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Determination of Metals, Fungi and Mycotoxins in Cat Meal Samples used in Saudi Arabia

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The cat food samples viz Canned Food, Pouched Food, Raw Food, Lightly Cooked Food, Dry Food were analysed for the presence of essential and non-essential metal ions in addition to fungal infection determination. All the food samples were found to have adequate amount of carbohydrates, proteins, lipids, vitamins and folic acid. The concentration of essential elements in all the analysed samples are within the intake range of cats for their normal growth and development. Although the presence of heavy metals makes some concern about the quality of the food samples, but all these heavy metals are below the toxic level. All the food samples showed the presence of microbial fungi to some extent, but the release of micro-toxin from these fungal species are below the level of their profound health related issues among the cats. Among all the samples analysed the best food samples with very good health quality seems the dry food, followed by pouched food.

Keywords: Essential Elements; Heavy Metals; Ochratoxin; Total aflatoxin; Zearalenone.

Domestic cats (*Felis silvestris catus*) are very curious about the feeding behavior and have particular nutritional demand [1–3] which is related to their carnivore metabolism, like, needs high crude protein content which are enriched with particular type of essential amino acids (like, arginine and taurine), and vitamins particularly Vitamin A, D, and B complex as well as arachidonic acids[4–6]. As seen cats mostly eat in little quantity, throughout the day showing their feeding habits as same as their ancestor cats (*Felis silvestris lybica*), which are dependent upon small-sized preys[5, 7]. Cats bear a capacity to select their meal on the basis of taste, smell, temperature, and texture [6, 8, 9] related to self-regulation consumption of particular type of food to make it sure that accurate and an adequate consumption of good amount of nutrients, thus balancing their diet[10,11]. In particular cats usually show less interest for food items which are sugar rich and even in some studies mentioned that that cats did not recognize the taste which is sweet[5], so cats did not go for sweet foods and excess their

energy limit intake not more than 250 kJ/day, and in case of the consumption goes higher than this amount, they vomit typically and thus display diarrhea their after[12]. Cats bear the capacity to show different behaviour for new ingredient presence in the diet related to the origin, experience, and metabolic status towards food, in order to avoid intoxication or any sort of harm[13], to neophilia which may result towards more consumption of new and little readily available diets [14,15]. The food preparing industry carry out many tests for food preparations in cats with the intuition to understand the mutability of their choices, which is ordered in big range of odors, constituents and the presentation making. In addition, the industries try to understand the food product ranges, from catering to nutritional values required for the cats as per their intrinsic variations, like, age, breed, reproductive status, weight, also as per the specific health conditions[5]. Furthermore, many scientific studies showed that intrinsic variables could change the amount of preference for variable commercial cat diets[5].

The development of feline obesity is not made more susceptible by high carbohydrate intake when dry food is consumed, although obesity is a significant risk factor for type II diabetes in cats[16, 17].

Cats have the tendency to check and maintain their everyday intake of calories, even if given the commercial diets, they neglect to consume[18, 19].

In Saudi Arabia, cats are largely being cultivated in large scale. Almost every house does have at least one cat, so the food analysis related to cats becomes the need of an hour. In that context the large used food being given to cats are Canned Food, Pouched Food, Raw Food, Lightly Cooked Food, Dry Food. So accordingly, the study in reference was conducted to measure the presence of metals, microorganisms and the toxins in cat food samples.

MATERIALS AND METHODS

Sample collection

Some total of 05 samples of cat meal, viz, Canned Food, Pouched Food, Raw Food, Lightly Cooked Food Dry Food have been collected from fodder market of Dammam, Saudi Arabia. Fifty gram of each sample was collected for the analysis. The samples were collected in airtight bottles and were kept at 4^0C for further analysis. The samples were kept in airtight bottles and were stored in research laboratory at 4°C until analyzed. As per the analysis, the samples are made, like for metal analysis the samples were taken directly in solid form, and for fungal analysis the samples were dissolved in distilled water in the ratio 1:2 (w/v). The dilution is done as per the analysis. The recommended value of the energy protein and other dietary contents are shown in Table 1.

