ruminants are superior in fibre digestion compared to non-ruminants in general, indicating that NDF digestibility in hindgut fermenters is 44% vs. 59% in ruminant foregut fermenters. Subsequently, it is known that within the non-ruminants the rhinoceroses (*Ceratotherium simum* and *Rhinoceros unicornis*) and equids are superior to tapirs and elephants in fibre digestion and within the ruminants, in respect to feeding types, the GR are superior to CS. From our results, we can confirm that CS had higher NDF concentrations than GR and IM and as well as elephants had higher

concentration of NDF (however not statistically significant), ADF and lignin (for both $P< 0.001$) to equids. However, for higher concentration of fibre fraction in elephants we should take into account that elephants are intermediate feeders, in contrary to equids, and according to Cerling et al. (1999) which conducted the stable isotope analysis from extant African elephants from majority of African localities (also from Zambia), the browse species strongly dominated in elephant diet. Thus, African elephants had diet higher on lignin and plant secondary compounds concentrations than equids, which can be reflected in our results.

However, from both African ruminants and non-ruminants elevated concentration of Ca was determined and zebra species had particularly low concentration of K and high concentration of Mg in faeces. We supposed that it should be in line with the poorer quality diet and particularly, the elevated concentration of Ca in CS can reflect the effect of presence of antinutrive compounds, i.e. plants' secondary metabolites in browse (e.g., tannins), which are known to be responsible of reduced utilization of nutrients, especially Ca and Fe, in animals' metabolism (Lavin, 2012).

According to Wrench et al. (1967) confirmed by Grant et al. (2000), for most herbivores the threshold concentrations for FN and FP are 14 g kg^{-1} and 2 g kg^{-1} respectively, meaning that values below the threshold indicate diet deficiency and poor nutritional status. Applying this on our data, several animal species, i.e. zebra, moose and springbok were below of this threshold concentration. Zebra species were under the threshold of FN concentrations, with achieved concentrations around 13 g kg^{-1}, and slightly above threshold concentration of FP (2-3 g kg^{-1}), indicated poor condition of those animals, which also corresponds with determined imbalances in other faecal nutrients, such was low concentration of K and high concentration of Mg compared to other animals. Particularly low concentration of FP had moose (1.83± 0.39 g kg^{-1}) from Norway. Despite of there is no other available research for moose faecal data comparison and moreover there are some indication that tannin in browse can cause the decrease of faecal nutrients (e.g. Stapelberg et al., 2008), Ohlson and Staaland (2001), suggested in their study of the mineral ecology of moose in Norway, that moose experienced nutritional stress because the browse plant were generally characterized by low macronutrients concentrations. Springbok antelopes have also low concentrations of FN (14.33± 0.29 g kg^{-1}) and FP (2.13± 0.19 g kg^{-1}), compared to FN (16±0.03 g kg^{-1}) and FP (3.5± 0.16 g kg^{-1}) concentrations determined for springbok from Kalahari desert

by Stapelberg et al. (2008). However, according to Grant et al. (1995) the critical FN concentration of 11-12 g kg^{-1} is essential to maintain rumen fermentation, thus according to that, all investigated ruminants were above the threshold of nutritional deficiency, indicating that they did not undergo of any metabolic stress during the sampling period.

Finally, the functional link between plants available N, P in soils and FN, FP from investigated large herbivores was not proved, however this functional link between soil N and FN of wisents (*Bison bonasus*) in Cherga breeding station in Altai was proved in bachelor thesis (Karafiátová, 2014). We supposed that major role of this difference between these two results is due to enlarged sampling of this study, where investigated animals inhabited larger area and because the soil sampling in such a large area is highly demanding, insufficient soil samples were taken. Therefore, deeper investigation aimed on available nutrients in soil in connection of diet quality of free ranging herbivores would be applicable (appropriate) in order to better comprehension of soil-plant- herbivores interactions.

6 Conclusion

In conclusion, large herbivores from Africa had poorer diet quality in comparison with herbivores from Europe, resulting in lower concentrations of faecal macronutrients (except for Ca) and higher concentrations of ratios of N:P and Ca:P in African herbivores. From all investigated animals, the highest concentrations of lignin had herbivores with CS foraging strategy. Subsequently, within the ruminants higher concentration of majority of macronutrients had GR and IM from Europe compared to CS from Africa. Non-ruminants from Europe (i.e. horses and asses) had higher concentrations of FN, FP and lower concentration of faecal Ca in comparison with elephant and zebra from Africa, with the more balanced fibre concentrations than in ruminants, with significantly highest concentrations of lignin and ADF in elephants (Figure 10). Within the non-ruminants zebras significantly differ in lowest concentration of K and highest concentration of Mg (Figure 8-9). Finally, the relationship between plants available N, P in soil and FN, FP in investigated herbivorous animals was not proved, implying that more investigation in this area is needed to be done to verify this results. Furthermore, due to determined differences in diet quality between animals in Africa and Europe, more investigations should be focused in diet requirement of animals inhabiting those areas which are generally poorer on nutrients supply in order to better habitat managements and animals health.

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Appendices

Appendix 1. The landscape of Židlov game park in Czech Republic (Source: www.vls.cz).

Appendix 2. The faeces sampling in Židlov game park in Czech Republic (Source: photo by Pavla Hejcmanová)

Appendix 3. The faeces samples collection of cattle in pasture in the surrounding of Srbeč in Czech Republic (Source: Photo by Pavla Hejcmanová).

Appendix 4. The pasture situated in open landscape in Iceland (Source: Photo by Pavla Hejcmanová).

Appendix 5. The Hungarian Grey cattle grazing in puszta in Hortobágy national park (Hungary) (Source: photo by Pavla Hejcmanová)

Appendix 6. The konik horses in Kraansvalk national park (Netherlands) (Source: photo by Pavla Hejcmanová).

Appendix 7. The landscape of Kraansvalk national park, were samples of European bison and konik horses were collected (Source: photo by Pavla Hejcmanová).

Appendix 8. The semi-natural pastures in Obzor in Bulgaria, where samples from horses, goats and cattle were collected (Source: photo by Michaela Stejskalová)

Appendix 9. Plains zebra in Bandia resereve during the hot dry season (Source: photo by Pavla Hejcmanová)

Appendix 10. The landscape of Bandia reserve in hot dry season (Source: photo by Pavla Hejcmanová)

Appendix 11. The Bandia reserve where faecal samples of Derby elands were collected (Source: Derbianus conservation team)

Appendix 12. The landscape of Fathala reserve with Derby elands foraging (Source: photo by Pavla Hejcmanová)

Appendix 13. The roan antelopes in Fathala reserve (Source: photo by Pavla Hejcmanová).

Appendix 14. The landscape of Mosi-Oa- Tunya national park in Zambia, where faecal samples from elephants were collected (Source: photo by Lucie Stoklasová).