Online ISSN: 2249-4626
Print ISSN: 0975-5896

Global Journal

of Science Frontier Research: D

Agriculture and Veterinary

Physical Properties of Ginger

Mineral Phosphorus Fertilizer

Highlights

Conventionally Milking Systems

Agro-Ecological Zone of Ghana

Discovering Thoughts, Inventing Future

VOLUME 14 ISSUE 8 VERSION 1.0

Global Journal of Science Frontier Research: D
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Volume 14 Issue 8 (Ver. 1.0)

Open Association of Research Society

Incorporation No.: 0423089
License No.: 42125/022010/1186
Registration No.: 430374
Import-Export Code: 1109007027
Employer Identification Number (EIN):
USA Tax ID: 98-0673427

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USA Toll Free: +001-888-839-7392
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Contents of the Issue

Global Journal of Science Frontier Research: D
Agriculture and Veterinary
Volume 14 Issue 8 Version 1.0 Year 2014
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Physical Properties of Ginger (Zingiber Officinale)

By E. A Ajav & C. A. Ogunlade
University Of Ibadan, Nigeria

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GJSFR-D Classification : FOR Code: 060799

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I. Introduction

Ginger (*Zingiber Officinale* Roscoe) is a tropical monocotyledon and herbaceous perennial specie belonging to the order *Scitamineae* and family *Zingiberaceae*. It is the oldest rhizome widely domesticated as a spice. The cultivation of ginger commenced in Nigeria in 1927 and the locations include Southern Zaria, Jemma Federated districts and neighboring parts of Plateau but today, ginger is cultivated nationwide (Okwuowulu, 1997). NRCRI (2005) confirmed that ginger grows well in the rainforest region of the country where rainfall is above 2000 *mm* and altitudes ranging from 0 - 800 meters above sea level within a temperature range of 25 ^{0}C – 35 ^{0}C (Njoku *et al.,* 1995). Nigeria's production in 2004 was 117,000 tonnes (FAOSTAT, 2012), 10 % of which is locally consumed as fresh ginger and 90 % dried primarily for the export markets (Ayemibo, 2009) Ginger is a plant with leafy shoots, finger-like perennial underground part or rhizomes called hands and grows to a height of about 1.5 *m* with an aerial part as high as 0.8 *m* depending on cultivars and growing environment (Entrepinoys, 2010). It can be grown on sandy loam and clay loam soil with good drainages and a lot of organic matter. Ginger is popular for its distinct sharp and hot flavor due to an oily substance called *gingerol*. The knobby rhizome is ready for harvesting and dug up when all the leaves and stems of the plant wither, which occurs between 6 and 12 months after planting, harvesting of ginger starts from October and normally continues until April/May, depending on market situation as ginger can be left on the ground (not harvested) for two years. Yields of up to 20-30 tonnes per hectare are possible under improved cultural management. Ginger is in three (3) forms namely: fresh or green ginger, whole dry ginger and split dry ginger. The fresh or green ginger refers to the newly harvested ginger with little or no loss in moisture content; this type of ginger is not in hot demand in the international market because of the length of time it takes for the product to dry up. Dry whole and dry split ginger are the most sort-after ginger in the international market though the whole dry ginger commands higher price because of the longer time it takes for the product to get dried-up and ready for sales.

Ginger is a spice and medicinal plant gaining attention in the pharmaceutical, food and chemical industries. A remarkable increase in the use of medicinal plant products has been observed in the past decade. Due to their properties, medicinal plants are used as primary health care aid among 80 % of the world's population in the form of plant extracts or their active components (WHO, 2008). Today, herbs are still found in 40 % of prescriptions, and the interest for use of herbal remedies instead of chemical drugs is increasing because of lesser side effects (Craig, 1999). Eze and Agbo (2011) reported that processing of ginger in Nigeria has not been standardized consequent upon which low quality ginger which falls short of importer's specifications are produced. In West Africa and Nigeria in particular, this important rhizome is subjected to local processing method which includes cleaning, sorting, peeling, grading, drying, splitting (slicing), size reduction and storage which is labour intensive and generally has a low output. The aspect which is of interest to the engineer (food processor) is the physical properties, mechanical properties, electrical properties, and thermal properties. This gives the engineer guidelines for

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designing machines that will be suitable for the processing of the biomaterial. Most important among these properties is the physical property which is the first consideration in the design of the post-harvest handling and sorting equipment. Jayan and Kumar (2004) designed a planter in relation to the physical properties of certain seeds.

II. Materials and Method

Sampling: Whole fresh ginger rhizomes were procured from Bodija market, Ibadan, Oyo state. The rhizomes were cleaned manually by hand to remove all foreign matter such as dirt, pieces of stone and broken rhizomes. Measurement of physical properties was thereafter followed at the central laboratory of Federal College of Agriculture, Moor plantation, Ibadan. The rhizomes were labeled and numbered as shown in Figure 1, for the purpose of identification of samples and the total number of rhizome used for the research is forty-one (41).

Figure 1 : Numbered Ginger Rhizomes

Moisture Content Determination: The moisture content of the ginger rhizomes was obtained according to ASAE Standard S358.2 (1983). The sample was dried in an electric oven at a temperature of 105 °C for 24 hours and weighed using a weighing balance at every 6 hours interval to obtain four different levels of moisture content. The moisture content of the sample in percent dry basis was calculated using Equation 1.

$$Ms = \frac{100\,(W_i - W_f)}{W_f} \qquad (1)$$

Where: Ms is the Moisture Content of Ginger rhizomes (in % dry basis), *Wi* is the Initial Mass of ginger rhizomes before oven drying (in grams) and *Wf* is the Final Mass of the rhizomes after oven drying (in grams).

a) Physical Properties

i. *Determination of Axial Dimensions*

Alphabets x, y, z are used to represent axial dimensions; major, intermediate and minor diameters respectively however, this can also be referred to as *the* length, width and thickness respectively. Vernier calliper (0.001 mm accuracy) was used in taking the measurement of length, width and thickness. Figure 2 shows the measurement of major (x), intermediate (y) and minor (z) diameters.

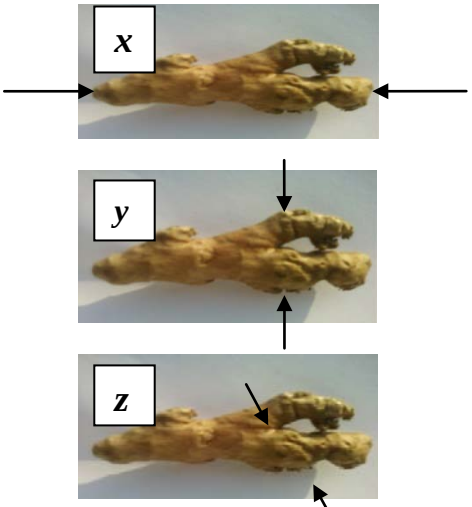

Figure 2 : Measurement of Major (x), Intermediate (y) and Minor Diameter (z) of a Ginger Rhizome

ii. *Determination of Geometric Mean*

The geometric mean was calculated using Equation 2 described by Mohsenin (1986).

$$Gm = (xyz)^{\frac{1}{3}} \qquad (2)$$

Where: Gm is the Geometric Mean, *x* is the Major Diameter of the rhizome, *y* is the Intermediate Diameter of the rhizomes, *z* is the Minor Diameter of the rhizomes (all in *mm*).

iii. *Determination of Sphericity*

The higher the sphericity value of a material, the closer its shape to a sphere, this property is useful in the design of hopper and dehulling equipment for agricultural products, it determines the tendency of a material to roll when placed on a particular orientation. The degree of sphericity of the ginger rhizomes was calculated using Equation 3 described by Mohsenin (1986).

$$\Phi = \frac{(xyz)^{1/3}}{x} = \frac{Gm}{x} \qquad (3)$$

Where: Φ is the Sphericity in decimal and other parameters remain as defined above.

iv. *Determination of Bulk Volume*

The bulk volume of the ginger rhizomes was determined using Archimedes's principle as described by Nelkon (2005). The sample was weighed and immersed in a measuring cylinder containing a known volume of water thus leading to an increase (rise) in the water volume, the difference between the new level of

water in the measuring cylinder and the initial level of water is the bulk volume of the seed.

v. *Determination of Bulk Density*

The bulk density of the ginger rhizomes was determined as the ratio of bulk weight of ginger to the bulk volume.

vi. *Determination of Surface Area*

The surface area S in mm^2 was estimated by the relationship given by Asoiro and Anthony (2011) as:

$$S = \pi G m^2 \quad (4)$$

Where: *Gm* is the geometric mean diameter (*mm*) and S is the surface area of the ginger rhizomes (mm^2).

vii. *Determination of Coefficient of Friction*

The static coefficient of friction was determined with respect to each of the following three structural materials on the tilting table: stainless steel, plywood and glass. The ginger rhizomes were placed parallel to the direction of motion and the table is raised gently by a screw device, the angle at which the rhizomes begin to slide (the angle of inclination) was read from a graduated scale on the tilting table, this was repeated three times for each structural material. The coefficient of friction was calculated as the tangent of this angle as shown in Equation 5 (Olaoye, 2000; Adejumo, 2003; and Pliestic *et al.,* 2006).

$$\mu = \tan\theta \quad (5)$$

Where: μ is the Static Coefficient of Friction (decimal), θ is the Angle of Inclination (degrees).

viii. *Determination of Angle of Repose*

The angle of repose was evaluated by using a specially constructed topless and bottomless box made of plywood, with a removable front panel (Dutta *et al.,* 1988; Olaoye, 2000). The box was filled with rhizomes of ginger and placed on the floor, the front panel was quickly removed allowing the rhizomes to slide down and assume natural slope. This value is used in the design of agricultural machine hopper and other conveying equipment. The angle of repose was calculated from the measurements of the height (*h*) of the free surface of the seeds and the lenght (*l*) of the heap formed outside the box using the relationship described by Bamgboye and Adejumo, (2009):

$$\theta = tan^{-1}(\frac{h}{l}) \quad (6)$$

Where: θ is the Angle of Repose (degrees), *h* is the Height of the free surface of the rhizomes and *l* is the Length of the heap formed outside the box.

III. Results And Discussion

The number of samples used for the research, the range, the mean value and the standard deviations of some physical properties of ginger are presented in Table 1; these properties include the major diameter, minor diameter, intermediate diameter, geometric mean, sphericity, bulk volume, surface area and the bulk density of ginger rhizomes.

Table 1 : Summary of Physical Properties of Ginger (*Zingiber officinale*)

Property	Number of Samples	Range	Mean value	Standard Deviation
Major Diameter *(mm)*	41	76.1 – 133	112	25.5
Intermediate Diameter *(mm)*	41	60 – 82.0	72.3	9.8
Minor Diameter *(mm)*	41	28 – 44.0	38.3	7.1
Geometric Mean *(mm)*	41	50.4 – 78.3	67.6	12.4
Sphericity *(dec)*	41	0.66 – 0.59	0.61	0.03
Surface Area *(cm^2)*	41	79.8 – 192.6	147	49.8
Bulk Volume *(cm^3)*	41	660 – 1120	832.5	201.2
Bulk Density *(g/cm^3)*	41	0.96 – 0.87	0.92	0.048

a) *Bulk Weight and Moisture Content*

The bulk weight of ginger rhizomes at 6 hours interval of drying ranges from 0.64 kg to 0.97 kg, the percent moisture content dry basis varied from 10.9 - 51.6 % with a mean value of 24.5 % (+ 18.4). For wet basis, the moisture content mean value was lower than the value obtained for the dry basis; the percent moisture content wet basis ranges from 8.6 - 34% with a mean value of 18.2% (+11.04), this implies that ginger has a high moisture content and it is liable to deteriorate

quickly after harvesting therefore, processing or post-harvest handling should not be delayed because dried foods in general keep longer if held at low moisture contents. This can be attributed to their reduced oxidation and lowered chemical reaction (Bolin, 1980). This value is in the same range with the moisture content of green gram plant ranging from 8.39 to 33.40 % wet basis (Nimkar and Chattopadhyay, 2001). A similar trend was reported by Bande *et al.* (2012) for *Egusi* melon (*Citrullus colocynthis* lanatus) seeds having a moisture content range between 7.11 - 38.7 % wet basis; Zareiforoush *et al.* (2009) for paddy grains having a moisture content range of 8 - 21 % dry basis, Li Ma *et al.* (1998) for foods and other biological materials. However, Alakali and Santimehin (2009) reported that the equilibrium moisture content (EMC) of the ginger powder increases as the water activity increases at constant temperature and an increase in temperature causes more activation of water molecules due to increase in energy level.

b) Axial Dimensions

The major diameter (length), intermediate diameter (width), minor diameter (thickness), and geometric mean of ginger are significantly different and increases with increasing moisture content. It was observed that Within the moisture content range of 10.9 % dry basis to 51.6 % dry basis, the length of ginger rhizomes increased from 76.1 *mm* to 133 *mm* (75 % increase in length), the width increased from 60 *mm* to 82 *mm* (37 % increase in width) and the thickness increased from 28 *mm* to 44 *mm* (84% increase in thickness) while the geometric mean increased from 50.4 *mm* to 78.3 *mm* (55 % increase). However, the relationship observed between values of length (*x*), width (*y*), thickness (*z*) and geometric mean diameter (*Gm*) and the percent moisture content (*Mc*) of ginger in dry basis are given in equations 7, 8, 9 and 10 respectively.

$$x = 0.977Mc + 87.83\ (R^2 = 0.497) \quad (7)$$

$$y = 0.424Mc + 61.85\ (R^2 = 0.635) \quad (8)$$

$$z = 0.266\,Mc + 31.73\ (R^2 = 0.471) \quad (9)$$

$$Gm = 1.08Mc - 48.34(R^2 = 0.526) \quad (10)$$

A similar trend was reported by Asoiro *et al.* (2011) having a major diameter range of 6.7 cm to 10.32 cm with mean value of 8.1778 cm, the intermediate diameter range of 5 cm to 7.7 cm with mean value of 6.712 cm, minor diameter range of 5.17 to 7.44 cm with mean value of 6.3025 cm and geometric mean diameter of 5.910 cm to 8.057 cm with mean value of 7.013cm.

c) Sphericity

The sphericty of ginger rhizomes decreases with an increase in moisture content, as moisture content of ginger increased from 8.6 to 34% wet basis, the average sphericity of ginger rhizomes decreased from 0.66 to 0.59 (10% decrease), this is similar to findings of Bande *et al.* (2012) on *egusi* melon seed (having a 9 % decrease), Simonyan *et al.* (2009) for *Ronghai lablab* seeds having a sphericity value decreasing from 0.78 to 0.76 between 9.7 and 29 % moisture content wet basis and *Highworth Ronghai* seeds decreasing from 0.659 to 0.653 between 10.2 and 22.6 % wet basis. The relationship between moisture content and the sphericity of ginger rhizomes is given in equation 11.

$$\Phi = -0.001Mc + 0.635(R^2 = 0.312) \quad (11)$$

Sphericity value of most agricultural produce has been reported to range between 0.32 and 1.00 (Mohsenin, 1970; Irtwange and Igbeka, 2002).

d) Bulk volume

The bulk volume of ginger rhizomes increases as the moisture content increases. It was observed that as moisture rises from 10.9 to 51.6 % moisture content dry basis and the bulk volume rises from 0.66 cm^3 to 1.12 cm^3 respectively as shown in Figure 3.