Heavy metal analysis

The metal analysis (essential and heavy) has been carried by acid digestion and was detected using atomic absorption spectroscopy (PERKIN ELMER), as per AOAC (1995) guidelines. 1 g or 1ml of each sample was digested by 3 ml mixture of nitric acid and perchloric acid (1:1, v/v) using hot plate till a clear solution formation. The experimentation was carried out in triplicates for every sample and mean value was determined.

Identification and isolation of Fungi

20 ml of the each sample was added to 0.18L sterile deionized water having 0.02% Tween 80, shacked on rotary shaker with a speed of 0.85 RCP for half of an hour for the determination of colony-forming units (CFU/g). The serial dilutions (10^{-1} to 10^{-5}) with de-ionized water using 0.02% Tween 80 were made and 1 ml aliquot of each sample inoculation was done for each of three plates of Czapek-Dox agar having 1% streptomycin as standard, was added for the inhibition of microbial growth. The Petri plates were inoculated with a spread-plate technique, and incubation was done at 25°C. The Total fungal counts (CFU/g) of every sample was determined after incubation of 10 days as per Pitt and Hocking[21] and Samson et.al.[22]. The fungi after isolation was identified as per their characteristics both macro- and microscopic as per Raper and Fennell[23], Moubasher[24] and Samson et.al.[25]. The total counts (TC) of each fungi was calculated in relation to the gram of sample used (TC/g).

Mycotoxins Quantification by Fluorometry Method

The meal samples have been analyzed for the determination of total aflatoxins; ochratoxin and zearalenone with the help of immune affinity method as per the (AOAC), Association of Official

Analytic Chemists methodology [26,27]. Nearly 100 ml of every sample was extracted and mixed with 100 ml of MeOH/H_2O mixture (80:20 v/v) solution. The whole mixture was filtered after shaking, using Whatman no. 1filter paper, and the obtained filtrate was diluted (1:4) by water, from which 2 ml of the sample mixture was analysed for total aflatoxins determination by Aflatest P immuno affinity column (VICAM, Watertown, MA, USA). Ochratoxin A and zearalenone have been analysed with the help of Ochraprep and Zearala Test Immuno affinity Column Procedures (VICAM, Water- town, MA, USA). Total aflatoxins, ochratoxin A and zearalenone quantities were calculated with the help of recalibrated VICAM Series 4 fluorometer set at 360 nm (excitation) and 450 nm (emission) [28].

RESULTS AND DISCUSSION

The cats feed mostly on the food items which are enriched with adequate amount of nutrients and the nutrients with mixed composition including vitamins, essential elements etc. the samples analysed also show the good concentration of nutrients. The meal for cats must sustain long life and should be healthy one as per the requirements of cats. Cats can digest starch which is cooked in an efficient way and can consume and release glucose at molecular level. The regular dry food, is effective in terms of calories, as is full of proteins, fats and carbohydrates in 30:40:30 ratio approximately. The canned foods contain 80% water and possess an energy ratio of 40:50:10. However the wet food of cats is highly enriched with animal proteins and less carbohydrate content. Also some study showed that dry foods, do not make obesity and diabetes in cates in comparison to moist foods. The obtained results related to the study of the available cat meal have been analysed for the metal ions and other nutrient ions.

Metal ions in Cat Meals

The cat meal samples (05), used mainly as feeding materials, in Saudi Arabia were analysed

Table 1. [20]Daily Recommended Allowance of Energy/Proteins/Lipids/Minerals for Cats (a)

Nutrients	Kittens	Adult Cat	Nursing Cats
Calories/ Day (Kcals per day*)	200	310	440
Crude Protein	10 g	12.5 g	41 g
Total Fat	4 g	5.5 g	12 g
Vitamin A	30 µg	50 µg	63 µg
Vitamin D	0.4 µg	0.5 µg	0.7 µg
Vitamin E	2.5mg	3mg	5mg
Vitamin K	50 µg	70 µg	80 µg
Vitamin B1	0.21mg	0.33mg	0.40mg
Riboflavin	0.17mg	0.20mg	0.30mg
Vitamin B6	0.07mg	0.16mg	0.20mg
Niacin	1.8 mg	2.5 mg	3mg
Pantothenic acid	0.2mg	0.4mg	0.5mg
Vitamin B12	1 µg	1.4 µg	1.7 µg
Folic acid	30 µg	45 µg	50ìg
Calcium	0.15g	0.18g	22g
Phosphorus	0.12g	0.17g	0.21g
Magnesium	21mg	25mg	30mg
Sodium	35mg	42mg	45mg
Potassium	0.25g	0.33g	0.35g
Chlorine	50mg	60mg	65mg
Iron	3mg	5mg	5mg
Copper	0.2mg	0.4mg	0.5mg
Zinc	3mg	4.6mg	5mg
Manganese	0.2mg	0.3mg	0.5mg
Selenium	15 µg	20 µg	22 µg
Iodine	70 µg	90 µg	95 µg

