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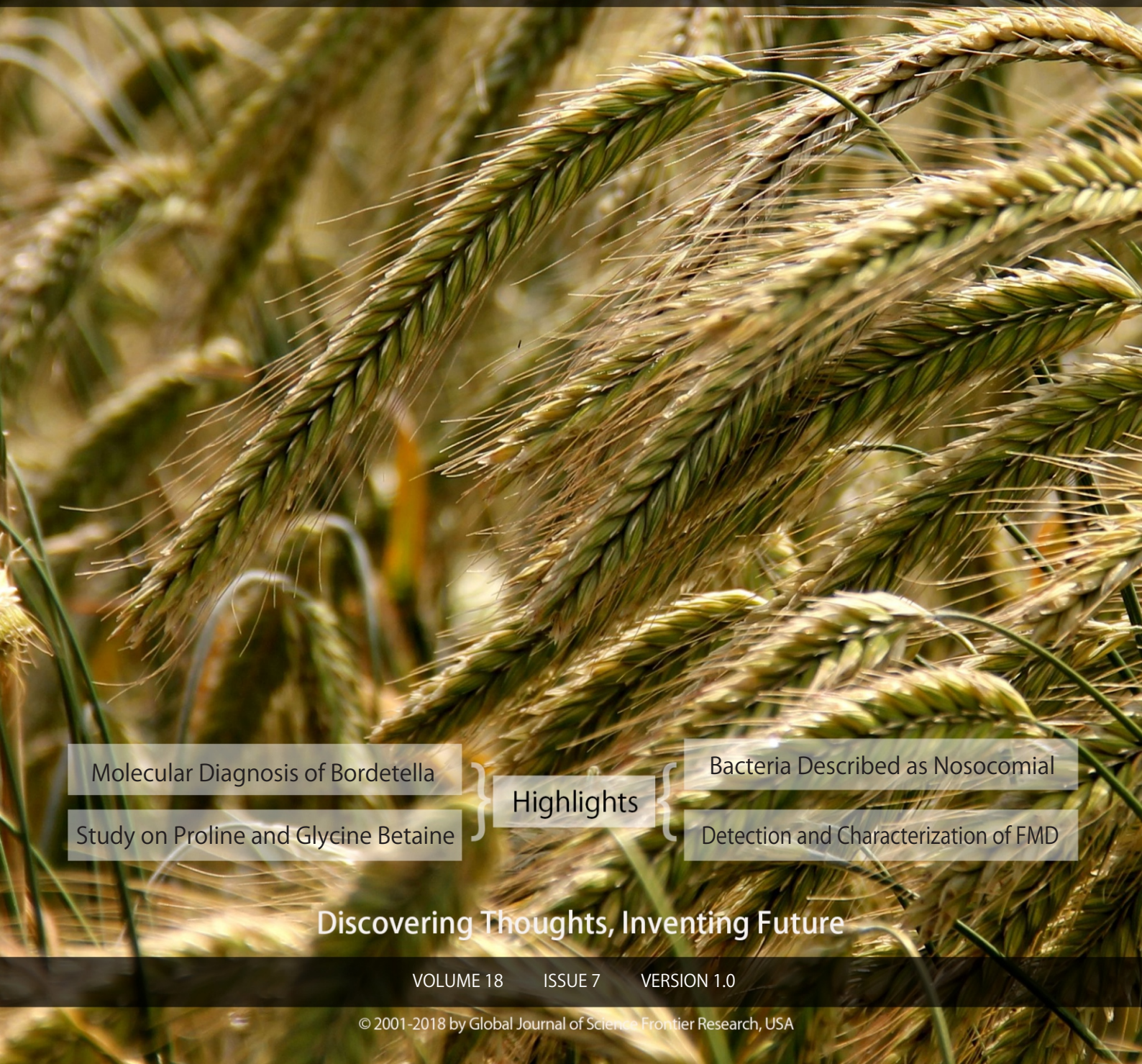

Molecular Diagnosis of Bordetella

Study on Proline and Glycine Betaine

Highlights

Bacteria Described as Nosocomial

Detection and Characterization of FMD

Discovering Thoughts, Inventing Future

VOLUME 18 ISSUE 7 VERSION 1.0

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Assesing the Hematological Parameters of Rabbit Fed Graded Levels of *Cassia Tora* Seed Meal