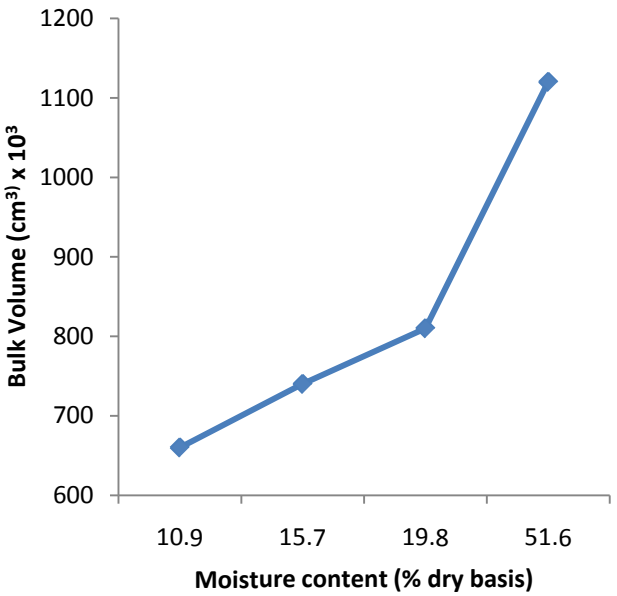

Figure 3 : Effects of Moisture Content on Bulk Volume of Ginger

However, a linear relationship between the bulk volume and moisture content of ginger rhizome was observed to be:

$$Y = 10.85X + 566.6(R^2 = 0.987) \quad (12)$$

Where: Y is the bulk volume (cm^3) and X is the moisture content (% dry basis) and R is the regression analysis.

The volumetric rise may be attributed to the expansion in the axial dimensions which contributed to

weight increase of the rhizomes thereby resulting to displacement of more liquid. The variation of bulk volume with moisture content is similar to the trend reported by Bamgboye and Adejumo (2009) for Roselle (*Hibiscus sabdariffa* L.) seeds with the mean volume of the seeds increasing from 29.7×10^{-6} m^3 to 40×10^{-6} m^3 as the moisture content increased from 8.8 to 17 % dry basis; Desphande *et al,* (1993) for soybean seeds; Ozarslan (2002) for cotton seeds; Altuntas and Demirtola (2010) for some grain legume seeds, Simonya *et al.* (2009) for *Ronghai lablab* Seeds increasing from 0.24cm^3 to 0.54cm^3 within 9.7 to 29% moisture content wet basis and *Highworth Lablab* seeds increasing from 0.108 cm^3 at 10.2 % moisture content wet basis to 0.54 cm^3 at 29 % moisture content wet basis, Zareiforoush *et al.* (2009) for paddy grains with the volume of both varieties of paddy grains observed to increase from 26.91 to 29.94 mm^3 and 23.12 to 25.11 mm^3 for *Alikazemi* and *Hashemi* varieties respectively within a moisture content increase from 8 to 21% (d.b.); Asoiro and Anthony (2011) for African yam Beans and Ndukwu (2009) for *Brachystegia eurycoma* seeds.

e) Bulk Density

The bulk density of ginger decreased with an increase in moisture content as shown in figure 4. It decreased from 0.96 g/cm^3 at 10.9 % d.b. to 0.87 g/cm^3 at 51.6 % d.b. The regression equation for the bulk density (B) of ginger was of the form:

$$B = -0.002x + 0.971 (R^2 = 0.701) \quad (13)$$

Similar trend was reported for chickpea seeds by Konak et al. (2002), African yam bean by Irtwange and Igbeka (2002), *lablab* seeds by Simonyan *et al.* (2009), arecanut kernels by Kaleemullah and Gunasekar (2002) and round red lentil grains by Isik (2007). Bulk density has been reported to have practical applications in the calculation of thermal properties in heat transfer problems, in determining Reynolds number in pneumatic and hydraulic handling of materials and in predicting physical structure and chemical composition (Irtwange and Igbeka, 2002).

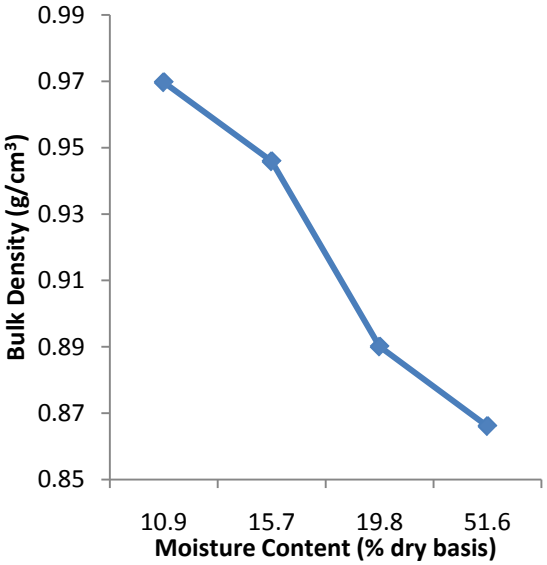

Figure 4 : Effects of Moisture Content on the Bulk Density of Ginger

f) Angle of Repose

The angle of repose is also determined at different moisture levels, it was observed to increase as the moisture content increases as shown in Figure 5. However, the angle of repose of ginger ranges from 41.3^0 at 10.9 % moisture content dry basis to 57.6^0 at 51.6 % moisture content dry basis, a linear relationship observed between the angle of repose and moisture content of ginger is given in equation 13:

$$A = 0.340\ B + 40.68\ (R^2 = 0.873) \quad (14)$$

Where: A is the angle of repose (degrees) and *B* is the moisture content (percent dry basis) and R is the regression analysis.

This increasing trend of angle of repose with moisture content occurs because surface layer of moisture surrounding the particle hold the aggregate of grain together by the surface tension (Pradhan *et al.,* 2008) and it implies that friction increases on the surface of the rhizomes as water content increases, thereby making the seeds less able to flow on one another. The experimental values were seen to be higher than that of oilbean seed (Oje and Ugbor, 1991). However, similar trend was reported by Zairefoush *et al.* (2009) for different varieties of paddy grains, he observed an increase from 35.67^0 to 41.23^o and 38.27^o to 44.37^0 respectively for *Alikazemi* and *Hashemi* cultivars in the moisture range of 8–21% (d.b.). Bamgboye and Adejumo (2009) reported a similar trend for Roselle

(*Hibiscus sabdariffa* L.) seeds increasing with an increase in moisture content from 20.13° to 24.85°; Fraser et al. (1978) for fababeans, Gamayak *et al.* (2008) for Jatropha seeds increasing from 35^0 to 43^0; Kalamullah and Gunasekar (2002) for *arecanut* kernels, Kerababa (2006); Gursoy and Guzel (2010) for grains; Karaj and Muller (2010) for *Jatropha curcas*.

The effect of moisture content on the angle of repose of ginger rhizomes is shown in figure 5.

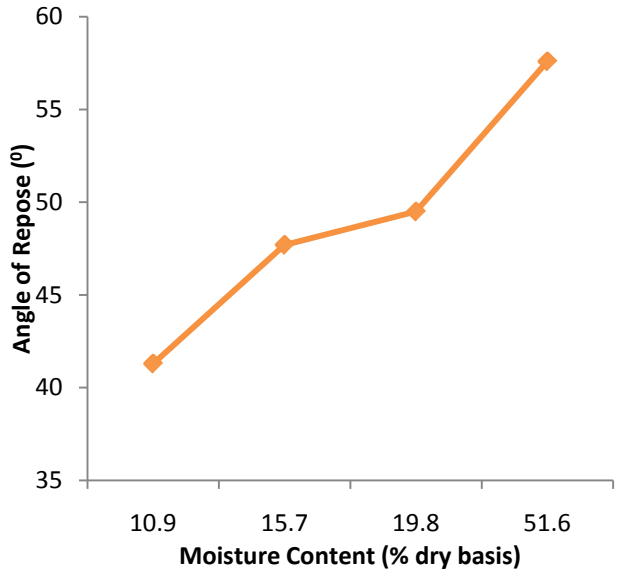

Figure 5 : Effect of Moisture Content on the Angle of Repose of Ginger

Coefficient of Friction: The coefficient of friction of seeds is required in the design of silos and hopper for processing machines thus, it was determined with respect to glass, stainless steel and wood surfaces, it was observed that the coefficient of friction was highest on wood and least on glass and it increases with an increase in the moisture content. A similar trend was reported by Zareiforoush *et al.* (2009) for two cultivars of paddy grains. He reported that the least static coefficient of friction may be owing to smoother and more polished surface of the glass than the other materials used. It was observed that the coefficient of friction for ginger rhizomes increased with increasing moisture content on all surfaces. The reason for the increased friction coefficient at higher moisture content may be owing to the water present in the rhizomes, offering a cohesive force on the surface of contact. As the moisture content of ginger increases, the surface of the samples becomes stickier. Other researchers reported a similar trend that coefficient of friction increases as the moisture content increase (Pradhan *et al.,* 2008; Altuntas and Erkol, (2010), Elfawal *et al.,* 2009).

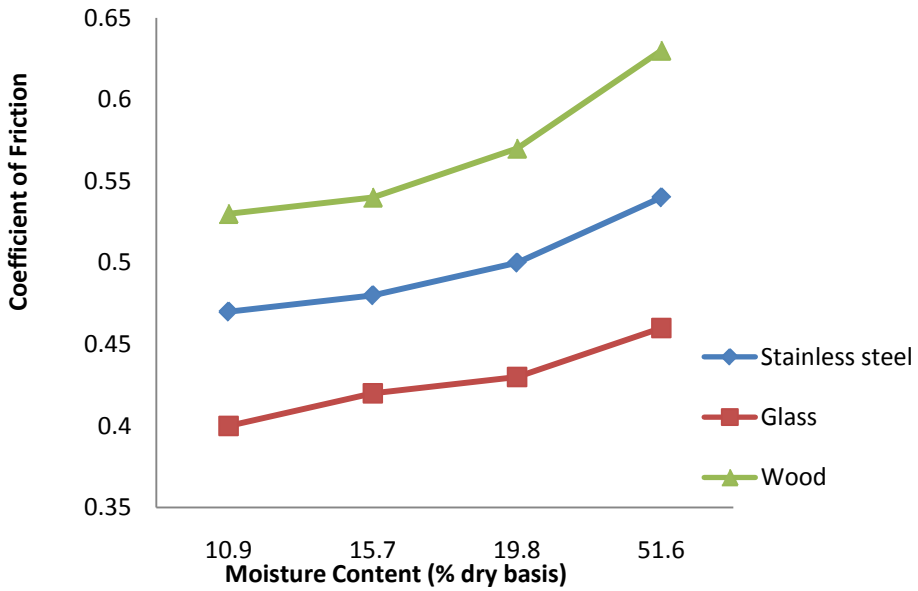

Figure 6 : Coefficient of Friction of Ginger on Glass, Steel and Wood

IV. Conclusions

The research looked at some selected physical properties of *Zingiber officinale,* such as axial dimensions (length, width and thickness), geometric mean, sphericity, bulk density, volume, bulk density, surface area, angle of repose and the coefficient of friction which are essential in the design and construction of the processing and handling equipment of *Zingiber officinale* and plays an important role in

selecting the proper sorting, grading and cleaning equipment. The main dimensions are considered in selecting and designing the suitable size of the screen perforations. The physical properties of ginger measured showed some deviations from the mean value which is typical of biomaterials. The following conclusions were drawn from the research:

1. The physical properties of the seed determined as a function of moisture content varied significantly with the moisture content.
2. The axial dimensions, geometric mean diameter, angle of repose, surface area, bulk density, and coefficient of friction showed an ascending relationship with moisture rise while bulk density and sphericty which has a descending relationship on moisture gain. These properties would provide important and essential data for efficient process and equipment design.
3. The coefficient of friction varies from one material to the other (0.4 for glass, 0.49 for stainless steel and 0.55 for wood). However, glass or stainless steel which has the lowest values of coefficient of friction should be used when constructing seed hopper in ginger processing machines with side inclination of about 450 to allow easy sliding of the ginger rhizomes.

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Global Journal of Science Frontier Research: D
Agriculture and Veterinary
Volume 14 Issue 8 Version 1.0 Year 2014
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Quantitative Behavior in Dairy Cows under the Conditions of Automatically and Conventionally Milking Systems

By Berit Füllner, Anne Harzke & H. Scholz
Anhalt University of Applied Sciences, Germany

Introduction- Automatically milking systems increased in the last years in the European Union. Behavior of lactating dairy cows in the barn can be used to evaluate the effects of changing in the procedures of milking like Forced Cow Traffic (AMS and VMS) and the design of management in the waiting area (AMR).

GJSFR-D Classification : FOR Code: 670105

Strictly as per the compliance and regulations of :

Quantitative Behavior in Dairy Cows under the Conditions of Automatically and Conventionally Milking Systems

Berit Füllner [α], Anne Harzke [σ] & H. Scholz [ρ]

I. Introduction

Automatically milking systems increased in the last years in the European Union. Behavior of lactating dairy cows in the barn can be used to evaluate the effects of changing in the procedures of milking like Forced Cow Traffic (AMS and VMS) and the design of management in the waiting area (AMR).

II. Material and Methods

Conventional milking systems (CONV; 5 farms) were used in this investigation as "standard". Data's from 4 farms with AMS /VMS and 1 farm with AMR provided the basis for the automatically milking systems. In this investigation were used 18-25 dairy cows in each farm for measurement of quantitative behavior (time for lying and lying + ruminating, standing and standing + ruminating, milking and others) over 24 hours. The behavior of cows was observed directly with Time-Sampling-method (5 minutes). In the farm with AMR24 data collection of behavior was every month over a period for more than 1 year.

