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AFLATOXIN VARIATIONS IN MAIZE FLOUR AND GRAINS COLLECTED FROM VARIOUS REGIONS OF KENYA

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ABSTRACT

In Kenya, maize remains an important staple food in every household. Unfortunately, the fungus *Aspergillus flavus* can infect the maize and produce aflatoxins. While government efforts to remove contaminated maize from circulation are well intentioned, there remain concerns that consumers are still being inadvertently exposed to aflatoxin. The aim of this study was to sample maize in different parts of Kenya and determine if consumers were inadvertently being chronically exposed to aflatoxins. Seventy-five maize samples and 27 samples of maize flour from three regions of Kenya (Nairobi, Eastern and Western) were analysed using an ELISA assay followed by microtiter plate reader (Neogen model) where the optical density of each microwell was read using a 450nm filter. There was a significant difference in aflatoxin levels in maize grains between the three regions and five stores ($P<0.05$). Samples from Eastern Kenya had the highest contamination at 22.54±4.94 ppb, while those from Nairobi had the lowest (7.92±1.57 ppb). There was no significant difference in the total aflatoxin in maize flours from Nairobi, Western and Eastern regions ($P>0.05$) at 95% confidence interval. Aflatoxin in maize flours were slightly above international upper limit of 5ppb but all the results were lower than the Kenya standard whose upper limit is 10ppb, indicating good manufacturing practices (GMP) by the millers. Samples of maize flours from Eastern Kenya had the highest aflatoxins concentrations at 6.98± 0.53 ppb. In summary, the study found aflatoxin contamination in maize grains especially in Eastern Kenya. The study concluded that measures put in place by government agencies for millers appear to be working. However, samples of maize grains showed variation among the regions and between stores, perhaps due to storage practices, with some levels far exceeding health limits. Due to higher levels of aflatoxin contamination in maize grains in relation to maize flours, the government and relevant stakeholders need to establish further measures to protect consumers.

Key words: Maize grains, maize meal, aflatoxin, aflatoxicosis, maize consumption, *Aspergillus flavus*

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INTRODUCTION

Maize is an important staple food for more than 1.2 billion people in sub-Saharan Africa (SSA) and Latin America [1]. Maize and its products have long been part of the African culture, and they form part of everyday meal in most homes [2]. Over the past decade, Kenyans have grown more concerned with the threat of aflatoxin poisoning. Aflatoxicosis, mainly from consumption of contaminated maize, has resulted in deaths in the Eastern region of Kenya [3, 4]. Aflatoxins can also be found in groundnuts and other crops [5]. *Aspergillus flavus* (mould) is the primary contaminant and producer of aflatoxin [2] and it is present in soil in many parts of the world, including the southern United States, Eastern Europe, and many developing countries [6, 7]. Agricultural produce is prone to aflatoxin contamination, particularly during harvesting, threshing, and drying. Contamination can also occur when grains are in storage due to pest infestation and the poor conditions that lead to accelerated growth rates of *Aspergillus* [8, 9].

Although often in minute concentrations, aflatoxin potency, prevalence and the ease with which they can permeate farmers' fields and storage areas make these highly carcinogenic metabolites particularly dangerous. These compounds cannot be seen, smelt, felt, or tasted in grains [10]; laboratory testing is required to detect them. These compounds are immunosuppressive, carcinogenic, teratogenic and mutagenic, and acute exposure to high levels of aflatoxin leads to aflatoxicosis, which can result in rapid death from liver failure. Symptoms of acute aflatoxicosis include, but are not limited to, swollen stomach, fatigue, swollen legs, and eyes turning yellowish [11,12]. In 2004, during the worst known outbreak of aflatoxicosis in Kenya, 317 cases were reported of which 125 died [13]. Chronic exposure to aflatoxin increases the incidence and severity of many infectious diseases, including hepatitis B and A [5].