for the nutrient constituents and for the essential metal ions by various chemical and spectroscopic methods and the obtained results are shown in tables. The mean values of essential nutrients found in the samples analysed are shown in Table 2.

The amount of essential mineral elements (Calcium, Phosphorus, Magnesium, Sodium, Potassium, Chlorine, Iron, Copper, Zinc, Manganese, Selenium, Iodine) were analysed by Atomic Absorption Spectroscopy and the obtained values are shown in Table 3, and the comparison was made with Current European Official Regulations (1mg/kg). All the values obtained have been found within the range that is necessary for the daily metabolic functions of the cats.

The meal samples were also analysed for the estimation of heavy metals by AAS and

Table 2. The Chemical Composition of Various Cat food Meal Samples

Tested material	Canned Food	Pouched Food	Raw Food	Lightly Cooked Food	Dry Food
Carbohydrates	210 g	209 g	205 g	202 g	230 g
Soluble sugars	65.0g	64.0g	61.0g	63.5g	75.0g
Total fat	11.0 mg	13.0 mg	11.4 mg	12.0 mg	15.0 mg
Saturated fat	4.00mg	4.5 mg	3.5 mg	4.2mg	6.00mg
Unsaturated fat	9.0mg	9.5mg	9.1mg	8.5 mg	12.0mg
Crude Protein	34 g	35.5 g	34.9 g	33.7 g	37 g
Total Fat	9 g	9.4 g	8.9 g	10 g	11 g
Vitamin A	55 µg	59 µg	55.9 µg	56 µg	58 µg
Vitamin D	0.1 µg	0.07 µg	0.09 µg	0.11 µg	0.3 µg
Vitamin E	3 mg	3.7 mg	4.1 mg	4.2 mg	5 mg
Vitamin K	65 µg	67 µg	63.5 µg	65.7 µg	75.5 µg
Vitamin B1	0.20 mg	0.21 mg	0.23 mg	0.25 mg	0.29 mg
Riboflavin	0.15 mg	0.15 mg	0.15 mg	0.15 mg	0.15 mg
Vitamin B6	0.10 mg	0.12 mg	0.11 mg	0.13 mg	0.19 mg
Niacin	2 mg	3 mg	2.5 mg	3 mg	5 mg
Pantothenic acid	0.3 mg	0.4 mg	0.5 mg	0.4 mg	0.7 mg
Vitamin B12	0.7 µg	0.8 µg	0.9 µg	0.8 µg	1.5 µg
Folic acid	30 µg	29.7 µg	30.1 µg	30.7 µg	45 µg

*Mean of 20 samples

Table 3. Concentration of Essential Elements in Various Cat food Meal Samples

Tested material	Canned Food	Pouched Food	Raw Food	Lightly Cooked Food	Dry Food
Calcium	12 g	13 g	14 g	17 g	19 g
Phosphorus	0.11g	0.12g	0.13g	0.15g	0.17g
Magnesium	20 mg	23 mg	24 mg	25 mg	29 mg
Sodium	35 mg	35.9 mg	36.4 mg	37.5 mg	40 mg
Potassium	0.25 g	0.27 g	0.29 g	0.31 g	0.37 g
Chlorine	55 mg	57 mg	56 mg	55.5 mg	65 mg
Iron	3 mg	5 mg	4.5 mg	5.6 mg	7.5 mg
Copper	0.3 mg	0.32 mg	0.4 mg	0.5 mg	0.7 mg
Zinc	3 mg	5 mg	4.5 mg	5.5 mg	6.7 mg
Manganese	0.3 mg	0.4 mg	0.5 mg	0.4 mg	0.7 mg
Selenium	15 µg	19 µg	17 µg	16 µg	20 µg
Iodine	78 µg	79.5 µg	80 µg	79 µg	85 µg