III. Results and Discussion

Under the condition of AMR and most of other farms delivery of feed were once a day. The average feeding time of all cows were 275 ± 73 minutes per cow and day and show the same level such as DLG (2012) and HOY et al. (2009). Between milking systems there are significant differences in feeding time (figure 1).

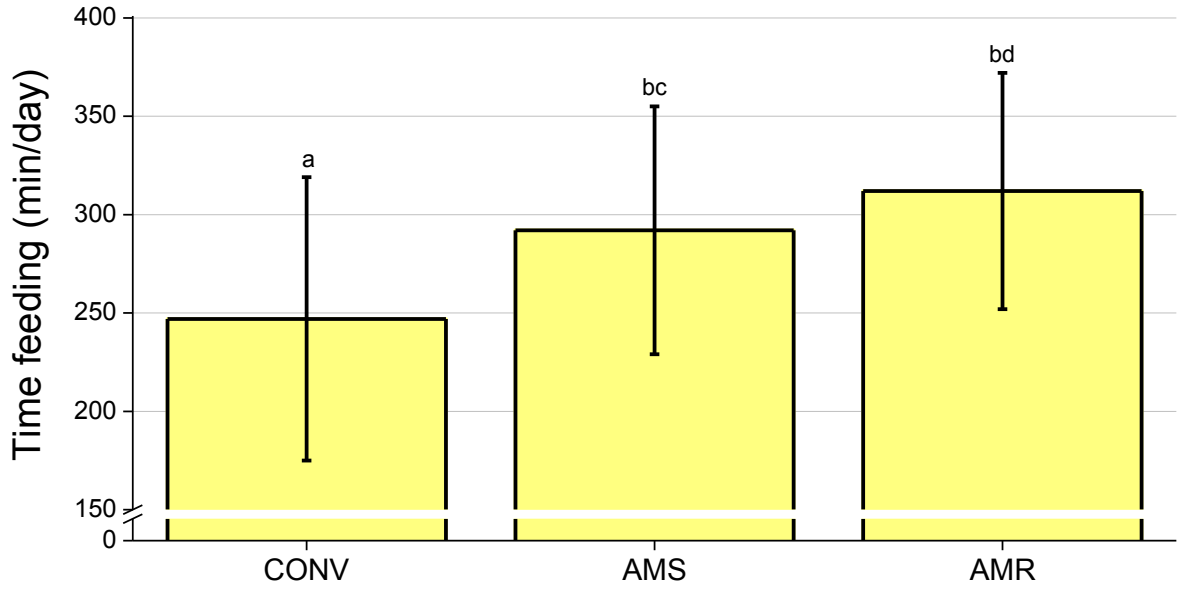

Figure 1 : Time duration for feeding time per cow and day

In the system of AMR were found an average lying time of 702 ± 107 minutes per day and in the conventional milking systems of 652 ± 139 minutes per day. Cows under the condition of AMS / VMS show an average lying time per day of 593 ± 149 minutes, while between free cow traffic and forced cow traffic no significant difference (SHAHHOSSEINI, 2013). JENSEN et al. (2005) and MUNKSGAARD et al. (2005) found an average lying time per cow and day of 12-14 hours, in contrast to HOY et al. (2009) with 7-14 hours per cow and day and 9-11 hours of cows at pasture (PHILLIPS and RIND, 2001; TUCKER et al., 2007). Ruminating time in the investigation was 501 ± 78 minutes per day while ruminating during lying was 69 % and ruminating during standing was 31 %.

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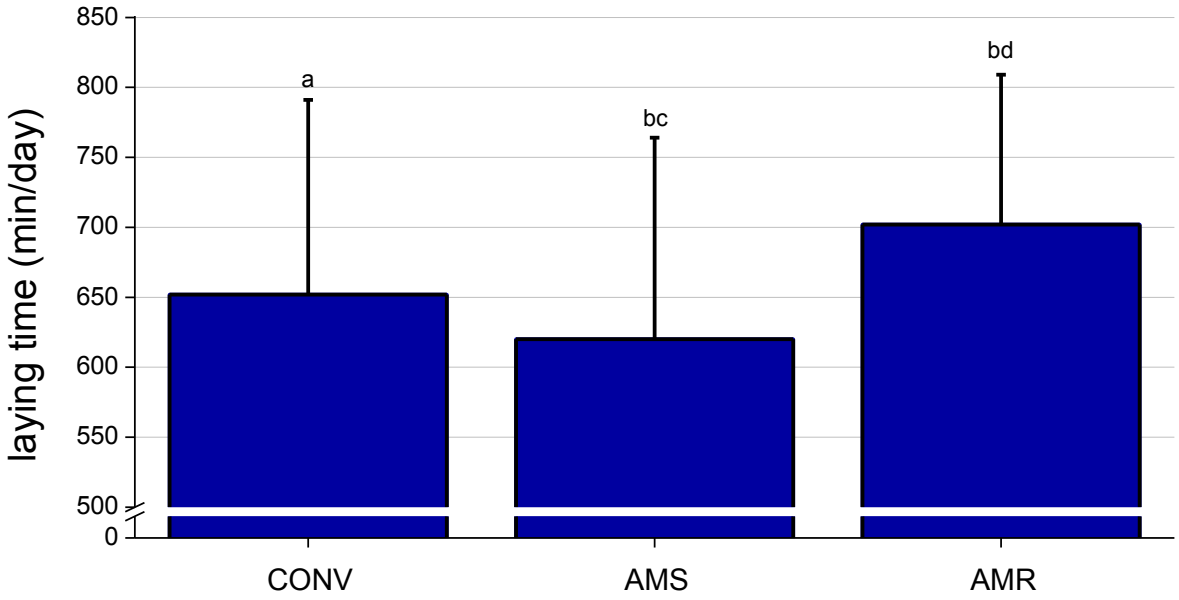

Figure 2 : Time duration for lying per cow and day

Calculation by means of univariate analysis of variance with fixed effects of milking system and farms showed no significant effect of milking system with significant effect of farms for lying time of cows per day (table 1). This shows very clearly the influence of management in dairy production for behavior of cows.

Table 1 : lying time of cows per day independent from the farms

System	AMR	AMS				CONV				
	1	1	2	3	4	1	2	3	4	5
Time (h/d)	702	572	617	651	646	669	716	549	596	798

The maximum time duration of the cows for standing (standing in the alley or stall and during the milking) were found under conventional milking systems with an average of 554 minutes per cow and day observed. The two automatically milking systems (AMS and AMR) showed significant lower time budgets (figure 3). GOMEZ and COOK (2010) found in 17 freestall barns in Wisconsin's an average time budget for standing + milking with 474 minutes per day which is comparable with AMR (458 minutes per cow and day).

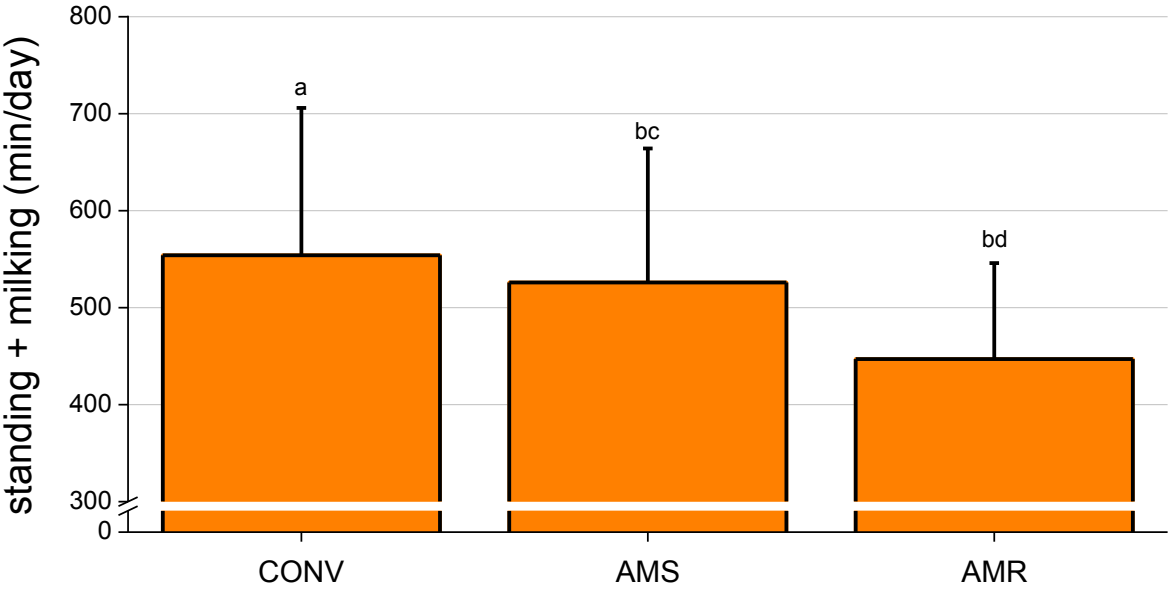

Figure 3 : Time duration for standing + milking per cow and day

IV. Conclusion

The investigation showed a significant influence of milking systems to the animal welfare - criteria's such as lying time and the time for standing and milking of dairy cows. An average lying time of 11 hours per cow and day can be founded. Further investigations with fixed effects such as season or number of lactations are planned and should be improve the results.

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Global Journal of Science Frontier Research: D
Agriculture and Veterinary
Volume 14 Issue 8 Version 1.0 Year 2014
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
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By Williams. K. Atakora, Emmanuel Oppong, Rufai Mahama, Henry Oppong Tuffour & Sandra Adu Gyamfi

CSIR-Savanna Agricultural Research Institute, Ghana

Abstract- Phosphorus, although not required in large quantities, is very critical to cowpea production because of its multiple effects on nutrition; shoot development and its influence on nodulation. Phosphorus application stimulates plant root growth, early flowering, enhances fruiting, initiate nodule formation as well as increase in yield of cowpea. In spite of this immense importance of P in soils, its availability to cowpea growth and development is influenced by several factors, which include level and time of application. Knowledge in level of P and time of application to facilitate timely availability of the P fertilizer to improve cowpea yield is critical in order to avertthe continues reduction of cowpea yield by farmers. As a result a 3x4 factorial experiment arranged in split-plot design was conducted at the University for Development Studies experimental site, Nyankpala near Tamale in the northern region of Ghana campus to determine the appropriate P fertilizer levels and timing of application. Three levels of P fertilizer; 24, 48 and 60 kg ha^{-1} P was applied at 30, 15 days before plating. However, a third application was implemented at sowing.

Keywords: *phosphorus, nodule number, nodule weights, time of application.*

GJSFR-D Classification : *FOR Code: 070301*

Strictly as per the compliance and regulations of :

Cowpea Yield as Affected by Level and Time of Mineral Phosphorus Fertilizer Application in the Guinea Savanna Agro-Ecological Zone of Ghana

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Abstract- Phosphorus, although not required in large quantities, is very critical to cowpea production because of its multiple effects on nutrition; shoot development and its influence on nodulation. Phosphorus application stimulates plant root growth, early flowering, enhances fruiting, initiate nodule formation as well as increase in yield of cowpea. In spite of this immense importance of P in soils, its availability to cowpea growth and development is influenced by several factors, which include level and time of application. Knowledge in level of P and time of application to facilitate timely availability of the P fertilizer to improve cowpea yield is critical in order to avertthe continues reduction of cowpea yield by farmers. As a result a 3x4 factorial experiment arranged in split-plot design was conducted at the University for Development Studies experimental site, Nyankpala near Tamale in the northern region of Ghana campus to determine the appropriate P fertilizer levels and timing of application. Three levels of P fertilizer; 24, 48 and 60 kg ha^{-1} P was applied at 30, 15 days before plating. However, a third application was implemented at sowing.

Results of the experiment indicated no significant affect (P>0.05) of P fertilizer rate applied on grain yield, root biomass and nodulation. However, there was significant effect (P<0.05) of P fertilizer on biomass yield. Similarly, time of application of P fertilizer had no significant effect (P>0.05) on grain yield, biomass weight, nodulation and root biomass. Furthermore, there was no interaction effect on P levels and time of application. However, application of 24 kg ha^{-1} P produced the highest grain yield of 1021 kg ha-1but was not significantly different from where 48 and 60 kg ha^{-1} P was applied.

Keywords: phosphorus, nodule number, nodule weights, time of application.

I. Introduction

Cowpea (*Vigna unguiculata* (L.) Walp. is a major food crop in Africa because of its high protein content, acceptable palatability and low cost of production. Cowpea have the potential to contribute substantially to human and animal health, income creation, food security and agricultural sustainability of less developed countries such as Ghana, Nigeria, Zambia and several others in sub-Saharan Africa (Muimui, 2010). Cowpea possesses the capacity to fix atmospheric nitrogen in poor soils and it is very adapted to arid areas and support high temperature and tolerates droughts (Ehlers and Hall, 1997). Sub Saharan Africa accounts for 84% of the world's cowpea grain production. Nigeria produces more than 45% followed by Niger that is nearly 15% of the world cowpea grains of 6.7 million metric tons produced each year covering an area of about 14.5 million hectares (Abate *et al.,* 2012). In Ghana 143,000 MT of cowpea is produced annually on about 156,000 ha making Ghana the fifth highest producer in Africa (MoFA, 2012). Economically, Ghanaian households generate annual income of about GH¢760 - 800 through increased production due to two or three cycles of production per year. And in Northern Ghana an additional income of between GH¢ 15 to GH¢ 16 million is generated yearly, at least 40% of this directly going to women farmers (ICRISAT, CIAT and IITA, 2012).

Cowpea is among the lowest yielding crops in the Africa averaging 310kg\ha (Ofosu Budu *et al.,* 2007) and this is because of declining soil fertility mainly N, P and K. Phosphorus is an important plant nutrient involved in several energy transformation and biochemical reactions including biological nitrogen fixation. However phosphorus availability is low in the moist savanna zone of West Africa and considerably limits cowpea crop production (Fox and Kang, 1977).Soil phosphorus deficiencies primarily result from either inherent low levels of soil phosphorus or depletion of phosphorus through cultivation. P fertilizers have low efficiency of use due to chemical fixation in soil (Gaur, 1983) and poor solubility of native soil phosphorus, sometimes there is a buildup of insoluble phosphorus as a result of chemical phosphorus application (Dubey, 1997). Furthermore, phosphorus can be fixed into forms unavailable to plants by Fe and Al oxides found in

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tropical soils (Sample *et al.,* 1980). Ineffective application of inorganic phosphorus fertilizer can therefore not be relied upon to adequately alleviate phosphorus deficiency for improved cowpea production (Devi *at al.,* 2013).

