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Evaluation of Chemical Quality of Camel's and Cow's Milk with Special Reference to Some Heavy Metal Residues

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ABSTRACT: In Egypt, several detailed studies have been carried out on chemical quality and composition of buffalo, cow, and goat milk but it is rare for camel milk. Consequently, the present study was carried out to evaluate the chemical quality and composition of protein, fat, and minerals of camel's milk compared to cow's milk to fill the gap in this respect, monitoring the level of heavy metals residues to ensure the availability of these milk for consumption. Random fifty milk samples collected from healthy camel and cow at Marsa Matrouh and Alexandria Governorates. The samples were analysed for titratable acidity, pH and acid values as a chemical quality index. Also, for total protein, fat, Ash, Cholesterol content, amino acids and fatty acid pattern, minerals, and some heavy metal residues. The obtained results revealed high chemical quality index of camel's and cow's milk. Total percentage of fat, protein, ash and cholesterol content were (4.6, 2.72, 0.79% and 9 mg/100 ml) and were (3.7, 3.5, 0.8%, and 5.5 mg/100ml) for camel's and cow's milk, respectively Amino acids pattern showed higher significant percentage of threonine, serine, glutamic, proline, cystine, methionine, leucine, histidine, lysine, ammonia, and total essential amino acids in camel's milk. Tyrosine and isoleucine in cow's milk showed highly significant percentage. Fatty acids profile showed the presence of 11 various fatty acids, in camel's milk and 13 in cow's milk, respectively. Butyric ($C_{4:0}$) and Caproic acids ($C_{6:0}$) were not detected in camel's milk, and pentadeconoic acid ($C_{15:0}$) was not detected in cow's milk. Short chain fatty acids showed significantly higher percentage in cow's milk but medium chain of high nutritive value also, linoleaic and linoleic acids which have potential health benefits and have potent anticarcinogenic effect were significantly higher in camel's milk. Minerals concentration (Fe, Mg, Ca, P, Na, and K) showed insignificantly higher camel's than cow's milk. Heavy metal residues declared that cow's milk showed significant higher concentration of zinc, cobalt, lead, and chromium than camel's milk with mean values (100.02, 11.792, 8.038, and 0.98mg/kg), respectively. Generally, 100% of cow's milk samples exceeded permissible limit of lead, zinc and Cadmium percentage in cow's and camel's milk exceeding permissible limit in (80%, 20%) of samples, respectively.

INTRODUCTION

Milk is an almost ideal and easily digestive food with high nutritive value. It supplies body building proteins, bone forming minerals and health giving

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vitamins and furnishes energy giving lactose and milk fat, supplying certain essential fatty acids, it is important for pregnant mothers, growing children, adolescents, adults, invalids, convalescents, and patients alike.[1,2]

Milk differs widely in its compositions, depending on species, breed, individual variation, season, age, milking interval, completeness of milking, irregularity in milking, yield, lactation effect, exercise, and excitement.[1,3,4]

Although, the word “Milk” fit for human consumption refers only to cow’s milk, in certain parts of the world, buffaloes, goat, sheep, and camels are kept for milk for human consumption.[5]

In Egypt, cow’s and buffaloes milk were most commonly used as human food, camel’s milk is often the only regular source for its owner, bedouins are used to drink milk and to make white soft cheese. Camel’s milk in U.S.S.R. is used for the preparation of various fermented products, butter gurag and cheese. Camel milk is mainly consumed in the raw state by nomads. Camel milk had a considerable long self life up to twelve days at room temperature without any obvious coagulation.[6-8] Lactation periods of camel vary from 9 to 18 months with annual milk yields of between 2-6 liters under desert condition and up to 12 to 20 liters under more intensive breeding systems.[7]

Camel’s milk is generally opaque, white has a sweet and sharp taste, with unpleasant smell but sometimes, it is saltish. It is more viscous than cow’s milk.[9,10]

Several reports showed that camel milk could have medicinal properties, which suggests that it contain antimicrobial components, could provide a potential antibacterial activity against diarrhea–causing bacteria,

Helicobacter pylori, Tuberculosis, and hepatitis C. Also high titer of antibodies against rotavirus were found in camel milk.[11-14]