In a disease that mostly affects children [14], the minimum level of aflatoxin exposure required to cause aflatoxicosis is not known. According to the International Food Policy Research Institute, 38% of samples from the Eastern region of Kenya from farmers' stores (post-harvest) were found to have aflatoxin levels greater than 10 ppb (parts per billion) [15]. Another study confirmed that maize in Western Kenya was contaminated with aflatoxin above 10 ppb [16]. Other areas have also been affected but to a lesser extent. Maize milling firms rarely segregate maize based on its source and, therefore, highly contaminated products reach the consumer market. The 10 ppb limit is double the international limit, a concentration that is not realistic to reach as the cut-off in developing countries experiencing food shortage. Products surpassing the cut-off are quarantined and destroyed. If products contaminated with 5ppb were removed from circulation, many people would suffer from malnutrition unless another source of food was found, such as pearl millet. In spite of the research and regulatory work done by the Kenya government and other stakeholders, it is still feared that maize meal in the retail market is contaminated with aflatoxin [17]. If validated, then regulatory, monitoring and evaluation activities in this regard have not been adequate.

The aim of this study was to assess aflatoxin contamination in maize meal products in Nairobi, Eastern and Western parts of Kenya. The intent was to not only reinforce the

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extent of the problem, but to encourage use of fermented foods as alternatives to provide better nutrition while lowering exposure to the toxins [18]. Data from this study would inform future mitigation measures as well be used when checking the extent of previous activities meant to reduce the effect of aflatoxin contamination [18].

MATERIALS AND METHODS

Sampling

Twenty-seven maize flour samples of three different brands corded X, Y, Z, were sampled from leading supermarkets stores within Nairobi, Eastern and Western regions of Kenya. These regions were selected based on the levels of consumption and production of maize and maize products. At the same time, samples of maize grains were collected from the same regions for analysis; 75 samples of maize grains from the three regions where five (5) samples from five (5) stores in leading maize grains outlets were collected (Table 1). The samples were transported immediately in cool boxes to an ISO 17025 accredited laboratory at Unga Limited Company in Nairobi and stored at -20^{o}C upon arrival until analysis.

Sample extraction

Laboratory apparatus included beakers, conical flasks, funnels, filter paper (Whatman No. 1), the aflatoxin kit with its accessories and 70% methanol solution. Twenty grams each per sample were weighed into clean disinfected beakers and labelled. The three samples of maize grains required grinding to the texture of fine coffee for optimum extraction. The 70% methanol solution was prepared by mixing 70 parts of concentrated methanol (Analytical Grade) with 30 parts of distilled water. The samples were prepared by extraction with 100ml of the 70% methanol solution (ratio of sample to extraction solvent was 1:5) (Figure 1). The samples were mixed by stirring and then filtered into clean conical flasks using Whatman filter paper No.1. The residue on the filter paper was discarded and the filtrate preserved in the beaker for analysis.

Figure 1: Extraction of samples for aflatoxin analysis

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Aflatoxin Testing

There was introduction of 100µl of the conjugate to the coloured premixing micro wells using a micropipette, and small aliquots of 100µl from the filtrate added and mixed by priming with the micropipette. A standard sample of 20ppb concentration of aflatoxin was introduced as a control. One hundred micro litres of the sample plus conjugate mixture was then transferred to antibody-coated micro wells and the samples incubated for 15 minutes.

After incubation, the contents of the micro wells were discarded and the micro wells washed at least 5 times with distilled water to remove the non-toxin reactants. The water was drained and 100 µl of the substrate solution put into each of the micro wells and incubated for another five (5) minutes. The reaction in this process resulted in a colour change from clear to blue colouration, whose intensity indicates the aflatoxin content. A deeper colour indicated more reaction with the substrate and less aflatoxin concentration in the sample (Figure 2).