By Aljameel, K. M, Sani, H. M., N. Muhammad, Maina, B. M. & Umaru, U. A.
Usmanu Danfodiyo University Sokoto

Abstract- A study was conducted to evaluate the effect of varying levels of Cassia *tora* on the performance of rabbits. The animals were fed diets containing 0, 2.5,5 7.5 and 10% inclusion levels of C. *tora* in a completely randomized design replicated six times. Data were collected in ten consecutive weeks on feed intake and live weight gain. Blood sample evaluation was carried out at the end of the experiments. Results indicated a significant difference in hematological parameters ($P<0.05$), a significantly higher levels of platelets, Lymphocytes and MCHC was found for the animals fed diets containing higher levels of C. *tora*. It was concluded that up to 7.5% of C. *tora* could be incorporated in the diet of rabbits without any deleterious effect on the blood profile of rabbits.

Keywords: *rabbit, cassia tora, haematology, lymphocytes and MCHC.*

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Assesing the Hematological Parameters of Rabbit Fed Graded Levels of *Cassia Tora* Seed Meal

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I. Introduction

Hematological studies have been found useful for disease prognosis and the therapeutic and feed stress monitoring [1]. Hematological studies are important because the blood is the major transport system of the body, and evaluations of the hematological profile usually furnish vital information on the body's response to injury of all forms, including toxic injury [2]. Hematological studies represent a useful process in the diagnosis of many diseases as well as investigation of the extent of damage to the blood [3]. This is relevant since blood constituents' change about the physiological conditions of animals. The blood transports or conveys nutrient and materials to different parts of the body. Therefore, whatever affects the blood; either drugs, pathogenic organism or nutrition will certainly affect the entire body adversely or moderately regarding health, growth, maintenance and, reproduction [4]. A readily available and fast means of assessing the clinical and nutritional health status of animals on feeding trials may be the use of blood analysis because ingestion of dietary components have measurable effects on blood composition [5] and may be considered as an appropriate measure of long-term nutritional status [6]. The examination of blood provides the opportunity to clinically investigate the presence of several metabolites and, other constituents in the body and it plays a vital role in the physiological, nutritional and pathological status of the animal [7]. It also helps to distinguish the normal state from the state of stress which can be nutritional [8]. Hematological parameters are good indicators of the physiological status of animals [9]. They are also an excellent medium for the measurement of potential biomarkers because its collection is relatively non-invasive and it encompasses an enormous range of physiological process in the body at any given time [8]. Due to that, the experiment aimed at assessing different hematological parameters of a rabbit when fed *Cassia tora*

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II. Materials And Methods

a) Experimental Site

The experiment is carried out at the Livestock Teaching and Research Farm of the Department of Animal Science, Faculty of Agriculture, located at the main campus of Usmanu Danfodiyo University, Sokoto. Sokoto state was located in the North-western part of Nigeria between (latitude 14-15^{0}N and longitude 4-5 ^{0}E). The state has an average maximum temperature of 41^{0}C and minimum of 13^{0}C in April and January respectively [10]. Sokoto State was characterized by alternating rainy and dry seasons. The annual rainfall is about 700mm per annum, and an altitude of 350m above sea level, [11]. The harmattan season stretches from November to February, when there is dry and laden wind accompanied with dust [12].

Sokoto has two main seasons; the dry season, which lasts from October to May/June, and the rainy season that lasts from June to September/October. Sokoto state has abundant livestock resources, because the climate is more suitable for livestock production, due to the absence of Tse-tse fly on open grassland [13]. Sokoto state ranks second in a livestock production in Nigeria, with livestock population of over 8 million [13].

b) Experimental Feeds Sourcing

Experimental feed ingredients such as maize, wheat offal, soya bean, and salt were purchased from the Sokoto central market. *Cassia tora* pod were sourced within Sokoto and Kebbi state; the pods were crushed to obtain the seeds. The seeds were toasted, due to its anti-nutritional factors before mixing it with the other feed ingredients.

c) Experimental Design and Feed Formulation

A completely randomized experimental design (CRD) was used in this experiment with a number of animals representing replication and graded levels of *Cassia tora* representing treatments. Six animals were allocated to each treatment and were balanced for weight between the treatments. Each animal was housed in a pen which was disinfected. Each group was assigned to one of the experimental diets and fed *ad libitum* for 12 weeks. Water was offered *ad libitum*. Five complete experiment diets were formulated with graded levels of *Cassia tora* seed meal at 0, 2.5, 5, 7.5 and 10% inclusion levels. The five experimental diets were used to feed thirty (30) rabbit. The diets were designated as treatments 1, 2, 3,4 and 5 in the experiment. The gross compositions of the experiment diets are shown in table 1.