Heavy metals represent the chemical residues which have a major role in animal and human health. Environmental pollution with heavy metals leads to an increase interest in metal contamination of milk and feed stuffs. The toxic elements which are considered of major interest in food safety include lead, cadmium, and zinc. The presence of these metals in food at significant concentration is a potential health hazard through progressive irreversible accumulation, their presence in food to a great or lesser extent are as accidental contamination and a result of increasing industrialization and associated in pollution of the biosphere. On the other hand essential elements as copper, chromium and zinc are well defind for physiological function as activator of certain enzymes and as essential components of vitamins or hormones.[15-18]

Information about camel milk is mostly limited to some data on gross composition. Studies on individual components have received very limited attention. So, the present study attempts to evaluate the chemical quality and composition of proteins, fat, and minerals of camel's milk in compared to cow's milk to fill the gap in this respect, also at monitoring the level of heavy metals residue and trace elements to ensure the availability of these milk for consumption.

MATERIAL and METHODS

Sampling:-

Fifty individual milk samples of raw camel's and cow's milk (25 of each) were collected at random from Marsa Matrouh and Alexandria Governorates (each sample about 250 – 500 ml). The

samples were kept in isolated ice box until transported to the laboratory where they were mixed and each sample was divided into two parts, the first examined directly for determination of pH, titratable acidity, and acid values, the second part were deeply frozen (- 20ºC) until analyzed for chemical composition of fat, proteins, minerals, and to determine the concentrations level of some heavy metal residues.

Methods

A* Chemical quality index

1. **Titratable acidity:** according to AOAC, (1990)[19] expressed as lactic acid%.
2. **Hydrogen ion concentration (pH):** according to AOAC (1980),[20] it was estimated directly by using a digital genco pH meter.
3. **Acid Value:** according to AOCS (1994)[21]

B* Chemical composition:

1. **Total fat:** was can determined by using Rose – Gottlieb method according to American Public Health Association (1992).[22]
2. **Total protein:** was determined by the macro–kjeldahl apparatus, using the factor total N x 6.38 according to American Public Health Association (1992).[22]
3. **Ash content:** according to (AOAC, 1990)[19]
4. **Cholesterol content:** was determined colorimetrically according to Bohac, *et.al*., (1988).[23]

C* Amino acids pattern: according to Peter, (1984)[24] using amino acid analyzer Beckman, model (119CL).

D* Fatty acids profile: the methyl ester of extracted milk fat was prepared according to Radwan, (1978)[25] and estimated by using gas liquid chromatography (GLC), GC, mad Shimadz**o**y – 4 CM (PEE).

E* Estimation of minerals and heavy metals residues

Digestion: according to Agemain, *et al*., (1980)[26] using a mixture of concentrated nitric acid and hydrogen peroxide. Atomic absorption specrophotometric method was used for determination of heavy metals residues as described in Perkin Elmer catalogue of atomic absorption model 2380, U.S.A.

F* Determination of phosphorous: was determined colorimetrically using phosphomolybadate method AOAC, (1980).[20]

Results and Discussion

Chemical quality index:

1- Titratable acidity % and pH :

Milk acidity is an important indicator of milk quality and also used to monitor process making cheese and yoghurt . Also , hydrogen ion conc. is considered as one of the main factors which influences the physical properties stability of milk, natural acidity of milk mainly due to the presence of casein, acid phosphates, and citrates, and lesser to allumine globulins and CO_2. Bacteria multiply and utilize lactose to lactic acid there by increasing the acidity and deceased pH (real acidity). The sum of natural acidity and developed (real) acidity represented by titratable acidity, which expressed % of lactic acid. Milk behaves as a buffer solution, fresh milk has pH between 6.5 -6.7, pH higher than 6.7 indicate mastitis milk, but pH below 6.5 indicates the presence of colostrums or bacterial contamination [1,27,28]

Table (1) showed insignificant, higher mean value of titratable acidity percent in cow's milk rather than camel's milk. Also , our results revealed in significant different pH mean values of camel's and cow's milk which were 6.67± 0.182 and 6.61± 0.16 ; respectively. The fresh Camel's milk has lower acidity and pH

than those of cow's milk.[6,29,30] Nearly similar results were recorded by several previous studies.[1,27,29,31,32,] While higher values were reported in some studies.[9,33] However, other studies evaluated lower values of titratable acidity and pH.[10,34,35]