the comparison was done with Current European Official Regulations (1mg/kg), and the obtained values are shown in Table 4. As per the obtained results, the amount of lead (Pb^{2+}) varies from 0.1 to 0.31 mg/kg with a mean value of 0.11 mg/kg. The data analysis of showed that the meal samples have the concentration for below than the dangerous level 1 mg/kg, because as per the Current European Official Regulations the amount of lead should be below is 1 mg/kg. The obtained data for Cd^{2+} showed the range in between 0.15 to 0.33 mg/kg and the levels of cadmium is within the range as set by Current European Official Regulations. The nickel concentration was found in between 0.0 to 0.14 mg/kg. However the amount of Cr^{2+}, Ar^{2+} and Hg^{2+} have been found in the range 0.0 to 0.02, 0.0 to 0.04 and 0.0 to 0.04 mg/kg respectively and the values are shown in Table 4. All the obtained values for Chromium, Arsenic and Mercury are within the range set by Current European Official Regulations (1 mg/kg). The detected values for Chromium, Arsenic and mercury have been found[29]. Although

Table 4. Concentration of heavy metals (µg/g) in in Various Cat food Meal Samples

Sample Number	Pb^{2+}	Cd^{2+}	Ni^{2+}	Cr^{2+}	Ar^{2+}	Hg^{2+}
Canned Food	0.20	0.33	0.14	0.01	< 0.01	0.03
Pouched Food	0.30	0.23	0.21	< 0.01	0.02	0.02
Raw Food	0.10	0.31	< 0.1	0.002	0.04	0.04
Lightly Cooked Food	0.31	0.15	0.1	0.02	0.03	0.03
Dry Food	0.11	0.30	< 0.1	< 0.01	0.02	0.02

Table 5. The Detected Fungal Species in the Collected Cat Meal Samples

Isolated Species of Fungi	Canned Food	Pouched Food	Raw Food	Lightly Cooked Food	Dry Food
Eurotium amstelodami	√	x	√	x	√
Aspergillus candidus	√	√	x	x	√
Scopulariopsis	x	√	√	√	X
Alterneria tentuis	x	x	x	√	X
Mycelia sterilia	√	√	x	x	X
Aspergillus candidus	x	x	√	x	X
Penicillium sp	√	√	√	√	√
Aspergillus ochraceus	x	x	√	x	X
Geotrichum candidum	√	√	x	√	√
Cladosporium sp	√	√	x	√	X
Rhizopus oryzae	x	x	x	x	√
Eurotium chevalieri	√	√	x	x	√
Fusarium solani	x	√	x	x	√
Penicillium rubrum	√	x	x	x	X
Mucor circinelloides	x	x	x	√	√
Aspergillus versicolor	x	x	√	x	√
Fusarium graminearum	√	√	√	x	X
Aspergillus flavus	x	x	x	√	√
Penicillium citrinum	x	x	x	x	X
Absidia corymbifera	x	x	√	√	X
Botrytis cinerea	√	x	√	x	X
Aspergillus flavus	√	√	x	√	X
Humicola brevis	x	x	√	√	X
Mucor racemosus	√	x	√	x	X

Table 6. The Fungal isolate number, percentage toxigenic isolates and amount of aflatoxins, ochratoxin A and zearalenone (ppb) of Cat meal samples

Sample number	Total count of fungi x10^4	No. of fungal isolate	No. of toxigenic isolate	Quantity of the toxin detected (ppb)		
				AFT	OTA	ZON
Canned Food	2.5	09	1	1.3	2.2	1.0
Pouched Food	4.5	13	1	1.4	1.9	1.8
Raw Food	3.4	11	1	1.8	0.8	2.0
Lightly Cooked Food	5.1	15	2	2.0	3.1	3.0
Dry Food	1.5	07	1	<1	0.5	2.0

AFT= Total aflatoxin; OTA= ochratoxin A; ZON = zearalenone.

the concentration of heavy metals is so low but the contamination may occur during the process of processing of the grains of cat meal samples.