Table 1: Gross Composition of Experimental Diets

Ingredient	T1	T2	T3	T4	T5
Maize	37.6	37.1	36.45	35.86	35.34
Blood meal	10	9.5	9.3	9.3	9.3
Soya bean mea	1.26	1.26	1.21	0.77	0.33
Rice offal	12.53	10.83	9.23	7.5	5.96
Wheat offal	10.11	10.31	10.31	10.57	10.57
Cowpea hay	25	25	25	25	25
Salt	0.5	0.5	0.5	0.5	0.5
Premix	0.5	0.5	0.5	0.5	0.5
Cassia tora	0	2.5	5	7.5	10
Total	100	100	100	100	100
Energy	2500.097	2500.387	2500.077	2500.090	2500.439
Crude protein	17.4	17.2	17.2	17.3	17.3
Crude fiber	11.91	11.98	12.05	12.14	12.22

d) Experimental Animals and their Management

Thirty (30) male rabbits were purchased from reputable farms in and around Sokoto state. The rabbits were housed in a separate pen which was thoroughly disinfected before the commencement of the experiment.

All the experimental animals were identified, allowed two weeks pre-conditioning period, and medicated against common disease like coccidiosis and mange. They were given prophylactic coccidiostat (Ampro-tetracycline) via drinking water and dipped with cinatic powder based on manufacture's recommended doses. Daily washing of feeders and drinkers and disinfection of the pens was carried out. The animals were housed in pens of one m^2 per rabbit, as done by [14].

e) Chemical Analysis of the Experimental Diets

[15] Method was used to determine the Dry Matter (DM), Crude Protein (CP), Ether Extract (EE), Crude Fiber (CF) and Nitrogen Free Extract (NFE). The gross energy of the samples was determined by bomb calorimeter. Fiber fractions such nitrogen detergent fiber (NDF), acid detergent lignin(ADL) and, acid detergent fiber (ADF) were determined. Energy content was estimated using the formula: MEKcalkg= 37(%CP) + 81.1(%EE) + 35.5(%NFE) [16].

f) Statistical Analysis

The data generated from the experiment was subject to analysis of variance (ANOVA) using complete randomized design [17] using Statview Statistical Package [18]. Where significant differences exist, least significant differences (LSD) was used to separate the means as described by [17].

g) Proximate analysis of experimental diet

Table 4.1 shows the chemical composition of the experimental diets used in the study. It showed that crude fiber and moisture decreases with the increase in *Cassia tora* in the experimental diet. The dry matter on the other hand increases with increase in the level of *Cassia tora* in the diet(Table 2). The results obtain shows that the energy, crude protein and crude fiber are within the range required by rabbit for optimum growth as outline by [19, 20]. The dry matter of feeds increases with increase in *C. tora*, this could be attributed to the high level of dry matter in *C. tora*as shown from the results. So also the decrease in fiber across the treatment could be attributed to the decrease in rice offal which contains high fiber across the treatments from treatment 1 to treatment 5.

Table 2: Proximate analysis of experimental diets

Parameter	Treatment					
%	1	2	3	4	5	*Cassia tora*
Crude protein	16.62	17.34	18.24	18.89	16.8	22.37
Moisture	5.26	5.06	4.76	4.76	4.86	0.24
Fiber	9.46	9.63	7.86	7.53	6.93	13.08
Ash	10.60	10.02	9.20	9.05	8.60	12.75
Dry matter	94.74	94.94	95.24	95.24	96.14	99.76
Energykcal/kg	2886.7	2836.34	2858.57	2809.34	2857.79	3426.95