2- Acid value :-

The extent of lipolysis of milk fat is usually expressed as acid degree value which expressed as g of free fatty acid oleic / l00 g fat.[1] Table: 1 showed that acid values of camel's and cow's milk ranged between (0.09-0.15) and (0.1-0.35),respectively. Nearly similar results were recorded.[1] The flavor caused by free fatty acids is not always undesirable, free fatty acids contribute to the desirable flavour of several cheese varieties' e.g., Cheddar and Roguefrt.[1]

Main constituents of camel's and cow's milk :-

Publication dealing with the composition of camel milk are relatively scarce.[10] The Data summarized in table (2) verified insignificant and higher fat % of camel's milk (4.6%) and insignificant lower protein % (3.5%) than cow's milk. Also, it showed nearly the same level of ash % 0.79 .and 0.8, and camel's and cow's milk, respectively. The fairly wide range of values of the main constituents of camel's and cow's milk could be mainly related to the different genetic potential of breed, varying physiological, and feeding condition or to stage of lactation. One of the important factors that affects camel milk composition is the water.[36] These results nearly to somewhat the same ranges of fat , protein, and ash % it was ported in previous studies.[10,3135,37,38] showed that the main percent of protein, fat, and ash of camel's milk are generally similar to those of cow's milk.

Table (2) showed significantly higher cholesterol content of camels milk (9 mg/100 ml) compered to cow's (5.5

mg/100 ml. These results are in agreement with h other investigtor who found that cholesterol content of camel's milk was high, when compared with cow's milk, however, he detected more higher level of cholesterol l (31.32 and 25.63mg/100) for camel's and cow's milk, respectively.[37] Another study detected 40% less cholesterol in camel's milk than cow's milk.[4] In the main time bovine milk showed a level of cholesterol 0.2-0.4%.[1]

Amino acids

Milk is an excellent source of high biological value protein, it contains all of the essential amino acids in varing amounts.[39,2] Amino acids pattern of camel's and cow's milk were represented in table (3) that detected eight essential and nine non-essential amino acids beside ammonia. The essential amino acid cysteine was not detected in all examined samples. Highly significant higher percentage of tyrosine was detected in cow's milk than camel's milk, 24.573. While, amino acids pattern of camel's milk showed highly significant higher percentage of threonine, serine, glutamic, proline, cytstine, methionine, leucine, histidine, and lysine. Also ammonia was found in very significant higher percentage. Nearly similar amino acids pattern of camel's milk were reported in other studies.[29,40] However, different pattern of cow's amino acids was observed by others investigators.[40,4141] The present study showed that camel's milk protein has a highly biological quality compared to cow's milk, since it contains higher significant percentage of essential amino acids 44.816% compared to 38.111% of cow's milk.

Fatty acids profile :- Although the overall composition of camel milk is similar to cow's milk, some differences

exist in the molecular composition of proteins, lipids, and in the mineral balance.[7] Environmental and physiological factors as diet, stage of lactation, and genetic deference influenced fatty acids composition.[4] Table 4 revealed that the chromatographic pattern of examined cows milk fat has given 13 various fatty acids, but chromatogram of camel's milk fat was given 11 various fatty acids . Butyric (C4:O) and caproic (C6:O) acids were not detected in camel's milk. The most quantitative important fatty acids ($C_{18:1}$, $C_{16:0}$, $C_{14:0}$, $C_{16:1}$, and $C_{18:2}$) while the minor acid detected less than 3% were ($C_{6:0}$, $C_{8:0}$, $C_{18:0}$, $C_{10:0}$, $C_{12:0}$, $C_{15:0}$, and $C_{18:3}$. Pentadeconaic acid ($C_{15:0}$) was not detected in cow's milk. Individually the % of major fatty acids were within the range of variation of those previous studies.[29,42] But very different values were reported by other studies.[43,44,45] These result were in accordance with other investigator who observed that total saturated and unsaturated fatty acids showed the same percent in camel's and cow's milk.[37] Oleic acid and palmitic were the most abundant of unsaturated and saturated fatty acids, respectively. These results are in agreement with the previous studies.[37,1] Short chain fatty acid (C_4:C_{12}.) Table 4 revealed highly significant higher percentage in cow's milk but medium and long chain fatty acid ($C_{14:0}$, $C_{18:0}$) were relatively high in camel's milk. These agree with others,[4,7] they showed that the factor specific to camel milk fat is the low percentage of short chain fatty acids (C_4–C_{12}). Also, these results showed insignificant higher % of medium chain fatty acids in camel's milk (44.895%) which is useful from a nutritive point as they are more easily absorbed and metabolized than long chain fatty acids. Also, linoleaic

and linolenic showed significantly higher percentage in camel's milk compared to cows milk, these fatty acids are shown to have potent aniticarcinogenic and have potential health benefits.[46]