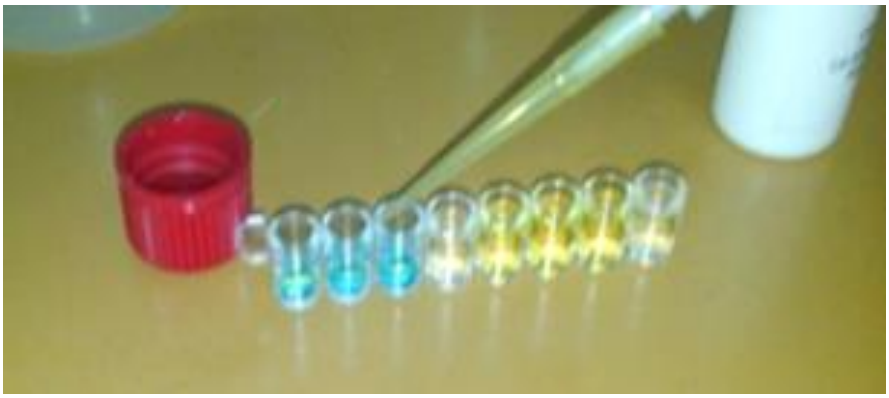

Figure 2: Final products after reaction of samples in aflatoxin kit

To stop the reaction from proceeding, an acidic stop solution was added, which resulted in colour change from blue to yellow, with varying intensities depending on the aflatoxin content. The resultant solutions in the micro wells were fed into a microtiter plate reader (Neogen model) where the optical density of each microwell was read using a 450nm filter, which gave the amount of total aflatoxin present in each sample quantitatively.

Statistical analysis

Frequencies and mode where necessary were generated using the Microsoft excel program while means, standard deviations, plots and significance differences of means were statistically calculated using IBM software package of social sciences (SPSS) version 21.

RESULTS

Aflatoxins were detected in all the samples. There was no significant difference in aflatoxin concentration in ppb between the three different brands of maize flours at 95%

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confidence interval (Table 2), or between the maize flours from different regions of Kenya (Table 3).

The aflatoxin levels in the maize flour samples were slightly higher than 5 ppb the internationally accepted limit of aflatoxin concentration (Figure 3).