h) Hematological profile of rabbits fed graded levels of Cassia tora

The results of hematology shows that hemoglobin, RBC, MCH, MCV, monocytes, neutrophils and eosinophil did not differ significantly ($P<0.05$) between the treatments. There is significantly higher values of PCV,MCHC, lymphocytes and WBC values for animals placed in tratment4. The results also show significantly lower values in treatment 1 regarding platelets, MCHC and WBC composition. The results show a decrease in PCV as the level of *Cassia tora*increased while MCHC increased. (Table 3). The mean values for Haemoglobin, PCV, RBC, MCV, MCHC, and neutrophils obtained in the study were within the normal reference values for rabbits as outlined by [21, 22 and 23]. However, the monocyte values obtained from the results showed a higher value from the normal range. This may indicate that the animals might be recovering from a certain bacterial infection, as indicated by [24] that the WBC values obtained were also within the normal range for healthy rabbits as reported by [25] though the Rabbits in treatment 1 have lower WBC below the normal reference range. The Hb is within the value of 9 -17.4 g/dl reported by [24]. [23] Found that there was a strong influence of diet on hematological traits with PCV and Hb being a very strong indication of the nutritional status of the animals.

The values for eosinophils were all within the reference value reported by [23]which is an indication that the rabbits are healthy. The platelets levels of the blood were varied among the treatments, with treatment 1 showing the lowest value below the normal range as reported by [26].

Differences observed in Packed Cell Volume (PCV) for animals in different treatment groups in this study may be attributed to the physiological status of the animals [27]. Higher WBC count may explain the reason for disease resistance which has been reported by [28] or the prevalence of disease condition. It may also explain longevity as reported by [29]. Lower than normal White Blood Cells (WBC) count suggests a greater challenge to the immune system of rabbits. [30] Noted that a decrease in WBC count, however, reflected a fall in the production of defensive mechanism to combat infection. [1] Reported that significantly lower lymphocyte count was an indication of a reduction in the ability of the experimental rabbits to produce and release antibiotics when infections occur. According to [31] Packed Cell Volume is involved in the transport of oxygen and absorbed nutrients. [24]Posited that MCV, MCH and MCHC are used in diagnosing anaemic conditions. [32]Observed that MCHC values decrease with increase in the level of protein which is contrary to the finding of this study.

Table 3: Hematological profile of rabbits fed graded levels of *Cassia tora*

	Treatment					
Parameter	T1	T2	T3	T4	T5	SEM
Haemoglobin (g/dl)	11.9	12.67	12.01	12.01	10.5	0.73
PCV (%)	38.53^{ab}	40.03^{a}	40.10^{a}	38.9^{ab}	32.93^{b}	1.95
RBC	5.12	5.30	5.10	5.05	4.45	0.36
MCH	5.33	7.33	7.0	6.0	4.0	1.41
MCV	75.27	75.5	75.57	77.03	74.27	1.15
MCHC	30.9^{b}	31.03^{ab}	31.33^{ab}	31.67^{ab}	31.9^{a}	0.29
WBC (x 10^9/L)	3.23^{b}	6.03^{ab}	7.67^{a}	7.23^{ab}	6.03^{ab}	1.05
Monocytes (%)	8.50	7.67	8.17	5.00	5.53	1.88
Neutrophils (%)	48.33	44.67	45.0	46.67	43.67	3.65
Lymphocytes (%)	44.67^{ab}	44.67^{ab}	42.67^{b}	44.0^{ab}	48.58^{a}	1.78
Eosinophil (%)	3.00	4.00	2.5.00	3.80	2.9	1.15
Platelets (x 10^9/L)	41.0^{b}	199.0^{a}	237.3^{a}	180.33^{ab}	208.33^{a}	48.85

a, b means values with different superscripts in a row denotes significant ($P<0.05$) difference between means within the same rows. PCV- Packed cell volume; RBC-Red Blood cells; MCV- Mean corpuscular volume; MCH- Mean Corpuscular Hemoglobin;

MCHC- Mean corpuscular Haemoglobin Concentration; WBC- white blood cells; Treatment 1- control-0, 2- 2.5% *C. tora diet*; 3- 5% *C. tora diet*; 4- 7.5% *C. tora,, 5- 10% C. tora*

III. Conclussion

It was concluded that up to 7.5% of *C. tora* could be incorporated in the diet of rabbits without any deleterious effect on the blood profile of rabbits.

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Molecular Diagnosis of *Bordetella spp.* by Means the Semi-Nested Polymerase Chain Reaction

By Nicolás Tamayo, Consuelo Borie, Maria A. Jara & Carlos Navarro
University of Chile

Abstract- Traditionally, the diagnosis of *Bordetella bronchiseptica (B. bronchiseptica)* is based mainly on clinical signs and epidemiological history. Although the bacteriological culture has diagnostic specificity, it is a technique where the microorganisms present slow development and where the phenotypic identification of the bacteria is complex. On the other hand, its sensitivity could be lower when compared with molecular diagnostic, since only a fraction of all bacteria can grow in an artificial environment. Therefore, implementing diagnostic techniques of high sensitivity and specificity can contribute to the detection and management associated with respiratory symptoms produced by this agent.