Minerals contents

Milk is an excellent source of minerals and supplies virtually all of the minerals required by human, it contains high level of calcium, phosphorous, and Magnesium. However, milk is a poor source of iron.[1]

Mineral concentration level of camel's and cow's milk are present in Table 5 showed insignificant higher level phosphorus, iron, potassium and sodium in camel's milk than cow's but calcium and magnesium showed insignificant lower levels. This result was in agreement with other studies.[29,47,37] But in contrast with the previous studies[4] who found low level of potassium and phosphorus in Egyptian camel's milk. The major salts of camel's milk seem to be similar to those of cow's milk.[40] Mineral contents found in the present study were nearly similar as reported by previous studies,[38,48] in camel's and cow's milk.[49,37] Other studies detected lower level of sodium and iron but showed higher level of potassium and nearly the same level of calcium and phosphorus.[29,37,49] The same level of iron but lower level of calcium, potassium, Magnesium and sodium in camel's milk were detected in other studies.[27,43,50]

Heavy metals residues

Milk constitute a major source of food for infants and children, and even relatively low levels of toxic elements in them can be expected to contribute significantly to dietary intakes. So present study was carried out to determine Cu, Zn, Mn, Cd, Pb, Co, Ni, and Cr concentrations in camel's and

cow's milk. Heavy metals concentration are presented in table (6) which revealed higher concentration mean values of all detected heavy metals in Cow's milk than camel's milk, which are highly significantly higher in (zinc & Cobelt) and significantly in (Lead & Chromium). This result disagrees with previous studies,[29,37,38,47] which reported higher concentration level of copper, zinc, and Manganese in Camel's milk than cow's milk.

This difference may be attributed to contamination of dietary sources, water effluents and surrounding circumstances. Fungicid, insecticide, chemical alloys, and sewage are important source of Cd and Zn. Industrial contamination of the water supply is the main source of Cd and Cr. Dietary plants near the heavily crowded road, phosphate fertilizers are the main source of lead.[51] Milk is a poor source of copper. Copper, Zinc, and Chromium were essential elements but they very toxic at a higher concentrations.[52]

Individual level of heavy metals in Cow's and Camel's milk in the present study were nearly similar to the level detected by several previous author.[29,37,50,53,54,55,56] The mean levels of Cd, Pb, Cu and Zn in raw cow's milk were, 0.299, 2.375, 2.120, and 5.75 mg/kg, respectively.[56] Also, 1.54, 0.69 and 106 mg/kg concentrations of Pb, Cd, and Cu were detected in cow's milk.[38,57] Meanwhile, 1.22, 5.5, and 0.14 mg/kg concentration levels of Cu, Zn, and Mn were detected in mature camel's milk.[38]

In general, metals concentrations can be arranged in the sequence Ni > Zn > Co > Fe > Pb > Cu > Cr > Mn > Cd in camel's milk and Zn > Co > Ni > Pb > Cu > Fe > Cr > Cd > Mn in cow's milk.

Generally, in the present study 100% of examined camel's and cow's milk

samples did not exceed Egyptian permissible limits ES. (1993)[58] of Cu (5 ppm) and Fe (20 ppm). 100% of cow's and 20% of camel's milk samples exceed ES. maximum limit of lead (0.1-0.5 ppm). However cadmium exceeded ES. (0.05 ppm) in 20% of camel's milk and 80% of cow's milk samples. Zinc level exceeded permissible limit (40 ppm) detected by Bulinski *et al*., (1992)[59] in 100% of cow's milk samples.

It could be concluded from the present study that examined camel's and cow's milk samples revealed high chemical quality regarding to titratable acidity, pH and acid value. Also, chemical composition and nutritive value of camel's milk revealed higher biological value of protein, fat, and various minerals required by human since it contain a highly significant percentage of essential amino acids and essential fatty acids. In the main time camel's showed lower level of heavy metal residues. However, camel's milk contain higher fat and cholesterol level than cow's milk.