Phosphorus is effective in enhancing the nodulation, yield components and yield of cowpea. Similarly agronomic efficiency of phosphorus, physiological efficiency and phosphorus use efficiency are also higher with application of triple super phosphate (Kumar and Kushwaha, 2006). Therefore the development of appropriate management strategies to overcome phosphorus deficiency and increase production of these important crops requires in-depth knowledge of phosphorus application and its utilization (Devi *at al.,* 2013). Therefore, this experiment was setup to determine the appropriate P level as well as the time of application that would increase growth, development and yield of cowpea.

II. Materials and Methods

a) Experimental area

The experiment was conducted at University for Development Studies, Nyankpala campus farming for the future site, Nyankpala near Tamale in the Northern Region of Ghana on the coordinate (latitude 9.4075°N and 0.85333°W). The area has a unimodal rainfall season starting from April/May to September/October with a peak season in July/August with an annual average of 1100 mm within 95 days of intense rainfall. Consequently, staple food crop farming is highly restricted by the short rainfall duration. The mean day temperature ranges from 33 °C to 39 °C while mean night temperature range from 20°C to 22°C. The mean annual day sunshine is approximately 7.5hours (SARI, 2001). The area is characterized by natural vegetation dominated by grasses with very few shrubs.

b) Experimental design

The experiment was a 3 by 4 factorial laid in a split-plot design with three replications. Each replication contained 12 plots with six rows with plot size of 15 m^2. An alley of 1.5m and 1m between separated each replication and plot respectively. P fertilizer levels applied were 24, 48 and 60 kg ha^{-1}P and a control plot, which was considered as the main factor and was assigned to the main plots. P was applied at 30 and 15 days before planting as well as the day of planting. Consequently, the time of application of P was assigned to the sub plots. All plots received 30 kg ha^{-1} nitrogen and potassium respectively. Source of P, K and N used were triple super phosphate, muriate of potash and sulphate of ammonia respectively.

Cowpea variety *Padi-tuya* was planted at 60 and 20 cm between rows and plants respectively, on 16th July 2013 after the land was ploughed, harrowed and ridged. Pre-emergence herbicide cymethox super was applied at 50 ml ha^{-1} immediately after planting. Further weed control was carried out manually as needed.

Spraying was achieved at pre-flowering, flowering, podding and browning of the pods using dimethoatghte to control aphids, trips and maruca and pod sucking insects respectively.

Harvesting was done when pods were fully matured and dried. Hand picking was done several times due to different drying periods. An area of 4m^2 was used for data collection throughout the experiment. After harvesting, the cowpea pod were bagged and threshed. Seeds were separated from the haulm through winnowing and cleaned. Seeds were dried to constant weight before it was bagged and stored.

Field characterization was done to determine the initial soil fertility status. Parameters measured include soil pH, available P was determine using Bray 1 extraction procedure, total nitrogen was estimated using Khedjal method and exchangeable cations were estimated using 1 N ammonium acetate solution.

III. Results and Discussion

a) Experimental site characterization

Rainfall was uniformly distributed throughout the growing season even though it started late. Highest rainfall was obtained in early August and September. Highest rainfall values recorded were 80 and 75 mm respectively (Figure 1).This however facilitated good crop establishment and the crop did not suffer from water stress during the growing season. However, higher intermittent down pours during flower initiation affected flowering as a result of flower abortion.

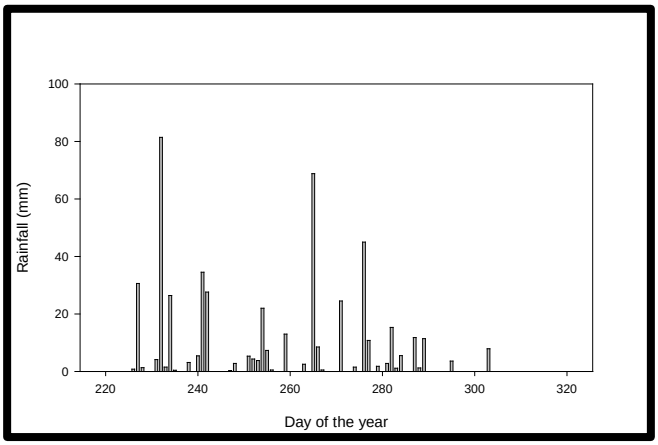

Figure 1 : Rainfall distribution during the growing season

Soil samples were taken from the experimental site and analyzed for pH, organic carbon, available P, total nitrogen and exchangeable cations before P fertilizer application. The results are however, presented in Table 1. Average pH measured was 5.19 indicating that the soil is acidic and this influences P availability due to fixation resulting in the limited P concentration of 2.26 mg/kg soil in the soil solution recorded. Similarly,

the soil was low in organic carbon, total nitrogen with a C:N ratio of 9. The soil was further characterized by low Effective Cation Exchange Capacity (ECEC). Particle size distribution analysis of the soil using the hydrometer method indicated sandy loam.

Table 1 : Physical and chemical properties soil at the experimental site

Parameter	
pH (2:1 water)	5.19
% OC	0.461
Available P.(Bray 1) mg P/kg soil	2.26
Total. Nitrogen (%)	0.048
Al+H (Cmol/kg soil)	0.15
Ca	1.27
Mg	1.16
K	0.32
ECEC	3.87
Sand	64.89
Silt	34.34
Clay	0.77

b) Effect of P application on nodulation, root biomass, stover and grain weight

Six (6) plants were randomly selected from each plot within an area of 4 m^2marked for the data collection. Application of 48 kg ha^{-1} P resulted in higher number of effective nodules. On the contrary, application of 60 kg ha^{-1} P produced lowest number of effective nodules compared to application of 24 kg ha^{-1} P. However, where no P fertilizer was applied produced 90 effective nodules, which is less than where 60 kg ha^{-1} was applied. This however implies that, native rhizobia were affected by P application. Statistical analysis indicated significant difference between effective nodules produced where 48 kg ha^{-1} P was applied and the control. Values recorded were 121.3 and 90.3 with and least significant difference of 28.81 respectively. This indicated that, application of P did not have any effect on number of effective nodules produced. Each plant on the average produced 16, 18, 20 and 16 effective nodules from the control, 24, 48 and 60 kg ha^{-1} P plots respectively. Statistical analysis showed no significant difference ($P<0.05$) among treatments (Figure 2). Furthermore, statistical analysis showed no significant ($P>0.05$) interaction effect on number of effective nodules produced (Figure 4). Values recorded are presented in Table 2.

Table 2 : Interaction effect of time and levels of P application on cowpea nodulation

Fertilizer (kg/ha P)	Day of Application (Before)	A	B	C
0		90.7	95.7	84.7
24		96.3	115.7	114.7
48		95	132	137
60		91.7	87.7	115.3

Lsd = 43.61

Table 3 : Interaction effect of time and levels of P application on cowpea dry root biomass

Fertilizer	Day of Application (Before)	A		C
0		8.46	8	5.71
24		5.04	6.24	8.74
48		6.01	6.68	9.16
60		7.07	6.08	7.68

Lsd = 3.863

Table 4 : Interaction effect of time and levels of P application on cowpea grain yield

Fertilizer	Day of Application (Before)	A	B	C
0		819	993	794
24		826	1171	1066
48		935	847	1156
60		967	941	1096

Lsd = 389.2

Table 5 : Interaction effect of time and levels of P application on cowpea stover

Fertilizer	Day of Application (Before)	A	B	C
0		217.8	217.1	185.9
24		259.7	260.5	221.2
48		285	233	323.6
60		317.7	228.5	250.6

Lsd = 199.2

Similarly, there was no significant effect ($P>0.05$) on dry root biomass. However, contrary to number of effective nodules, application of 24, 48 and 60 kg ha^{-1} P reduced root biomass compared to controlled ploteven though there was no significant difference ($P>0.05$), (Figure 2). Values recorded were 18.47, 16.68, 18.21 and 7.36 kg ha^{-1} for control, 24, 48 and 60 kg ha^{-1} P plots respectively (Figure 2). Statistical analysis indicated a least significant difference value of 3.206.Similar to nodulation, there was no interaction effect on days of P application and P applied on dry root biomass. Statistical analysis showed no significant difference ($P>0.05$) among interactions (Figure 5). Recorded values are presented in Table 3.On the contrary, application of P affected stover yield significantly ($P<0.05$), (Figure 2). Stover weight

recorded were 517, 618, 701 and 664 kg ha^{-1} from control, 24, 48 and 60 kg ha^{-1} P plots respectively (Figure 2) with a least significant value of 118.4 Although, application of P did not affect dry root biomass significantly, it however improved effective root nodulation although it was not significant. However, the improvement in stover weight was as a result of improved N fixation and use efficiency catalyzed by the application of P. However, there was no interaction effect. Values recorded are presented in Table 5. Similarly, grain yield was improved as a result of improved nodulation. Again statistical results indicated no significant difference (P>0.05), (Figure 2). Highest grain yield of 1021 kg ha^{-1}was recorded with application of 24 kg ha^{-1} P. Least significant value recorded was 266.4. Similar to above, statistical analysis did not show any significant difference (P>0.05) on stover yield and grain weight respectively, (Figures 6 and 7). Furthermore, there was also no interaction effect. Again values recorded are presented in Table 4.

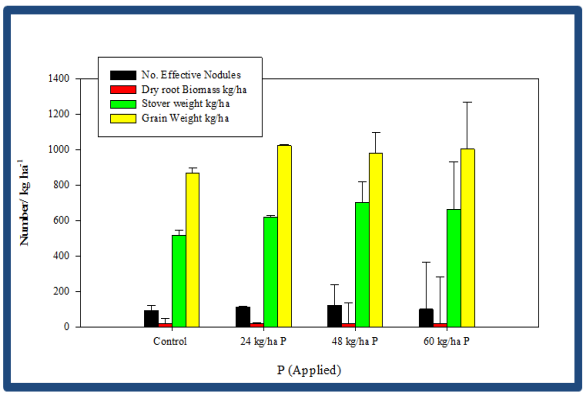

Figure 2 : Effect of P on Nodulation, root biomass, stover and grain weight

c) *Effect of day of P application on nodulation, root biomass, stover and grain yield*

Time of application of P fertilizer did not have significant effect (P>0.05) on effective root nodulation. However, application of P at 15 and 30 days after application improved cowpea nodulation compared to application at planting. Values recorded were 93.4, 107.8 and 112.9 respectively (Figure 3). This confirms the idea that P is slow releasing coupled with it fixation under soil with acidic conditions. Similarly, time of application of P did not influence dry root biomass significantly (P>0.05), (Figure 3). It however improved dry root biomass compared to where no P fertilizer was applied. 19.56, 16.87 and 16.61 kg ha^{-1} dry root biomass were recorded from plots where P was applied at planting, 15 and 30 days after planting, respectively. Furthermore, time of P application did not have significant effect on grain yield as well as stover (Figure 3). Although, time of application of P had no significant (Figure 3) effect on grain yield and stover, it improved yield compared to where no P was applied.

Positive correlation was established between effective nodule numbers and grain yield (Figure 8). Increasing number of nodules resulted in marginal increase in grain yield. R^2 value obtained was 0.581. On the contrary, root biomass did correlate with effective nodule numbers (figure 9). Higher root biomass did not result in nodule numbers.

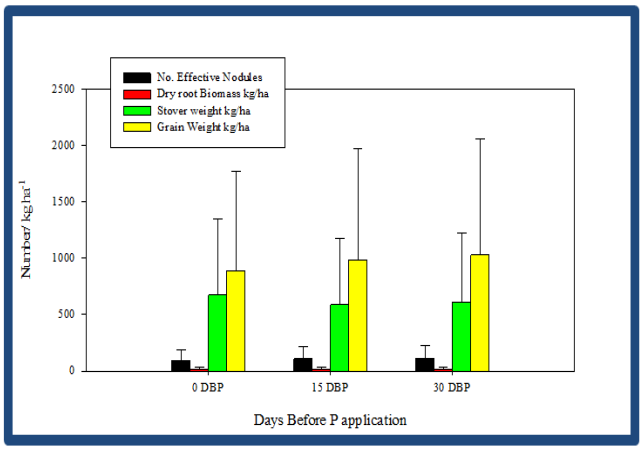

Figure 3 : Effect of day of P application onnodulation, root biomass, stover and grain yield

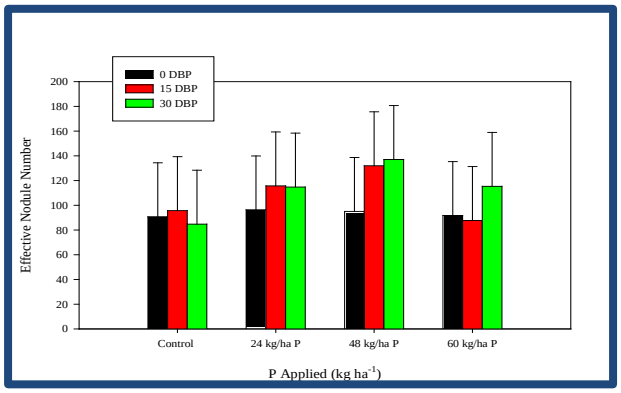

Figure 4 : Interaction effect of day and rate of P application on number of effective nodules

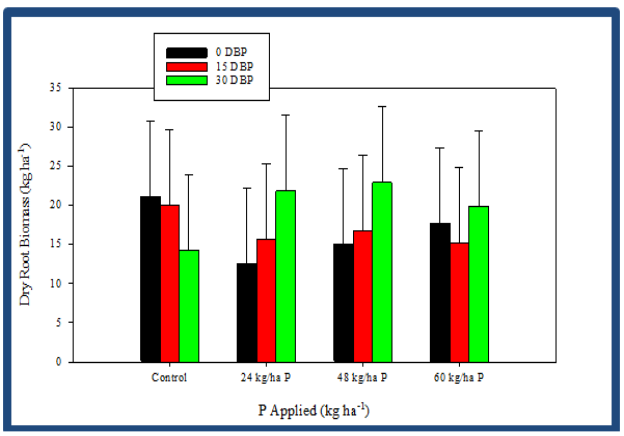

Figure 5 : Interaction effect of day and rate of P application on dry root biomass