The objective of this work is to implement a diagnostic protocol for Bordetella spp. by the semi-nested Polymerase Chain Reaction (sn-PCR)by means the detection of the gene which encodes the structural protein flagellin. For this, three in silico primers were designed using the free access Oligo Perfect ™ program, which was later synthesized by the BIOSCAN company.

Thus, using three strains of *B. bronchiseptica* as samples, fragments of the expected sizes were obtained (362 bp and 170 bp) and, after the sequencing of the larger amplicon, a percentage of 95% nucleotide identity was determined by the Clustal Ω program with respect to the official data registered in GenBank®. This value was corroborated when entering the same sequence to the BLAST online program, which also delivered 95% nucleotide identity percentage concerning to the flagellin protein gene of Bordetella spp.

GJSFR-D Classification: *FOR Code: 060506, 060599*

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Thus, using three strains of *B. bronchiseptica* as samples, fragments of the expected sizes were obtained (362 bp and 170 bp) and, after the sequencing of the larger amplicon, a percentage of 95% nucleotide identity was determined by the Clustal Ω program with respect to the official data registered in GenBank®. This value was corroborated when entering the same sequence to the BLAST online program, which also delivered 95% nucleotide identity percentage concerning to the flagellin protein gene of Bordetella spp.

I. Introduction

B. bronchiseptica is a Gram-negative, strict aerobic coccobacillus. This microorganism can be found in the respiratory tract of dogs andother species such as cats, pigs, laboratory animals and humans. Its transmission is produced by direct contact, by the generation of contaminated aerosols or fomites. There is evidence of occasional transmission between species, particularly between dogs and cats it is associate with respiratory symptoms, where its role as the primary or secondary agent is still discussed(Molina *et al*., 2006). This is because there is evidence that this bacterium is involved in the presentation of acute cases of respiratory diseases, as well as that it is an etiologic agent secondary to a viral infection (Molina*et al*., 2006).

B. bronchiseptica is considered the etiological agent of canine infectious tracheobronchitis (CIT), known as "a kennel cough". Also, that canine parainfluenza virus, adenovirus type-2, canine herpesvirus, reovirus, fungi and *Mycoplasma sp*also participate in this scenario(Keil and Fenwick 2000).

In most cases, the infection remains localized in the respiratory tract. The congregation of dogs of different ages and different levels of susceptibility to the agents involved in the status predisposes to the presentation of the same. Because, the canine respiratory complex is a disease to prevent in human societies, veterinary hospitals, research institutes and places where exhibition dogs are housed or shown (Keil and Fenwick, 2000).

The ability to colonize ciliated epithelial cells of the respiratory tract is recognized in bacteria. This is due to the presence of fimbrial and non-fimbrial adhesins. Within the latter are hemagglutinin and pertectin, located mainly in the outer membrane of the bacteria. *B. bronchiseptica* produces endo and exotoxins, within the latter, a hemolysin adenylate cyclase, dermonecrotic toxin and tracheal cytotoxin. These factors affect the hair cells, inhibit the response of phagocytic cells, suppress the immune response and are associated with clinical signs in dogs with CIT (Keil and Fenwick, 2000).

In general, CIT is self-limiting and does not require antibiotic therapy. However, its prophylactic use is recommended in individuals who have been in contact with dogs that have presented the status (Keil and Fenwick, 2000).

Respiratory symptoms due to this bacterium occur in laboratory animals. The manifestations can vary from nasal discharge, sneezing, anorexia, and weight loss to bronchopneumonia and septicemia. Specifically, in rabbits it is known as the rabbit's catarrh (Matto and Cherry, 2005).

In humans, *B. bronchiseptica* is hardly found. However, there are cases of immunologically compromised people where this bacterium can cause upper respiratory tract infections, pneumonitis, endocarditis, peritonitis, meningitis and bacteremias. In humans with AIDS, this bacterium can cause interstitial pneumonia (Galeziok *et al*., 2009).

It is usually diagnosed clinically by symptomatology and the presence of predisposing factors, such as environments of high animal load such as exposures, breeding sites, hotels, hospitals or another group situation (Keil and Fenwick 2000).