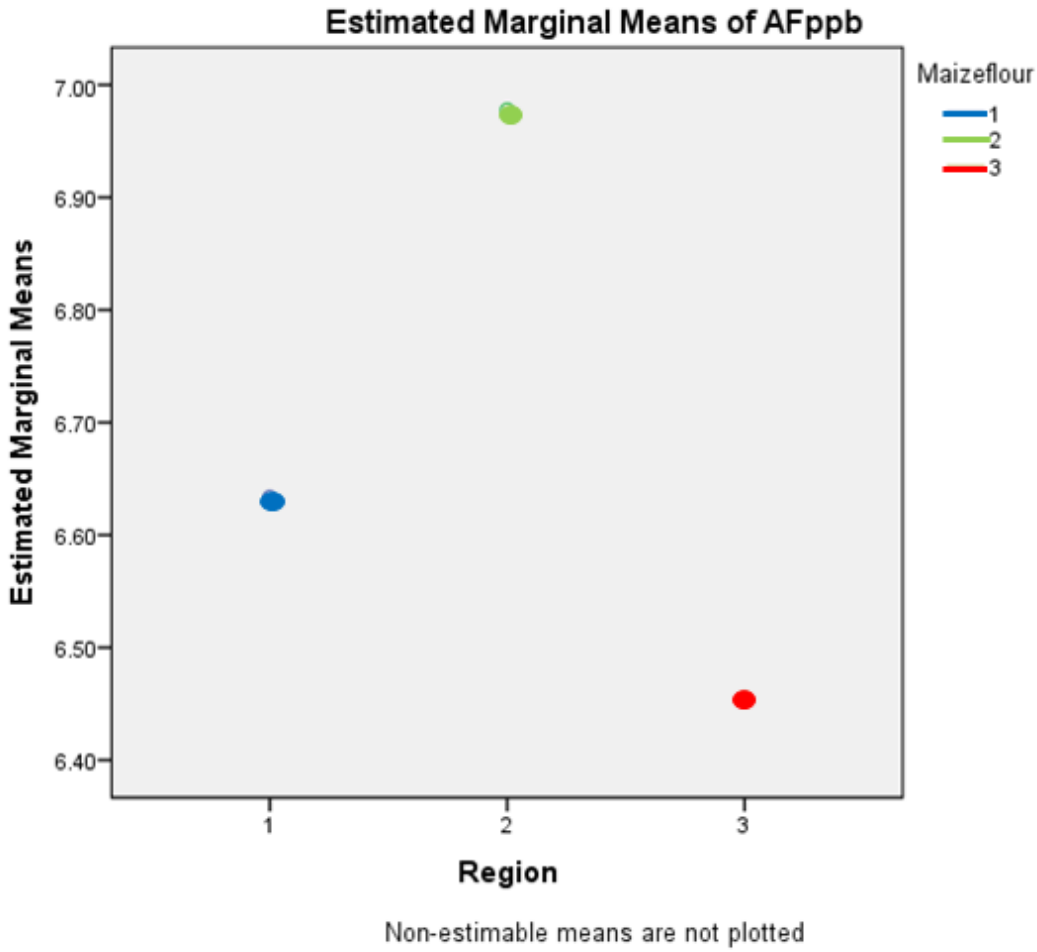

Figure 3: Levels of aflatoxin in ppb in the three selected maize flours*

*x-axis indicates the regions, 1-Nairobi, 2- Eastern, 3-Western; Y-axis indicates concentration in ppb while maize flours 1, 2, 3, represents brand x, y, z, respectively

Analysis of samples of maize grains gave results which were significantly different between Eastern and Nairobi regions ($P<0.05$), but not between Eastern and Western (Table 4; Table 5; Figure 4).

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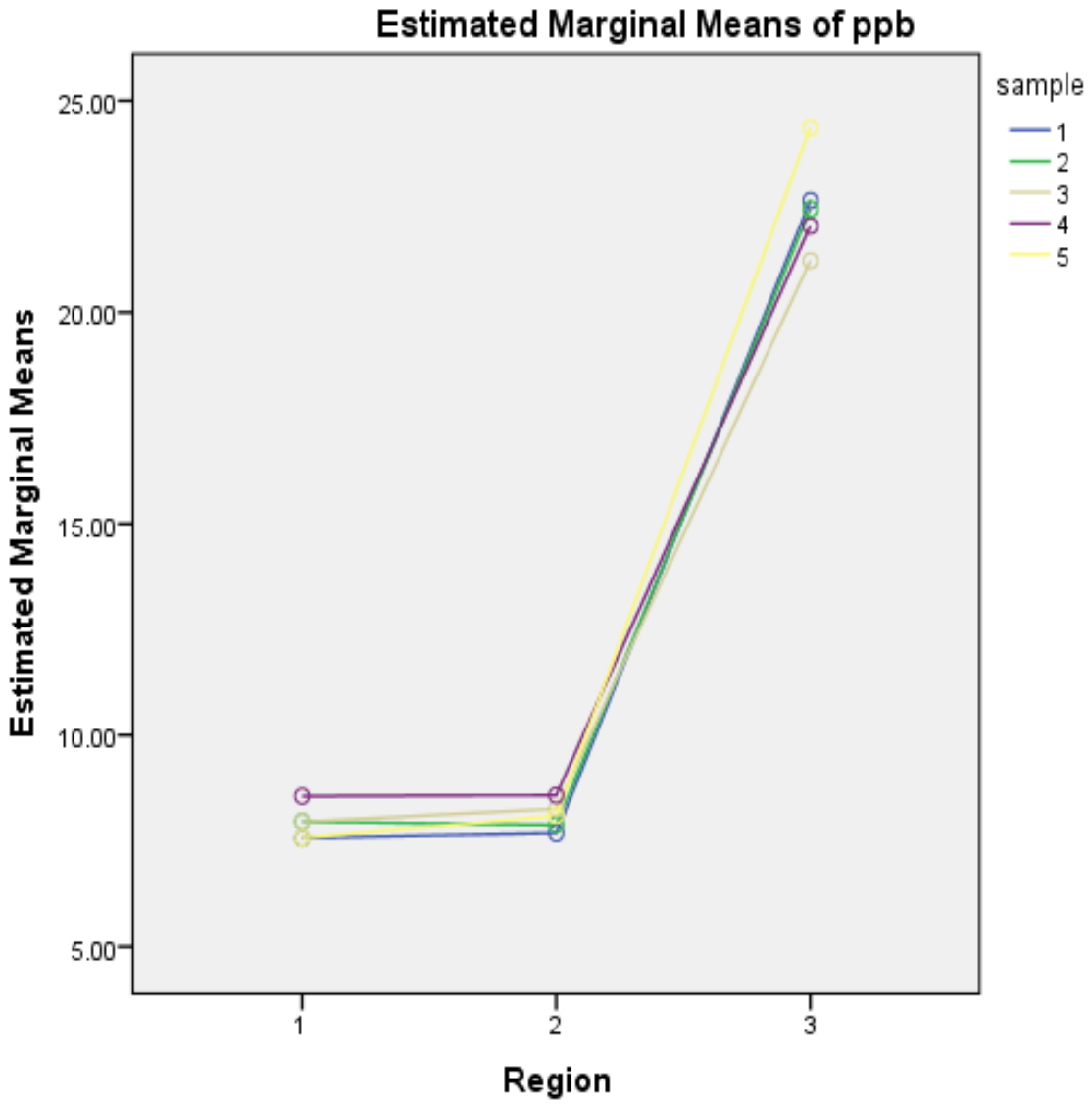