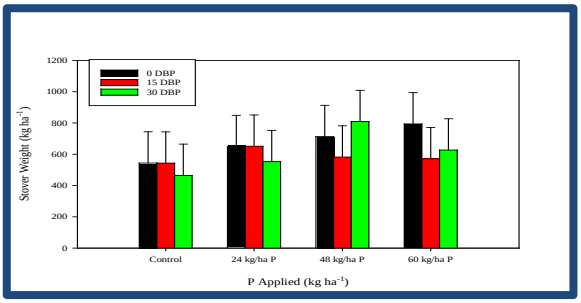

Figure 6 : Interaction effect of day and rate of P application on stover weight

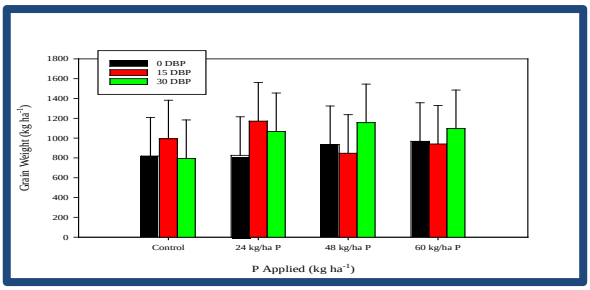

Figure 7 : Interaction effect of day and rate of P application on grain yield

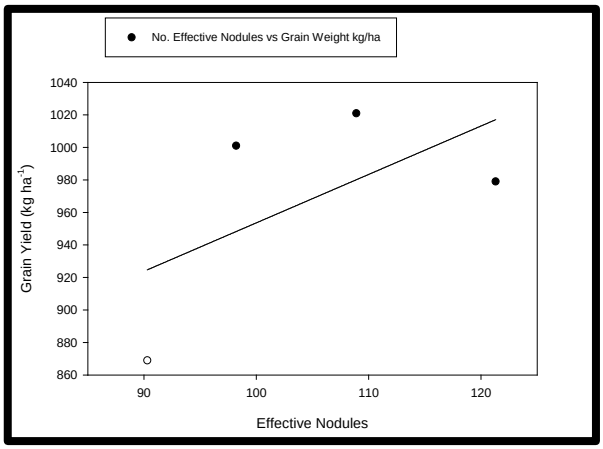

Figure 8 : Correlation between effective nodules and grain yield

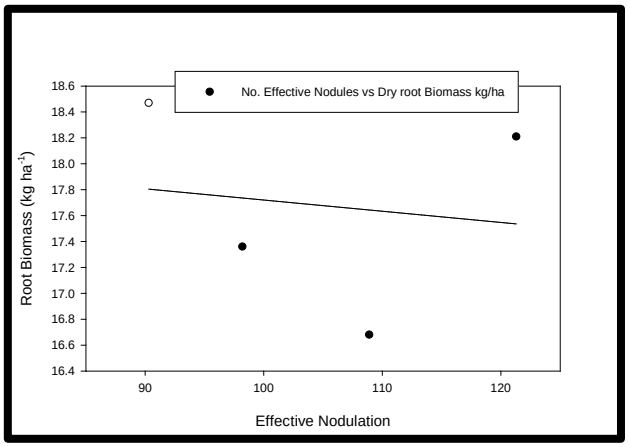

Figure 9 : Correlation between effective nodules and root biomass

IV. Conclusion

Application of 24 kg ha^{-1} P resulted in increased grain yield even thought it was not significantly different (P>0.05) from 48 and 60 kg ha^{-1} P. Similarly, application of 48 kg ha^{-1} P resulted in increased nodulation. Again it was also not significantly different (p>0.05) from 24 and 60 kg ha^{-1}. Days of P application did not affect nodulation, stover and grain yield. However application of P at 30 days before planting increased nodulation and grain yield although it was not significantly different from when P was applied 15 days before planting and also at Planting.

Finally, there was no interaction effect on days of P application and levels of P application on nodulation, stove and grain yield.

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Global Journal of Science Frontier Research: D
Agriculture and Veterinary
Volume 14 Issue 8 Version 1.0 Year 2014
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Changes of Selected Behavior in High Producing Dairy Cows in the Time of Heat

By Berit Füllner & H. Scholz
Anhalt University of Applied Sciences, Germany

Abstract- Successful Artificial Insemination (AI) requires that humans detect the receptive phase of oestrus. There are various ways to detect oestrus in cattle, and behavior plays a key role in each method. The aim of this study was to detect different behaviors using time sampling method in 5-minute intervals and analyzed. Given over a period of 1 week (24 hours) in 34 dairy cows of LLFG Iden recorded these behaviors, in which 11 cows were observed in oestrus. The behavior has been differentiated as follows: feeding, resting and lying including rumination, standing on the walkways or the cubicles and standing rumination, including the duration of milking. The activities presented as percentages of the daily sum shows that the largest changes in the „standing“ of the cows are observed. For standing in the time without oestrus 30 % of the day were used. In oestrus increased it to an average of 47 % of the day. The duration of the externally visible oestrus of cows averaged 944 minutes per day, with a very high variability could be determined.

Keywords: *heat, dairy cows, behavior.*

GJSFR-D Classification : *FOR Code: 079999*

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Berit Füllner [α] & H. Scholz [σ]

Abstract- Successful Artificial Insemination (AI) requires that humans detect the receptive phase of oestrus. There are various ways to detect oestrus in cattle, and behavior plays a key role in each method. The aim of this study was to detect different behaviors using time sampling method in 5-minute intervals and analyzed. Given over a period of 1 week (24 hours) in 34 dairy cows of LLFG Iden recorded these behaviors, in which 11 cows were observed in oestrus. The behavior has been differentiated as follows: feeding, resting and lying including rumination, standing on the walkways or the cubicles and standing rumination, including the duration of milking. The activities presented as percentages of the daily sum shows that the largest changes in the „standing" of the cows are observed. For standing in the time without oestrus 30 % of the day were used. In oestrus increased it to an average of 47 % of the day. The duration of the externally visible oestrus of cows averaged 944 minutes per day, with a very high variability could be determined.

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I. Introduction

Cattle are polyoestrous, meaning they are able to breed year-round. In pasture-based dairy systems and in beef cow calf operations, breeding is typically managed such that calves are born when grass growth begins. On barn-based dairy farms, cows calve year-round in order to produce a steady supply of milk for human consumption. The duration of the receptive phases of oestrus, where the cow will stand to be mounted, lasts approximately 12 h. Females 'on heat' will stand to be mounted by bulls, but cattle also show female-female mounting. In systems with bulls, female-female mounting can attract the attention of males. In farming systems without males (as is the case in many dairy farms, and in some beef operations), artificial insemination (AI) is used. Successful AI requires that humans detect the receptive phase of oestrus. There are various ways to detect oestrus in cattle, and behavior plays a key role in each method. Cows may be observed for femalefemale mounting to detect oestrus. Cows also show restless behavior during the receptive phase of oestrus. Automated measures of movement, such as a sensor on a collar or a pedometer attached to the leg, are used to record behavior and detect the increase in activity associated with oestrus.

II. Materials and Methods

The aim of this study was to detect different behaviors using time sampling method in 5- minute intervals and analyzed. Given over a period of 2 week (24 hours) in 34 dairy cows of LLFG Iden recorded these behaviors, in which 11 cows were observed in oestrus. The cows of the group of fresh cows reported a maximum of 60 days post partum, while the high performance of the cows had at least 60 days post partum (Table 1). The selection of the animals were observed for the theoretical oestrus in the corresponding periods of the investigation. The cows in the Fresh group were no inseminating in the time of heat because the time for the first insemination is day 60 of lactation.

The behavior has been differentiated as follows: feeding, resting and lying including rumination, standing on the walkways or the cubicles and standing rumination, including the duration of milking. Symptoms in the cows with the number of signs of heat and the number of stiffness jump tolerations acquired. From these records were calculated the length of oestrus. Each day were taken, milk sample from each cow to analyzed progesterone and estradiol.

Table 1 : Animal number and milk amount of the investigated milk cows

	Sum	Fresh group	High-capacity group
Number of animals (n)	34	18	16
Animals in oestrus (n)	11	6	5
Milk yield (kg)	-	$47^{a} \pm 8$	$42^{b} \pm 9$

III. Results and Discussion

The feed intake (kg DM / d) and the feeding behavior of the animals were not affected by the occurrence of oestrus significantly. The cows took in the period without oestrus for an average amount of feed of 25,8 ± 2,6 kg DM per day, which decreased during the oestrus to 25,1 ± 2,3 kg DM per day. In the time without heat the cows needs 217 ± 46 minutes for the feed intake and in the time with heat can we found a

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significant reduction to 171 ± 32 minutes per day. The correlations between time for feed intake and the daily dry matter intake of cows was r = -0,144 (p=0,203).

However significant differences between means were in the time period of the food intake, for the rumination, for lying and standing in the group to be determined (Table 2). The mean duration of resting of cows decreased by 189 minutes (more than 3 hours each day) with a simultaneous reduction in the length of time for rumination by 88 minutes per day. Comparable results for the changes in behavior by cows in Heat can be found by ROLOFF (2013), REITH and HOY (2012) and FÜLLNER (2012). The normal behavior (without oestrus) in the investigation shows an average of 636 minutes for resting and is on a comparable level then other results (SCHNEIDER et al., 2013).

Table 2 : Duration of the activities by milk cows with and without oestrus sign

	without oestrus	with oestrus
Feeding (min)	$217^{a} \pm 46$	$171^{b} \pm 32$
Resting (min)	$636^{a} \pm 107$	$447^{b} \pm 102$
Standing (min)	$430^{a} \pm 120$	$680^{b} \pm 129$
Rumination (min)	$532^{a} \pm 60$	$444^{b} \pm 78$

The activities presented as percentages of the daily sum shows that the largest changes in the „standing“ of the cows are observed (figure 1). For standing in the time without oestrus 30 % of the day was used. In oestrus, this figure increased to an average of 47 % of the day. For this, the lying by 13 % and the feeding period will be reduced by 4 % on average, while milking the animals themselves cannot be significantly affected. Between the both groups of fresh cows and high-capacity cows were found differences in the reduction or increasing of the selected behavior while the changes is in the high yielding dairy cows more pronounced. This data are not shown in the paper.

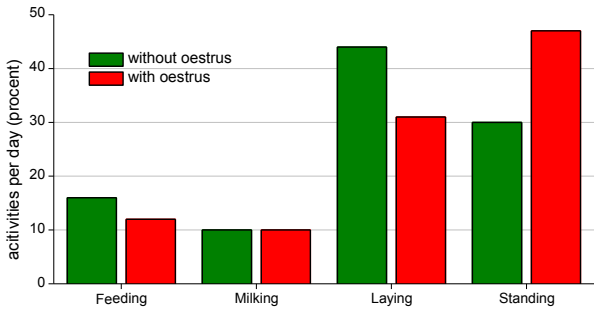

Figure 1 : Changes due to the oestrus

The duration of the externally visible oestrus of cows in the investigation averaged 944 minutes per day, with a very high variability could be determined (Table 3). It was found, however, that the number of stiffness jump can vary from 4 to 40 per day. The number of tolerations reported a variation of 5 to 26 days, depending on tolerance status (Table 3). Variation in estrus found WHITE et al. (2002) in beef cattle and other parameters that influence the heat detection gives RAO et al. (2013).

Significant correlations were not found between the oestrus symptoms and the daily milk yield (Table 4). It was found, however, that a significant positive correlation between the duration of both the oestrus and the number of passed tolerations stiffness jump per day. RAO et al. (2013) found that the cows are stands immobile for mounting on her, indicates the she is definitely in heat (NEGUSSI et al., 2002; ROELOFS et al., 2005).

Table 3 : Duration of oestrus, number of stiffness jump and toleration

	Mean ± s	Min - Max
Duration of oestrus (min)	944 ± 273	570 – 1.396
Number of stiffness jump (n)	22 ± 12	4 – 40
Number of toleration (n)	15 ± 7	5 – 26

Table 4 : Correlations between the characteristics

	Mkg	Heat - time	Jumping - high	Tolerations
Milk (kg)	1,000	-0,162	-0,001	-0,461
Heat - time (min)		1,000	0,481	0,590*
Jumping - high (n)			1,000	0,696**
Tolerations (n)				1,000

Progesterone (P4) was significant reduced in the time of heat from average 5.9 pg per ml to 1.1 pg per ml. Comparable results are in the investigations of RAO et al. (2013) and SAMAD et al. (2004). The content of estradiol in milk increased in the time of heat (Figure 2) and was also found by DOMENECH et al. (2011).

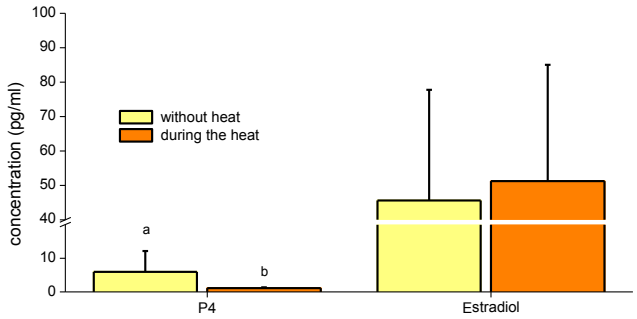

Figure 2 : progesterone (P4) and estradiol in milk of cows

IV. Conclusions

Behavior of cows in heat is significant different to the normally behavior. There were found a significant difference among the animals with extremely variations. The behavior „standing“ had shown the highest deviation between normally behavior and the behavior in heat. But it is remarkable that we cannot predict the oestrus season.

The development of the visible oestrus signs (in the case landings bounces and tolerances) is defeated by a very high animal-individual development. Nevertheless, the milk amount day showed no significant influence.

For continuing statements the milk tests are to be analysed and check for their practical conversion to. The increase of the data material shows another point of view.

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Global Journal of Science Frontier Research: D
Agriculture and Veterinary
Volume 14 Issue 8 Version 1.0 Year 2014
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Isolation and Molecular Characterization of Novel IBV Isolates from Broiler Chicken Farms in Egypt

By Ashraf M. Awad, Mahmoud E. Sediek & Mohamed E. El-Yamany

Alexandria University, Egypt

Abstract- A survey of infectious bronchitis virus (IBV) genotypes in 25 commercial broiler flocks of various ages (20-35 days) raised in Al-Behera and Kafr-Elsheikh governorates and suffering from respiratory symptoms and pathological changes in kidney associated with high mortality rate. All flocks were vaccinated against IB disease. Tissue samples (trachea, lung and kidney) were collected aseptically from these flocks. Then virus propagation was performed in embryonated SPF chicken eggs via allantoic sac inoculation. Results of virus isolation trails from the collected organs revealed 15 IBV isolates (60%) out of 25 flocks as judged by antigen detection in CAMs by the AGPT against reference IBV Beaudette antiserum after 2-5 chicken embryo serial passages. However, three of 15 IBV isolates were also positive by the slide HA test. Moreover, 5 flocks gave positive slide HA test and negative by the AGPT. The other 5 flocks gave negative slide HA test and AGPT. Then selected ten IBV field isolated strains in this study were characterized by RT-PCR (all of ten selected isolates are positive for S1 gene), and then sequence analysis of partial S1 spike glycoprotein gene of seven IBV field isolates in this study (11, 15, 21, 13, 19, 22, 24) were made. The seven IBV field isolates showed 97% to 98.3 % and 96.7% to 98.3% nucleotide sequence identity to IBV-CU-2-SP1 and Eg/12120s/2012 strain (variant 2 like strain), respectively.