Table 1: Statistical analysis of chemical quality index of camel's and cow's milk (n = 25)

		Titratable acidity %	pH	Acid Value
Camel's Milk	**Range**	(0.12 – 0.18)	(6.5 – 6.93)	(0.09 – 0.15)
	Mean ± S. E	0.144 ± 0.028	6.67 ± 0.182	0.11 ± 0.012
Cow's Milk	**Range**	(0.14 – 0.2)	(6.45 – 6.97)	(0.1 – 0.35)
	Mean ± S. E	0.167 ± 0.022	6.61 ± 0.169	0.15± 0.032
	t-test	0.646^{NS}	0.242^{NS}	1.17^{NS}

NS. Non-significant at $p < 0.05$

Table 2: Statistical analysis of main constituents of camel's and cow's milk (n=25)

		Fat %	Protein %	Ash %	Cholesterol (mg / 100 ml)
Camel's Milk	**Range**	(3.2 – 5.7)	(2.19 – 3.7)	(0.72 – 0.9)	(8 – 11)
	Mean ± S. E	4.6 ± 0.932	2.72 ± 0.522	0.79 ± 0.061	9 ± 1.414
Cow's Milk	**Range**	2.5 – 4.6	(2.7 – 5)	(0.7 – 0.9)	(4.5 – 6.5)
	Mean ± S. E	3.7 ± 0.792	3.5 ± 0.807	0.8 ± 0.08	5.5 ± 0.707
	t-test	0.736^{NS}	0.812^{NS}	0.099^{NS}	2.212*

NS. Non-significant at $p < 0.05$
* Significant at $P < 0.05$

Table 3: Statistical analysis of amino acids pattern and ammonia of camel's and cow's milk (n=5)

Amino acid	Camel's milk	Cow's milk	t-test
Aspartic acid	7.391±0.957	5.574±0.296	1.814^{NS}
Threonine	4.680±0.096	3.693±0.266	3.490**
Serine	4.487±0.042	3.417±0.311	3.410**
Glutamic acid	16.579±0.487	13.80±1.120	2.275*
Proline	11.036±1.162	6.937±0.178	2.410**
Glycine	1.291±0.11	1.363±0.231	0.281^{NS}
Alanine	2.098±0.212	2.330±0.11	0.971^{NS}
Cystine	0.968±0.133	0.293±0.031	4.943**
Valine	5.616±0.433	5.436±0.533	0.262^{NS}
Methionine	1.84±0.231	1.346±0.231	1.512^{NS}
Isoleucine	7.547±0.620	11.47±7.5	0.521^{NS}
Leucine	8.58±0.792	6.260±0.627	2.397*
Tyrosine	2.97±0.043	24.273±2.341	9.098***
Phenylalanine	3.906±0.256	3.396±0.243	1.445^{NS}
Histidine	4.164±0.279	2.660±0.214	4.277**
Lysine	8.483±0.057	3.845±0.391	11.738***
Ammonia	5.161±0.431	0.414±0.0312	10.984***
Arginine	3.26±0.023	3.689±0.401	1.068^{NS}
Essential amino acids	44.816±5.021	38.111±3.211	1.1250^{NS}
Non essential amino acids	50.092±5.092	61.477±6.451	1.385^{NS}

Each reading represents mean value of 5 trial anlaysis for polling samples of 5 individual milk samples.
NS. Non-significant at $p < 0.05$
* Significantly different at ($P<0.05$).
** highly significantly different at ($p<0.001$).
*** very highly significantly different at ($p<0.001$).

Table 4: Statistical analysis of Fatty acids profile (weight %) of camel's and cow's milk fat (n=5)