Figure 4: The mean levels of aflatoxin in ppb in maize grains samples from 5 selected leading maize outlets from three regions*

*x-axis indicates the regions, 1-Nairobi, 2-Western 3-Eastern; Y-axis indicates concentration in ppb while maize flours 1,2,3,4,5 represents samples of maize grains from 5 leading maize outlets, respectively

DISCUSSION

Maize remains the most popular staple food in Kenya and largely in East Africa. However, control of aflatoxin contamination remains a big challenge. In this study, aflatoxins were detected in 27 maize flour samples from three regions of Kenya, but these were within the statutory limit of 10ppb. Much higher aflatoxin contamination was detected in maize grains than flour from Eastern Kenya (22.54 v 6.98ppb). While it is not surprising to detect aflatoxin, it is disconcerting that despite governmental measures to reduce the problem, excessive levels are clearly still being ingested, more so in Eastern

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Kenya than Nairobi and Western Kenya. While differences could be due to variations in water stress and the nature of soils, the poison has clearly continued to infect the crops. Different methods of sorting, cleaning, bram removal and use of chemical and biological agents have attempted to reduce levels of aflatoxins in grains [19], but this has not been universally achieved.

The role that maize plays in the staple diet of people in Kenya indicates that consumers are likely to be exposed to aflatoxins and are at risk of disease if they consume maize grain. Presently, maize millers in Kenya are required to test for aflatoxin in incoming consignments prior to milling, where most of them treat this process step as a Critical Control Point (CCP) [20, 21]. Nevertheless, there are still many families who do not buy maize flours from the retail markets. They harvest maize from their farms or buy from their neighbours and mill it using hammer mills. This trend is common in the rural setup. In addition, some families occasionally dispatch packages of milled maize to their loved ones in the cities. This maize will not have undergone any quality assurance test and its aflatoxin concentrations would not be tested. This implies that regardless of the efforts put forth by big commercial maize millers to control aflatoxin, the oblivious consumers may still be feeding on maize laden with aflatoxin as often as they eat *ugali*, *githeri* or porridge. Moreover, the fact that aflatoxin was detected in products from three regions means that it is difficult to eradicate aflatoxin from the maize value chain [22].

The expansion of maize consumption has come at the expense of fermented foods such as millet and milk. The process of fermentation by lactic acid bacteria in itself has nutritious properties and can counter heavy metal toxin adsorption [23,24], and a recent study in Embu County, Kenya showed that this could reduce aflatoxin adsorption in school children consuming aflatoxin-contaminated corn [18]. This raises the issue of whether a return to affordable fermented food preparation might be a good strategy for families to pursue.

CONCLUSION

The study indeed confirmed that Maize flour from three regions of Kenya did not exceed the national limit of 10 ppb aflatoxin hence the measures put in place by government agencies for millers appear to be working. However, given the wide range of aflatoxin levels found in maize grains between stores, perhaps due to storage practices, the government and relevant stakeholders need to establish further measures to protect consumers.

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We report no conflict of interest.