GJSFR-D Classification : FOR Code: 079999

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The presence of these strains may account for vaccination failure against IBV, since all IBV isolates were from vaccinated chickens. This study demonstrates a constant evolution of IBV in Egypt that necessitates continuous monitoring to control the spread of infections, and the development and use of vaccines based on indigenous viruses.

I. Introduction

Infectious bronchitis (IB) disease is an acute, highly contagious and infectious disease of poultry in worldwide, possess a major threat to the poultry industry and was first reported in North Dakota, USA, as a novel respiratory disease by Schalk and Hawn in 1931. The disease is characterized by respiratory signs including (sneezing, cough, tracheal rales, gasping and nasal discharge), reduction the growth rate of broilers, nephropathogenic strains causing acute nephritis, urolithiasis and may be associated by high mortality (The Merck Veterinary Manual, 2006).

IBV belongs to group III of the genus coronavirus of the coronaviridae family. It is an enveloped, non-segmented, positive sense single stranded RNA virus (Cavanagh, 2003). The nucleocapsid (N) protein is one of the major structural proteins of the virion, and since the N gene is highly conserved even among IBV isolates of different serotypes, it is often the target for nucleic acid based virus identification in diagnostic laboratories. The spike (S) glycoprotein is another major structural protein of the virion, and it is post-translationally cleaved into S1 globular and S2 stalk polypeptides (Cavanagh, 2007).While the S2 subunit is conserved, the S1 subunit generally varies by up to 23% at the amino acid level among viruses of the same serotype (Cavanagh et al., 2005). Three hypervariable regions (HVRs) have been identified in the S1 subunit (Moore et al., 1997). Diversity in S1 probably results from mutation, insertions, deletions, or RNA recombination of the S1 genes (Jackwood et al., 2012).

Detection of IBV infection in poultry flocks, as well as differentiation from other upper respiratory diseases, is amajor challenge and necessitates the use of appropriate diagnostic methods. Virus isolation in specific pathogen free (SPF) eggs, the reference standard, is time consuming and may require more than one passage before obtaining a result (Sediek, 2005). Reverse transcriptase-polymerase chain reaction (RT-PCR) assays are rapid, specific, and accurate, and when targeting the viral S1 gene, the amplification products can be used for further classification of the virus (Gelb et al., 2005 and Lee et al., 2000). Sequence analysis of the S1 portion of the genome of hundreds of isolates belonging to the many different serotypes of IBV worldwide has been carried out to study and determine phylogeny, evolution, antigenic, and genetic relatedness and virulence of this important poultry pathogen (Jackwood et al., 2007; Cavanagh, 2007).

IBV strains related to the Massachusetts, D3128, D274, D-08880 and 4/91 genotypes have been detected at different poultry farms in Egypt (Abdel-

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Moneim et al., 2006; Sultan et al. 2004; Sediek, 2010). The Egyptian variants which were closely related to the Israeli variant strain were isolated from different poultry farms (Abdel-Moneim et al. 2002; Sediek, 2010). The Egyptian variant IBV-CU-2-SP1and Eg/12120s/2012 were isolated by Afifi et al. (2013) and Arafa et al. (2013), respectively.

Control of the disease is mostly through the use of live attenuated vaccines, but antigentically different serotypes and newly emerged variants from field chicken flocks sometimes cause vaccine breaks. The generation of genetic variants is thought to be resulted from few amino acid changes in the spike (S) glycoprotein of IBV (Adzhar et al., 1997).

The aim of this study is to isolate and molecular characterization of novel IBV isolates from broiler chicken farms in Egypt. This is important for implementation of control measures especially for the future vaccination strategies.

II. Materials and Methods

a) Samples

Tissue samples (trachea, lung and kidney) were collected aseptically from 25 suspicious IBV chicken flocks (4-5 tissue samples per flock) of various ages (20-35 days) raised in Al-Behera and Kafr-Elsheikh governorates and suffering from respiratory symptoms and pathological changes in kidney associated with high mortality rate. All flocks were vaccinated against IB disease.

b) Virus propagation and isolation

Virus propagation was performed in 9–11 day-old embryonated SPF chicken eggs (Kom Oshim, Fayoum), Procedures were performed according to ***OIE terrestrial manual (2008)***. The allantoic fluid and CAM were harvested 48 h post-inoculation (PI). Three to five serial blind passages were performed in order to induce lesions typical of IBV in the chicken embryo.

c) Slide haemagglutination (HA) test

Slide HA was carried according to (Beard, 1980) on harvested AF collected from eggs inoculated at each serial passage with suspected field samples to rule out pathogens with HA activity. This test was done by placing one drop of 10% washed chicken RBCs suspension in sterile saline (0.8% sodium chloride) onto a clean microscopic slide and thoroughly mixed with one drop of the harvested AF. The result was recorded within one minute.

d) Agar Gel Precipitin Test

The test was carried out on a homogenate of the CAM of infected chicken embryos. The test was performed as described by (Lohr, 1980, 1981). Six peripheral wells surrounding a central well in a hexagonal form were made in the agar medium by a special appliance, the well size was 4 mm in diameter, and the distance between the central well and the evenly spaced peripheral wells was 4 mm. 30 µl of IBV Beaudette reference antiserum was placed into the central well, while 30 µl of antigens to be tested for precipitinogen were placed into the peripheral wells. The last peripheral two wells (NO. 6,5) in each slide served for positive control antigen (Beaudette antigen) and negative control (PBS) respectively. Readings were recorded after 24 h by observing the plate against an illuminated indirect light source with a dark background. Final readings were recorded after 48 h. An opaque precipitin line between the antigen- antibody wells was considered as a positive result.

e) Viral RNA Extraction

Extraction of viral RNA was carried out on allantoic fluids of selected ten IBV isolates according to the instructions for the QIAamp Viral RNA Mini Kit (Qiagen, Germany)

f) RT-PCR amplification of S1 gene

i. *-Oligonucleotide primers*

The RT-PCR was done for these selected ten IBV isolates using oligonucleotide primers encoding for S1 gene (Adzhar et al., 1997).

Table (1) : Primers for S1 gene amplification in conventional PCR

Primer ID	Nucleotide Sequence	Length	Position in S1 gene	Reference
IB-F	5'CACTGGTAATTTTTCAGATGG-3'	21 nt	729-749	Adzhar et al., 1997
IB-R	5' –CAGATTGCTTACAACCACC-3'	19 nt	1093-1111	Adzhar et al., 1997

ii. - *Qiagen one step RT – PCR kit:* was supplied by Qiagen, Germany (Cat. NO. 210212).

Thermal profile used in one step RT-PCR.

Stage	Temperature	Time	Cycles
Reverse transcription	50 oC	30 min	1
Primary denaturation	95 oC	15 min	1

Amplification a) Secondary denaturation	95 oC	30 sec	40
b) Annealing	56 oC	45 sec	
c) Extension	72 oC	2 min	
Final Extension	72 oC	10 min	1
Storage		4 oC	

After the end of PCR run, Amplification products run in agar gel 1.5% which give specific band at 500 pb in weight measured against 100 pb ladder (Qiagen – Germany).

g) Sequencing of the S1gene

Seven purified PCR products were sent to NLQP, Animal Health Research institute, Egypt for sequencing.

h) Genetic analysis

A BLAST analysis of raw sequence data was initially performed to exclude sequence redundancy with the existing Gen Bank entries.

i. ***For sequence analysis:*** Bioedit software was used for analysis for the sequence of S1 gene of the isolates of this study.
ii. ***For Phylogenetic analysis:*** Software *MEGA* version 5 with a bootstrap resampling method (500 bootstraps) to make alignment for of S1 sequence and make a phylogenetic analysis for these isolates.
iii. ***Calculate the Sequence Distances:*** to display the divergence and identity percent values of each sequence pair in the alignment. Divergence is calculated by comparing sequence pairs in relation to the phylogeny reconstructed using MegAlign software. Percent Identity compares sequences directly, without accounting for phylogenetic relationships.

III. Results

a) Clinical signs and gross pathology

The clinical examination of investigated flocks revealed general signs of illness, respiratory signs and diarrhea in some flocks. The respiratory signs ranged from mild to severe and included conjunctivitis associated with lacrimation, gasping, sneezing, rales and coughing. Mortality rates during three days before flock visits (0.3-3.5 %). The examined affected chickens flocks aged 20- 35 days.

Gross pathological examination generally revealed mild to severe congestion of respiratory mucosa of trachea and small areas of pneumonia. Some flocks frequently showed mucous or caseated material in trachea and bronchi, and other showed fibrinous pericarditis, perihepatitis and airsaculitis. Pale or congested and enlarged kidneys with prominent tubules with urates deposition and slight to moderate distention of the ureters with urates were seen in some flocks.

b) Virus isolation and identification

Results of virus isolation trails from the collected organs revealed 15 IBV isolates (60%) out of 25 flocks as judged by antigen detection in CAMs by the AGPT against reference IBV Beaudette antiserum after 2-5 chicken embryo serial passages. However, three of 15 IBV isolates were also positive by the slide HA test and these 3 isolates were exclude from further identification. Moreover, 5 flocks gave positive slide HA test and negative by the AGPT against reference IBV Beaudette antiserum after 5 chicken embryo serial passages. The other 5 flocks gave negative slide HA test and AGPT against reference IBV Beaudette antiserum after 5 chicken embryo serial passages.

The 15 IBV isolates caused variably low embryonic death and or curling and dwarfing after 3-5 serial passages.

c) Results of conventional RT-PCR for S1 gene

All of selected ten isolates are positive for S1 gene (Fig1).

d) Results of sequence and Phylogenetic analysis

A phylogenetic tree was constructed from the nucleotide sequences of the S1 glycoprotein gene showing that the seven selected Egyptian IBV isolates (11, 13, 15, 19, 21, 22 and 24) present in the same group with IBV-CU-2-SP1, Eg/12120s/2012-SP1, Eg/12197B/2012-SP1 and Egypt/01-13. D274 strain is the nearest vaccinal strain present in Egypt to seven isolate (Fig 2).

e) Results of percent identity and divergence of seven Egyptian IBV isolates in this study in comparison to other Egyptian strains, reference strains and vaccinal strains present in Egypt

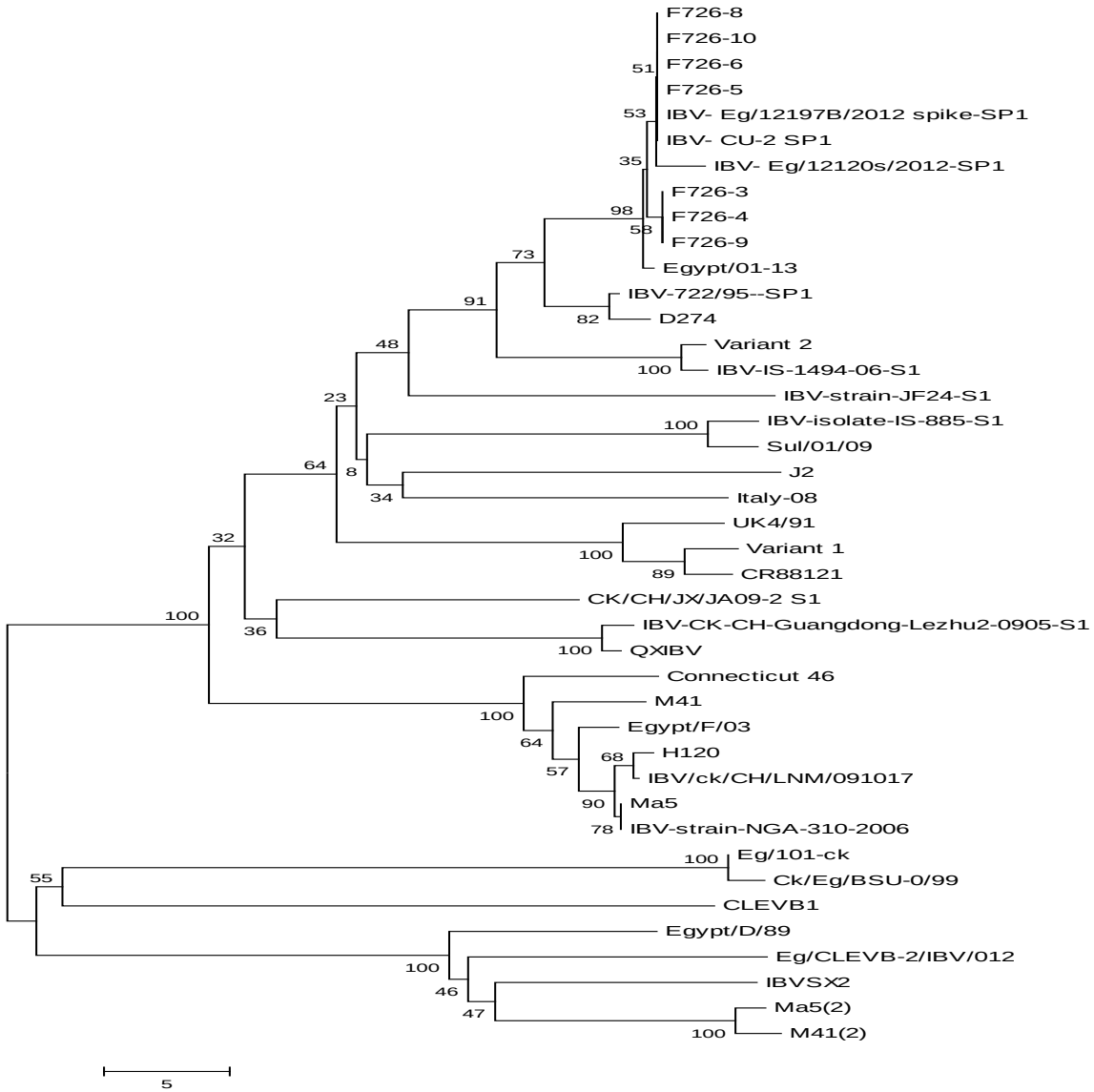
The seven IBV field isolates showed 97% to 98.3 % and 96.7% to 98.3% nucleotide sequence identity to IBV-CU-2-SP1 and Eg/12120s/2012 strain (variant 2 like strain), respectively. Nucleotide identity between these seven IBV isolates ranged from 97.7% to 99% and between these isolates and vaccinal strain used in Egypt (M41, H120, Ma5, 4/91, CR88 and D274) ranged from 64.7% to 65.7%, 65.3% to 66.3%, 65.7% to 66.7%, 67.3% to 68.3%, 68.6% to 69.6% and 84.2% to 84.8%, respectively table (2).