Fatty acids	Camel's milk	Cow's milk	t-test
Butyric C $_{4:0}$	-	3.3 ± 0.412	8.01***
Caproic C $_{6:0}$	-	1.6 ± 0.181	8.84***
Caprylic C $_{8:0}$	0.59 ± 0.371	1.37 ± 0.121	1.998^{NS}
Capric C $_{10:0}$	0.79 ± 0.112	0.96 ± 0.753	0.223^{NS}
Lauraic C $_{12:0}$	1.39 ± 0.052	1.15 ± 0.133	1.681^{NS}
Myristic C $_{14:0}$	7.512 ± 1.133	5.64 ± 0.61	1.455^{NS}
Pentadecanoic C $_{15:0}$	0.77 ± 0.511	-	1.507^{NS}
Palmitic C $_{16:0}$	31.19 ± 1.985	33.74 ± 2.91	0.724^{NS}
Palmiloletic C $_{16:1}$	5.44 ± 1.092	1.04 ± 0.052	4.023**
Stearic C $_{18:0}$	9.49 ± 0.981	8.53 ± 0.641	0.819^{NS}
Oleaic C $_{18:1}$	34.53 ±3.07	37.52 ± 3.072	0.932^{NS}
Linoleaic C $_{18:2}$	4.751 ± 2.182	3.36 ± 0.711	0.453^{NS}
Linolenic C $_{18:3}$	3.55 ± 0.713	1.79 ± 0.432	2.116*
Total Saturated	51.74 ± 4.431	56.29 ± 5.065	0.676^{NS}
Total unsaturated	48.27 ± 3.092	43.71 ± 1.851	1.265^{NS}
Mono loneic fatty acids	39.97 ± 4.212	38.56 ± 3.034	0.271^{NS}
Poly loneic fatty acids	8.3 ± 2.891	5.15 ± 1.151	1.012^{NS}
Short chain fatty acids	2.77 ± 0.311	8.38 ± 0.734	7.037***
Medium chain fatty acids	44.895 ± 3.921	40.42 ± 3.823	0.85^{NS}
Long chain fatty acids	52.321 ± 5.432	51.2 ± 6.112	0.137^{NS}

Each reading represents mean value of 5 trial analysis for polling samples of 5 individual milk samples.

- Short chain fatty acids ($C_{4:0}$ - $C_{12:0}$)
- Medium chain fatty acids ($C_{14:0}$ - $C_{17:0}$)
- Long chain fatty acids ($C_{18:0}$ - C 18:3)

NS. Non-significant at $p < 0.05$
* Significant at $P < 0.05$
** Highly significant at $P < 0.01$
*** Very Highly significant at $P < 0.001$

Table 5: Statistical analysis of minerals (mg/kg) of examined Camel's and Cow's Milk (n=25)

		Fe	Mg	Ca	P	Na	K
Camel's Milk	Range	(0.7 – 32)	(174 – 744)	(722 – 1320)	(560 – 1527)	(360 – 1400)	(600 – 1730)
	Mean	2.22	107	957.2	1045.4	748.0	972
	± SE	0.87	35.688	240.460	366.273	417.935	475.889
Cow's Milk	Range	(0.076 – 4.21)	(113.9 –180)	(1000 – 1400)	(750 – 1100)	(350 – 1250)	(500 – 1550)
	Mean	1.202	136.45	1180.6	933.6	716.0	920.0
	± SE	1.53	25.941	171.66	126.312	358.441	495.732
	t-test	0.578^{NS}	0.152^{NS}	0.756^{NS}	0.289^{NS}	0.058^{NS}	0.076^{NS}

NS non-significant

Table 6: Statistical analysis of heavy metal residues (mg/kg) of examined Camel's and Cow's milk (n=25).

		Cu	Zn	Mn	Cd	Pb	Co	Ni	Cr
Camel's Milk	Range	(0.076-1.412)	(18.0-4.3)	(0.075-0.322)	(0 – 0.01)	(0. 091 – 4.4)	(1.53 – 5.11)	(10.22-20.31)	(0.29 – 0.73)
	Mean	0.75	9.186	0.187	0.016	1.354	3.228	16.109	0.41
	± SE	0.551	5.122	0.146	0.027	1.639	1.2823	3.358	0.167
Cow's Milk	Range	(0.208 – 5.72)	(44.12 – 126)	(0-0.84)	(0.039 – 0.98)	(5.7 – 12.11)	(9.23 – 15.11)	(8.12 – 12.31)	(0.7 – 1.21)
	Mean	2.716	100.02	0.352	0.409	8.038	11.792	10.74	0.98
	± SE	1.793	29.103	0.369	0.325	2.398	2.105	1.524	0.177
	t- test	1.048^{NS}	3.08**	0.416^{NS}	1.205^{NS}	2.301*	3.475**	0.395^{NS}	2.342*

NS. Non-significant at p <0.05
* Significant at P <0.05
** Highly significant at P <0.01

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