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Table 1: Maize grains sample collection plan (n=75)

		N
Region	1	25
	2	25
	3	25
Store	1	15
	2	15
	3	15
	4	15
	5	15
Sample	1	15
	2	15
	3	15
	4	15
	5	15

Table 2: Analysis of variance at 95% C.I for 27 samples of three different brands of maize flour multiple comparison*

(I) Maize flour	(J) Maize flour	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-0.3444	0.30604	0.5080	-1.1087	0.4198
	3	0.1778	0.30604	0.8320	-0.5865	0.9420
2	1	0.3444	0.30604	0.5080	-0.4198	1.1087
	3	0.5222	0.30604	0.2230	-0.2420	1.2865
3	1	-0.1778	0.30604	0.8320	-0.9420	0.5865
	2	-0.5222	0.30604	0.2230	-1.2865	0.2420

Based on observed means. The error term is Mean Square (Error) = 0.421
*P>0.05 (Sg.) indicating insignificant differences

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Table 3: Analysis of variance at 95% C.I for 27 samples between regions 1, 2, 3 Nairobi Eastern and western, respectively*

(I) Region	(J) Region	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-0.3444	0.30604	0.5080	-1.1087	0.4198
	3	0.1778	0.30604	0.8320	-0.5865	0.9420
2	1	0.3444	0.30604	0.5080	-0.4198	1.1087
	3	0.5222	0.30604	0.2230	-0.2420	1.2865
3	1	-0.1778	0.30604	0.8320	-0.9420	0.5865
	2	-0.5222	0.30604	0.2230	-1.2865	0.2420

Based on observed means. The error term is Mean Square (Error) =0 .421
*P>0.05 (Sg.) indicating insignificant differences

Table 4: Analysis of variance at 95% C.I for 75 samples of maize grains between regions 1, 2, 3 Nairobi, Western and Eastern, respectively*

(I) Region	(J) Region	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-.1800	0.46447	0.9210	-1.2962	0.9362
	3	-14.6200*	0.46447	0.0000	-15.7362	-13.5038
2	1	0.1800	0.46447	0.9210	-0.9362	1.2962
	3	-14.4400*	0.46447	0.0000	-15.5562	-13.3238
3	1	14.6200*	0.46447	0.0000	13.5038	15.7362
	2	14.4400*	0.46447	0.0000	13.3238	15.5562

Based on observed means. The error term is Mean Square (Error) = 2.697

* The mean difference is significant at the 0.05 level

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Table 5: Analysis of variance at 95% C.I for 75 samples between stores in Nairobi, Western and Eastern Kenya, respectively*

(I) store	(J) store	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	.2600	0.59963	0.992	-1.4264	1.9464
	3	-2.2667*	0.59963	0.003	-3.9531	-.5802
	4	-3.5700*	0.59963	0.000	-5.2564	-1.8836
	5	-5.1900*	0.59963	0.000	-6.8764	-3.5036
2	1	-.2600	0.59963	0.992	-1.9464	1.4264
	3	-2.5267*	0.59963	0.001	-4.2131	-.8402
	4	-3.8300*	0.59963	0.000	-5.5164	-2.1436
	5	-5.4500*	0.59963	0.000	-7.1364	-3.7636
3	1	2.2667*	0.59963	0.003	0.5802	3.9531
	2	2.5267*	0.59963	0.001	0.8402	4.2131
	4	-1.3033	0.59963	0.204	-2.9898	0.3831
	5	-2.9233*	0.59963	0.000	-4.6098	-1.2369
4	1	3.5700*	0.59963	0.000	1.8836	5.2564
	2	3.8300*	0.59963	0.000	2.1436	5.5164
	3	1.3033	0.59963	0.204	-0.3831	2.9898
	5	-1.6200	0.59963	0.066	-3.3064	0.0664
5	1	5.1900*	0.59963	0.000	3.5036	6.8764
	2	5.4500*	0.59963	0.000	3.7636	7.1364
	3	2.9233*	0.59963	0.000	1.2369	4.6098
	4	1.6200	0.59963	0.066	-0.0664	3.3064

Based on observed means. The error term is Mean Square (Error) = 2.697

*. The mean difference is significant at the .05 level

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