IV. Discussion

The present study is a trail to investigate the current status of IBV infection among broiler chickens in

Al-Behera and Kafr-Elsheikh governorates. For this purpose, IBV isolation trails were accomplished by investigation of 25 broiler flocks, with history of vaccination against IB disease, suffering from general signs of illness, respiratory signs and diarrhea in some flocks. The respiratory signs ranged from mild to severe and included conjunctivitis associated with lacrimation, gasping, sneezing, rales and coughing. Mortality rates during three days before flock visits (0.3-3.5 %). The examined affected chickens flocks aged 20- 35 days. Gross pathological examination generally revealed mild to severe congestion of respiratory mucosa of trachea and small areas of pneumonia. Some flocks frequently showed mucous or caseated material in trachea and bronchi, and other showed fibrinous pericarditis, perihepatitis and airsaculitis. Pale or congested and enlarged kidneys with prominent tubules with urates deposition and slight to moderate distention of the ureters with urates were seen in some flocks. The clinical signs and gross lesions were suggestive of infectious bronchitis and were similar to those described by many authors (abdel- Moneim et al., 2002 and Sediek, 2005).

Trachea, lung and kidney were processed for virus isolation in SPF embryonated eggs. Five serial blind passages were performed to consider the sample to be IBV negative. Results of virus isolation trails from the collected organs revealed 15 field IBV isolates (60%) out of 25 flocks as judged by antigen detection in CAMs by the AGPT against reference IBV Beaudette antiserum after 2- 5 chicken embryo serial passages. However, three of 15 IBV isolates were also positive by the slide HA test and these 3 isolates were exclude from further identification. Moreover, 5 flocks gave positive slide HA test and negative by the AGPT against reference IBV Beaudette antiserum after 5 chicken embryo serial passages. The other 5 flocks gave negative slide HA test and AGPT against reference IBV Beaudette antiserum after 5 chicken embryo serial passages. The 15 IBV isolates caused variably low embryonic death and or curling and dwarfing after 3-5 serial passages, which were in agreement with those described by other researches (wang et al., 1997 and sediek, 2005).



F726-3= isolate No 11 F726-4= isolate No 15 F726-5= isolate No 21 F726-6= isolate No 13
F726-8= isolate No 19 F726-9= isolate No 22 F726-10= isolate No24

Figure (2) : Phylogenetic tree based on a partial sequence of the S1 gene, showing the relationship between the seven Egyptian IBV isolates in this study, vaccinal strain present in Egypt and other reference IBV world circulated strains.

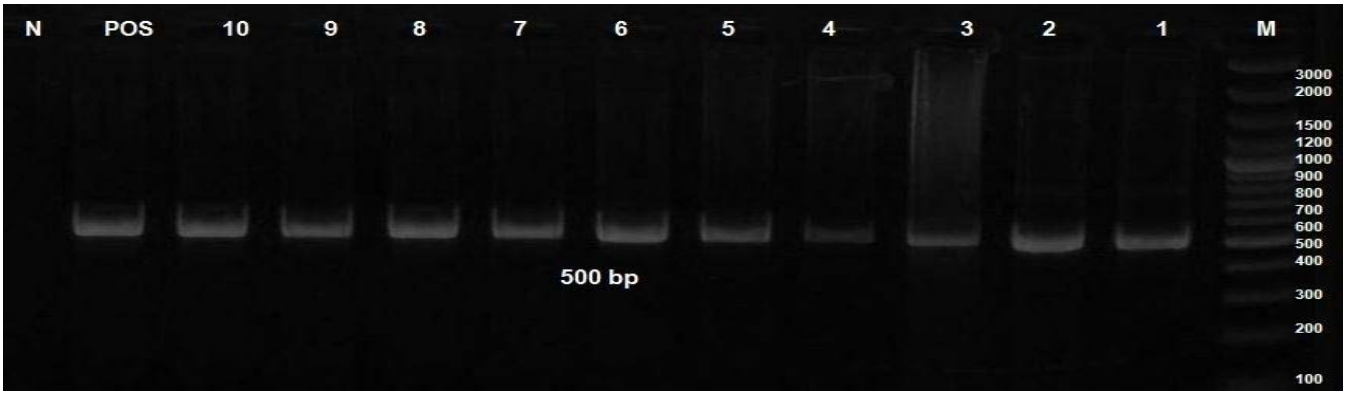


Figure 1 : Agarose gel electrophoresis of the 500 bp RT-PCR product of the selected ten isolates: lane 1, 2, 3, 4, 5, 6, 7, 8, 9, 10: the selected samples; lane M: DNA marker; Lane pos: positive control; lane N: negative control

Table 1 : Nucleotide identity percentage between the seven selected IBV isolates in this study and other reference IBV strains from gene bank

Isolates	Gene bank Accession No	Country	Year	Iden with 11	Iden with 15	Iden with 21	Iden with 13	Iden with 19	Iden with 22	Iden with 24
IBV CU-2 S1	KC985213.1	Egypt	2012	97%	97%	97%	97%	97%	97%	97%
Eg/12197B/2012 SP1	KC533683.1	Egypt	2012	97%	97%	97%	97%	97%	97%	97%
Eg/12120s/2012 SP1	KC533684.1	Egypt	2012	96%	96%	96%	96%	96%	96%	96%
IBV/Egypt/01-13/VIR9715/2012	KC527831.1	Egypt	2012	96%	96%	96%	96%	96%	96%	96%
IBV-11/99 S1	DQ449065.1	Russia	2006	91%	90%	90%	90%	90%	90%	90%
IBV-10/01 S1	DQ449064.1	Russia	2006	90%	90%	90%	90%	90%	90%	90%
NGA/295/2006.	FN182276.1	Nigeria	2006	90%	89%	89%	89%	89%	89%	89%
722/95	AF420320.1	Sweden	2001	90%	89%	89%	89%	89%	89%	89%
UK/123/82	X58067.1	UK	1991	90%	89%	89%	89%	89%	89%	89%
D3896	X52084.1	Netherlands	1990	90%	89%	89%	89%	89%	89%	89%
Egypt/D/89"	DQ487086.1	Egypt	1989	89%	89%	89%	89%	89%	89%	89%
D274"	X15832.1	Netherlands	1989	89%	90%	90%	90%	90%	90%	90%
RF/06/2008	HQ840489.1	Russia	2008	92%	92%	92%	92%	92%	92%	92%
IBV-05/00"	DQ449063.1	Russia	2006	89%	89%	89%	89%	89%	89%	89%
D207	M21969.1		1989	89%	89%	89%	89%	89%	89%	89%
RF/25/2009	HQ840496.1	Russia	2009	92%	92%	92%	92%	92%	92%	92%
RF/12/2008"	HQ840491.1	Russia	2008	92%	92%	92%	92%	92%	92%	92%
RF/11/2007	HQ840488.1	Russia	2007	92%	92%	92%	92%	92%	92%	92%
IS/378/97	AY789956.1	Israel	1997	84%	84%	84%	84%	84%	84%	84%
CU-4	KC985212.1	Egypt	2012	83%	83%	83%	83%	83%	83%	83%
variant 2"	AF093796.1	Israel	1998	83%	83%	83%	83%	83%	83%	83%
IS/572/98	AY789961.1	Israel	1998	83%	83%	83%	83%	83%	83%	83%
Eg/CLEVB-1/IBV/012	JX173489.1	Egypt	2012	83%	83%	83%	83%	83%	83%	83%
IS/1494/06"	EU780077.2	Israel	2006	83%	83%	83%	83%	83%	83%	83%
IS/223/96"	AY789950.1	Israel	1996	83%	83%	83%	83%	83%	83%	83%
IB VAR2-06	JX027070.1	Israel	2006	83%	83%	83%	83%	83%	83%	83%
Mans-1	KF856872.1	Egypt	2012	83%	83%	83%	83%	83%	83%	83%
Eg/CLEVB-2/IBV/012	JX173488.1	Egypt	2012	82%	82%	82%	82%	82%	82%	82%

Eg/1265B/2012	KC533682.1	Egypt	2012	82%	82%	82%	82%	82%	82%	82%
NGA/293/2006	FN182275.1	Nigeria	2006	84%	84%	84%	84%	84%	84%	84%
RF/01/2010	HQ840511.2	Russia	2010	81%	81%	81%	81%	81%	81%	81%
3374/05	DQ402364.1	Taiwan	2006	76%	77%	76%	77%	77%	76%	76%
JP/Iwate-1/2011	AB858434.1	Japan	2011	77%	77%	77%	77%	77%	77%	77%
1494/06	JX104082.2	Turkey	2011	90%	90%	90%	90%	90%	90%	90%
Md27	FJ008695.1	USA	1976	77%	78%	78%	78%	78%	77%	78%
53XJ-99II	KC577391.1	China	1999	75%	76%	76%	76%	76%	76%	76%
13-078037-0002	KJ196257.1	Canada	2011	80%	80%	80%	80%	80%	80%	80%

Iden= Identity percent

Percent Identity

Divergence

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
	78.9	77.9	78.2	80.5	95.0	77.1	74.9	75.2	74.9	74.1	76.3	86.2	74.4	77.9	77.6	77.6	78.2	77.6	78.2	77.9	1
21.6		97.7	98.0	89.8	79.9	71.0	68.6	66.0	66.3	65.0	70.0	85.1	67.3	97.4	97.4	97.0	98.3	97.7	97.4	97.7	2
23.0	2.4		98.7	89.8	78.9	70.3	68.0	66.0	66.3	64.4	69.3	84.5	67.0	97.7	96.7	98.0	97.7	97.4	98.3	98.3	3
22.6	2.1	1.4		89.8	79.2	71.0	68.6	66.0	66.3	65.0	70.0	84.8	67.3	97.7	97.4	98.0	98.3	97.4	98.3	99.0	4
19.5	11.4	11.4	11.4		80.2	70.3	68.0	68.6	69.0	68.0	70.0	94.4	67.3	90.1	88.8	89.4	89.4	89.1	89.8	89.4	5
5.1	20.3	21.6	21.2	19.9		76.3	74.7	73.8	73.6	72.7	75.8	85.7	73.6	78.9	78.5	78.5	79.2	78.5	79.2	78.9	6
27.4	33.5	34.6	33.5	34.7	28.5		92.8	70.2	70.5	70.0	96.1	75.8	71.1	70.0	69.6	69.6	70.6	70.0	70.3	70.3	7
30.7	37.3	38.4	37.2	38.6	31.0	7.6		71.3	71.6	71.1	93.9	72.7	73.6	67.7	67.3	67.3	68.3	67.7	68.0	68.0	8
30.3	42.0	42.0	41.9	37.3	32.4	38.4	36.6		99.2	95.9	71.1	72.7	69.4	65.7	65.3	65.3	66.3	65.7	66.0	66.0	9
30.7	41.4	41.4	41.3	36.7	32.9	38.0	36.1	0.8		96.4	71.3	73.0	69.7	66.0	65.7	65.7	66.7	66.0	66.3	66.3	10
32.0	43.8	45.0	43.8	38.4	34.2	38.9	37.1	4.3	3.7		70.8	72.7	68.9	65.0	65.0	65.0	65.3	64.7	65.7	65.0	11
28.8	35.3	36.4	35.3	35.3	29.4	4.0	6.3	37.1	36.7	37.6		74.9	70.8	69.0	68.6	68.6	69.6	69.0	69.3	69.3	12
15.4	13.4	14.2	13.8	2.8	16.1	29.6	34.4	34.3	33.9	34.3	30.9		71.3	84.8	84.2	84.2	84.8	84.2	84.8	84.5	13
31.8	39.5	40.0	39.5	39.6	33.1	36.8	32.8	39.4	38.9	40.4	37.2	36.6		66.3	66.7	67.0	67.0	67.0	66.7	66.7	14
23.1	2.8	2.4	2.4	11.0	21.7	35.2	39.0	42.6	42.0	43.8	37.0	13.8	41.3		98.7	98.7	98.0	98.3	99.0	98.7	15
23.5	2.8	3.5	2.8	12.6	22.1	35.8	39.6	43.3	42.7	43.9	37.7	14.6	40.7	1.4		98.7	98.3	98.7	98.3	98.3	16
23.5	3.1	2.1	2.1	11.8	22.1	35.8	39.6	43.3	42.7	43.9	37.7	14.6	40.1	1.4	1.4		97.7	98.7	99.0	99.0	17
22.6	1.7	2.4	1.7	11.8	21.2	34.1	37.8	41.4	40.8	43.2	35.9	13.8	40.1	2.1	1.7	2.4		99.0	98.0	98.7	18
23.5	2.4	2.8	2.8	12.2	22.1	35.2	39.0	42.7	42.1	44.6	37.1	14.6	40.1	1.7	1.4	1.4	1.0		97.7	98.3	19
22.6	2.8	1.7	1.7	11.4	21.2	34.6	38.4	42.0	41.4	42.6	36.5	13.8	40.7	1.0	1.7	1.0	2.1	2.4		98.7	20
23.0	2.4	1.7	1.0	11.8	21.6	34.6	38.4	42.0	41.4	43.9	36.5	14.2	40.7	1.4	1.7	1.0	1.4	1.7	1.4		21
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	

Variant-2
IBV- CU-2 (SP1)
IBV- Eg/12120s/2012 (SP1)
IBV- Eg/12197B/2012(SP1)
IBV-722/95-SP1
IBV-IS-1494-06-S1
Variant-1
4/91
H120
Ma5
M41
CR88121
D274
SUL/01/09
F726-3
F726-4
F726-5
F726-6
F726-8
F726-9
F726-10

F726-3= isolate No 11 F726-4= isolate No 15 F726-5= isolate No 21 F726-6= isolate No 13

F726-8= isolate No 19 F726-9= isolate No 22 F726-10= isolate No24

Table (2) : Nucleotide identities and divergences of the S1 partial sequence of seven Egyptian IBV isolated strains in this study with other Egyptian strains, reference strains and vaccinal strains present in Egypt.