Mycotoxins and fungal contamination of the collected cat meal samples

The concentration and the composition of microbial fungi in cat meal samples was analysed and calculated. The fungi amount counts in a range of 1.5 to 05 x10^4 CFU/g. The fungal species isolated from each meal sample was counted and then the percentage amount of toxigenic isolates was calculated. As per the results the highly contaminated cat meal sample was found the lightly cooked sample, with 15 different fungi species, followed by pouched sample, with 13 isolates of fungi, and least contaminated was dry food sample.

The samples analysed shows, 24 species that belongs to 11 genera of fungi isolated by Czapek-Dox agar and the identified species are shown in Table 5. The genera isolated are *Eurotium amstelodami, Aspergillus candidus, Scopulariopsis, Alterneria tentuis, Mycelia sterilia, Aspergillus candidus, Penicillium sp, Aspergillus ochraceus, Geotrichum candidum, Cladosporium sp, Rhizopus oryzae, Eurotium chevalieri, Fusarium solani, Penicillium rubrum, Mucor circinelloides, Aspergillus versicolor, Fusarium graminearum, Aspergillus flavus, Penicillium citrinum, Absidia corymbifera, Botrytis cinerea, Aspergillus flavus, Humicola brevis, and Mucor racemosus.* Among these genera, Aspergillus, Penicillium and Fusarium have been known be most dangerous fungi. The study carried out by Milicevic et al.[30] mentioned that *Aspergillus, Alternaria, Claviceps, Fusarium, Penicillium* and *Stachybotrys* genera occurs in food and feeding stuffs, before and after harvesting process of food stuffs and have adverse effect on humans of mycotoxins production. In addition, the wide occurrence of fungi includes *A. flavus, A. niger, A. tamarii* and *Penicillium chrysogenum* and can even exist in broad bean Saber [31], in coffee seeds (Bokhari and Aly [32]. The isolation and the characterization of *Alternaria species* shows that the isolation process was carried out in a very carious and in a scientific manner with highly used and sophisticated tools.

The amount of aflatoxins, ochratoxin A and zearalenone (ppb) in 05 cat meal samples were determined by ammino affinity methods as shown in Table 6. The concentration of total aflatoxins was found in between 0 to 1.8 ppb having a mean value of 1.5 ppb. The amount of ochratoxins A was found in between the range of 0.5 to 3.1 ppb with mean value of 2.1 ppb; and similarly the zearalenone concentration was found in between the range of 1.0 to 3.0 ppb with a mean value 2.0 ppb. The allowed mycotoxin limit in the fodder sample is < 20 ppb, and the amount more than this amount is not permitted for animal feeding meals[33]. From the results, it becomes clear that none of the feeding cat meal sample is contaminated with mycotoxins in a range fetal for cats. The study by Bokhari (2010) showed that feeding samples that are contaminated by higher amount of aflatoxins and ochratoxins than 20 ppb (regulatory limit) is not dangerous. Aflatoxins generally get formed by in food samples by contamination of with *Aspergillus flavus* and *Aspergillus parasiticus* and are carcinogenic and hepatotoxic in nature, but depends on the time and the level of exposure by Bennett and Klich[34, 35]. However, ochratoxins A is nephrotoxic and carcinogenic in nature and the toxin are produced by few strains of *Penicillium* and *Aspergillus* as shown by Fung and Clark[36]. Also the, zearalenone

is formed by many field fungi like, *Fusarium* which is very common in wheat, maize and in their bypro ducts (EFSA[37]).

CONCLUSION

The food and the feeding stuff of animals can be contaminated by various organic and inorganic compounds and by metals with the concentration level more or less than the required level. In addition, the foodstuff of cats may be effected by various microbial fungi species, which may release many mycotoxigenic substances in the form of aflatoxin, ochratoxin, zearalenone. These inorganic heavy metals and the presence of fungi species may cause serious health problems both in humans as well as in other animal species. As per the study of reference, the concentration of essential elements was found in a good quantity making these feeding items good for cats. In addition, the heavy metals were also found in the limits of tolerance. Although some fungi species were detected in the cat meal samples, but the concentration of micro-toxins released by these fungal species were found less than their concentration where they show some adverse effect in cats. Among all the meal samples analysed, dry food was found good for cats. Although the chance of decontamination of cat food seems limited and uneconomic, but prevention could be the most effective strategy for decontamination.

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