Embryo mortalities increased with further passage, these findings may suggest that the IBV isolate are field viruses since, they were not embryo adapted **(Difabio et al., 2000)**.

One of the major problems with IBV is the frequent emergence of new variants (**Abdel-Moneim et al. 2002; Sediek, 2010**). The detection and identification of these new variants is crucial to disease control ***(Nakamura et al., 1996)***. So in this study set of primers mentioned by **(Adzhar et al., 1997)** are used for amplification of S1 gene in AF of ten selected isolates of study, all of selected isolates are positive for S1 gene, which were in agreement with **Sarah, 2014**. And then seven selected purified PCR products (11, 15, 21, 13, 19, 22, 24) were sent to NLQP, Animal Health Research institute, Egypt for sequencing.

Phylogenetic analysis revealed that the sequences of seven selected Egyptian IBV field isolates in this study (11, 15, 21, 13, 19, 22, 24) found in the same group with IBV-CU-2-SP1 **(Afifi et al., 2013)**, Eg/12120s/2012-SP1 **(Arafa et al., 2013)**, Egypt/01-13**(Valastro et al., 2013)** and Eg/12197B/2012-SP1**(Arafa et al., 2013)**. The Phylogenetic analysis indicated that the seven selected Egyptian isolates are far from vaccine strains and D274 vaccinal strain is the nearest vaccinal strain present in Egypt to these seven isolates, this agree with **Ali, 2013**.

Nucleotides identity between the seven selected Egyptian IBV field isolates in this study (11, 15, 21, 13, 19, 22, 24) was ranged from 97.7% to 99%, between these isolates and vaccinal strain used in Egypt (M41, H120, Ma5, 4/91, CR88 and D274) ranged from 64.7%

to 65.7%, 65.3% to 66.3%, 65.7% to 66.7%, 67.3% to 68.3%, 68.6% to 69.6% and 84.2% to 84.8%, respectively. And between these isolates and IBV-CU-2-SP1 and Eg/12120s/2012 (variant 2 like strain) ranged from 97% to 98.3 % and 96.7% to 98.3%, respectively. Our results are in agreement with the concept that IBV mutates commonly and that endemic variants 1, 2 are cocirculating in Egypt (**Abdel-Moneim et al., 2012 and Sarah, *2014***).

The recent seven Egyptian IBV field isolates in this study were distinctly different from vaccinal strain used in Egypt, M41, H120, Ma5, 4/91, CR88 and D274, (**Abdel-Moneim et al. 2002; Sediek, 2010 and Sarah, 2014**). So, vaccination with one serotype does not ensure complete protection against herterologous serotype (**Sediek, 2005 and 2010)** which emerge by changes in the IBV genome by point mutation, deletions, insertions or RNA recombination **(Zenella et al., 2000; Thor et al., 2011; Hong et., 2012)** which were responsible for outbreaks of IBV in the vaccination chicken flocks. And also differences in as few as 5% of the amino acid in S1 can decrease crossprotection **(Cavanagh, 2007)**, so developing vaccines from local strains is necessary for IBV control in Egypt. In addition to serotype changes, the genetic variation may result in changes of the tissue tropism and pathogenicity of the virus which lead to the generation of new IBV pathotype.

These findings showed no geographical restriction in the distribution of these isolates and their related isolates but the great homology with Israeli isolates still present. Although there is no geographical restriction, many countries share some common antigenic types, IBV strains within a geographic region are unique and distinct; examples of this include Europe, the United States, and Australia (***Ignjatovic, et al. 2002, Callison, et al. 2001and Adzhar, et al. 1997***).

Further epidemiological surveillance studies are needed in order to explain the mechanism of emergence of variants and their biological properties, including pathogenicity, along with developing suitable vaccines from endemic virus strains. Continuous surveillance of new IBV strains is important for understanding the molecular evolution of different genotypes and for selecting candidate virus strains for vaccination regimes.

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Metric SI units are supposed to generally be used excluding where they conflict with current practice or are confusing. For illustration, 1.4 l rather than 1.4 × 10-3 m3, or 4 mm somewhat than 4 × 10-3 m. Chemical formula and solutions must identify the form used, e.g. anhydrous or hydrated, and the concentration must be in clearly defined units. Common species names should be followed by underlines at the first mention. For following use the generic name should be constricted to a single letter, if it is clear.

Structure

All manuscripts submitted to Global Journals Inc. (US), ought to include:

Title: The title page must carry an instructive title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) wherever the work was carried out. The full postal address in addition with the e-mail address of related author must be given. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining and indexing.

Abstract, used in Original Papers and Reviews:

Optimizing Abstract for Search Engines

Many researchers searching for information online will use search engines such as Google, Yahoo or similar. By optimizing your paper for search engines, you will amplify the chance of someone finding it. This in turn will make it more likely to be viewed and/or cited in a further work. Global Journals Inc. (US) have compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Key Words

A major linchpin in research work for the writing research paper is the keyword search, which one will employ to find both library and Internet resources.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy and planning a list of possible keywords and phrases to try.

Search engines for most searches, use Boolean searching, which is somewhat different from Internet searches. The Boolean search uses "operators," words (and, or, not, and near) that enable you to expand or narrow your affords. Tips for research paper while preparing research paper are very helpful guideline of research paper.

Choice of key words is first tool of tips to write research paper. Research paper writing is an art.A few tips for deciding as strategically as possible about keyword search:

- One should start brainstorming lists of possible keywords before even begin searching. Think about the most important concepts related to research work. Ask, "What words would a source have to include to be truly valuable in research paper?" Then consider synonyms for the important words.
- It may take the discovery of only one relevant paper to let steer in the right keyword direction because in most databases, the keywords under which a research paper is abstracted are listed with the paper.
- One should avoid outdated words.

Keywords are the key that opens a door to research work sources. Keyword searching is an art in which researcher's skills are bound to improve with experience and time.

Numerical Methods: Numerical methods used should be clear and, where appropriate, supported by references.

Acknowledgements: Please make these as concise as possible.

References

References follow the Harvard scheme of referencing. References in the text should cite the authors' names followed by the time of their publication, unless there are three or more authors when simply the first author's name is quoted followed by et al. unpublished work has to only be cited where necessary, and only in the text. Copies of references in press in other journals have to be supplied with submitted typescripts. It is necessary that all citations and references be carefully checked before submission, as mistakes or omissions will cause delays.

References to information on the World Wide Web can be given, but only if the information is available without charge to readers on an official site. Wikipedia and Similar websites are not allowed where anyone can change the information. Authors will be asked to make available electronic copies of the cited information for inclusion on the Global Journals Inc. (US) homepage at the judgment of the Editorial Board.

The Editorial Board and Global Journals Inc. (US) recommend that, citation of online-published papers and other material should be done via a DOI (digital object identifier). If an author cites anything, which does not have a DOI, they run the risk of the cited material not being noticeable.

The Editorial Board and Global Journals Inc. (US) recommend the use of a tool such as Reference Manager for reference management and formatting.

Tables, Figures and Figure Legends

Tables: Tables should be few in number, cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g. Table 4, a self-explanatory caption and be on a separate sheet. Vertical lines should not be used.

Figures: Figures are supposed to be submitted as separate files. Always take in a citation in the text for each figure using Arabic numbers, e.g. Fig. 4. Artwork must be submitted online in electronic form by e-mailing them.

Preparation of Electronic Figures for Publication

Even though low quality images are sufficient for review purposes, print publication requires high quality images to prevent the final product being blurred or fuzzy. Submit (or e-mail) EPS (line art) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Do not use pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings) in relation to the imitation size. Please give the data for figures in black and white or submit a Color Work Agreement Form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution (at final image size) ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs) : >350 dpi; figures containing both halftone and line images: >650 dpi.

Color Charges: It is the rule of the Global Journals Inc. (US) for authors to pay the full cost for the reproduction of their color artwork. Hence, please note that, if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a color work agreement form before your paper can be published.

Figure Legends: Self-explanatory legends of all figures should be incorporated separately under the heading 'Legends to Figures'. In the full-text online edition of the journal, figure legends may possibly be truncated in abbreviated links to the full screen version. Therefore, the first 100 characters of any legend should notify the reader, about the key aspects of the figure.

6. AFTER ACCEPTANCE

Upon approval of a paper for publication, the manuscript will be forwarded to the dean, who is responsible for the publication of the Global Journals Inc. (US).

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(Free of charge) from the following website:

www.adobe.com/products/acrobat/readstep2.html. This will facilitate the file to be opened, read on screen, and printed out in order for any corrections to be added. Further instructions will be sent with the proof.

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Before start writing a good quality Computer Science Research Paper, let us first understand what is Computer Science Research Paper? So, Computer Science Research Paper is the paper which is written by professionals or scientists who are associated to Computer Science and Information Technology, or doing research study in these areas. If you are novel to this field then you can consult about this field from your supervisor or guide.

TECHNIQUES FOR WRITING A GOOD QUALITY RESEARCH PAPER:

1. Choosing the topic: In most cases, the topic is searched by the interest of author but it can be also suggested by the guides. You can have several topics and then you can judge that in which topic or subject you are finding yourself most comfortable. This can be done by asking several questions to yourself, like Will I be able to carry our search in this area? Will I find all necessary recourses to accomplish the search? Will I be able to find all information in this field area? If the answer of these types of questions will be "Yes" then you can choose that topic. In most of the cases, you may have to conduct the surveys and have to visit several places because this field is related to Computer Science and Information Technology. Also, you may have to do a lot of work to find all rise and falls regarding the various data of that subject. Sometimes, detailed information plays a vital role, instead of short information.

2. Evaluators are human: First thing to remember that evaluators are also human being. They are not only meant for rejecting a paper. They are here to evaluate your paper. So, present your Best.

3. Think Like Evaluators: If you are in a confusion or getting demotivated that your paper will be accepted by evaluators or not, then think and try to evaluate your paper like an Evaluator. Try to understand that what an evaluator wants in your research paper and automatically you will have your answer.

4. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

5. Ask your Guides: If you are having any difficulty in your research, then do not hesitate to share your difficulty to your guide (if you have any). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work then ask the supervisor to help you with the alternative. He might also provide you the list of essential readings.

6. Use of computer is recommended: As you are doing research in the field of Computer Science, then this point is quite obvious.

7. Use right software: Always use good quality software packages. If you are not capable to judge good software then you can lose quality of your paper unknowingly. There are various software programs available to help you, which you can get through Internet.

8. Use the Internet for help: An excellent start for your paper can be by using the Google. It is an excellent search engine, where you can have your doubts resolved. You may also read some answers for the frequent question how to write my research paper or find model research paper. From the internet library you can download books. If you have all required books make important reading selecting and analyzing the specified information. Then put together research paper sketch out.

9. Use and get big pictures: Always use encyclopedias, Wikipedia to get pictures so that you can go into the depth.

10. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right! It is a good habit, which helps to not to lose your continuity. You should always use bookmarks while searching on Internet also, which will make your search easier.

11. Revise what you wrote: When you write anything, always read it, summarize it and then finalize it.

12. Make all efforts: Make all efforts to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in introduction, that what is the need of a particular research paper. Polish your work by good skill of writing and always give an evaluator, what he wants.

13. Have backups: When you are going to do any important thing like making research paper, you should always have backup copies of it either in your computer or in paper. This will help you to not to lose any of your important.

14. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several and unnecessary diagrams will degrade the quality of your paper by creating "hotchpotch." So always, try to make and include those diagrams, which are made by your own to improve readability and understandability of your paper.

15. Use of direct quotes: When you do research relevant to literature, history or current affairs then use of quotes become essential but if study is relevant to science then use of quotes is not preferable.

16. Use proper verb tense: Use proper verb tenses in your paper. Use past tense, to present those events that happened. Use present tense to indicate events that are going on. Use future tense to indicate future happening events. Use of improper and wrong tenses will confuse the evaluator. Avoid the sentences that are incomplete.

17. Never use online paper: If you are getting any paper on Internet, then never use it as your research paper because it might be possible that evaluator has already seen it or maybe it is outdated version.

18. Pick a good study spot: To do your research studies always try to pick a spot, which is quiet. Every spot is not for studies. Spot that suits you choose it and proceed further.

19. Know what you know: Always try to know, what you know by making objectives. Else, you will be confused and cannot achieve your target.

20. Use good quality grammar: Always use a good quality grammar and use words that will throw positive impact on evaluator. Use of good quality grammar does not mean to use tough words, that for each word the evaluator has to go through dictionary. Do not start sentence with a conjunction. Do not fragment sentences. Eliminate one-word sentences. Ignore passive voice. Do not ever use a big word when a diminutive one would suffice. Verbs have to be in agreement with their subjects. Prepositions are not expressions to finish sentences with. It is incorrect to ever divide an infinitive. Avoid clichés like the disease. Also, always shun irritating alliteration. Use language that is simple and straight forward. put together a neat summary.

21. Arrangement of information: Each section of the main body should start with an opening sentence and there should be a changeover at the end of the section. Give only valid and powerful arguments to your topic. You may also maintain your arguments with records.

22. Never start in last minute: Always start at right time and give enough time to research work. Leaving everything to the last minute will degrade your paper and spoil your work.

23. Multitasking in research is not good: Doing several things at the same time proves bad habit in case of research activity. Research is an area, where everything has a particular time slot. Divide your research work in parts and do particular part in particular time slot.

24. Never copy others' work: Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

25. Take proper rest and food: No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

26. Go for seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

29. Think technically: Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

32. Never oversimplify everything: To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print to the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects in your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form, which is presented in the guidelines using the template.
- Please note the criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of prior workings.

Writing a research paper is not an easy job no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record keeping are the only means to make straightforward the progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

Choose a revealing title. It should be short. It should not have non-standard acronyms or abbreviations. It should not exceed two printed lines. It should include the name(s) and address (es) of all authors.

Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

Introduction:

The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.

- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

Procedures (Methods and Materials):

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

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- The **major constraint** is that you must independently make all content, tables, graphs, and facts that are offered in the paper. You must write each part of the paper wholly on your own. The Peer-reviewers need to identify your own perceptive of the concepts in your own terms. NEVER extract straight from any foundation, and never rephrase someone else's analysis.

- Do not give permission to anyone else to "PROOFREAD" your manuscript.

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- To guard yourself and others from possible illegal use please do not permit anyone right to use to your paper and files.

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Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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ISSN 9755896