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Regarding the microbiological diagnosis, the samples may come from nasal swabs or transtracheal lavage. The tracheal samples are privileged, because the problem that can identify the *B. bronchiseptica* that is part of the normal microbiota of the oropharynx is eliminated (Keil and Fenwick 2000).

Although the bacteriological culture has a great diagnostic specificity, its sensitivity could be low when compared with molecular diagnostic techniques such as the PCR, which can give positive results despite the antibiotic therapy, it also allows to investigate samples with low counts of microorganisms and not necessarily viable (Osses *et al.* 2010). On the other hand, PCR is not limited by the ability of microorganisms to grow in culture media, in addition the result obtained can provide much more information than just indicating the presence of the bacteria, since it can provide usable data to determine epidemiological characteristics and phylogenetic (Couthino *et al.* 2009). In this regard, several PCR protocols have been established to identify different regions of the genome of several *Bordetella* species. However, most of them are focused on *Bordetella pertussis (B. pertussis)*, a microorganism that causes pertussis in humans (Couthino *et al.* 2009, Tatti *et al.*2011, Grogan *et al.*2011, Lanotte *et al*. 2011; Tatti and Tondella 2013).

Thus, in this work the molecular diagnosis of *Bordetella spp*. by the sn-PCR using the design of *in silico*primers to identify the *fla*A gene is already implemented. The gene that encode this protein are ideal candidates to act as biomarkers, since being a protein located on the surface of the bacterium show a much higher divergence rate in their amino acid sequence than those located inside the cell (Winstanley *et al.,* 1997). It has also been shown that the flagellum is necessary to initiate and potentiate the interaction between the bacterium and the cell surface, which is why the flagellum has the potential to distinguish between several host species (Nicholson *et al* 2012).

II. Materials and Methods

Samples. Three strains of *B. bronchiseptica* are used, two from the Institute of Public Health of Chile (ISP): (BB1, BB2) and another from the Microbiology Laboratory of FAVET: (BB3). DNA from *Salmonella* Enteritidis (SE) and from Canine Herpes Virus (CaHV-1) was used as a negative control. Nuclease-free water (NFW) was used as reagent control.

Bacterial DNA obtaining. The extraction of bacterial DNA was carried out using a commercial kit (Genomic DNA Purification kit, Fermentas®). From cultures of $8x10^6$ CFU / mL -obtained by turbidity compared to tube 0.5 of McFarland- 200 µL were taken, to which 400 µL of lysis solution was added, being incubated for five minutes at 65° C. After that time, 600. µL of chloroform were added, mixing gently and then centrifuged at 10,000 rpm for two minutes (Heraeus SepatechBiofuge®). Subsequently, the upper phase was collected in an Eppendorf tube and 800 µL of precipitation solution was added, to re-centrifuge at 10,000 rpm for two minutes. The supernatant was removed and the DNA was resuspended with 100 µL of 1.2 M Sodium Chloride solution and vortexed. To this mixture was added 300 µL of cold ethanol and then kept at -20 ° C for ten minutes. It was centrifuged again at 10,000 rpm, for three minutes, the supernatant was removed and resuspended in 100 µL of nuclease-free water (Winkler®), thus obtaining the DNA that was used in the PCR.

Detection of the *fla*A gene by the semi-nested polymerase chain reaction. Primers: Using the Oligo Perfect ™ program, two pairs of primers were designed whose target sequence is the *fla*A gene (Table 1, Table 2). Then they were sent to synthesize by means the company BIOSCAN. The sequence of the synthesized primers is as follows:

BOR 1F	ACCTGAACAAGTCCCAATCG
BOR 1R= BOR 2R	GACCTTGATGCCGTTGAAGT
BOR 2F	CGCTGAACGAAATCAACAAC

As you can see, only three primers are designed. It is expected that BOR 1F and BOR 1R generate an amplicon of 362 base pairs (Annex 2a), while BOR 2F and BOR 2R generate an amplicon of 170 base pairs (Annex 2b). For this, strictly speaking, this is a semi-nested PCR, since the BOR 1R (or BOR 2R) participates in both reactions.

Reaction mixture: 15 µL of the commercial Master Mix 2X PCR kit (Taq DNA polymerase, $MgCl_2$ and the deoxyribonucleotid triphosphates), fiveµL of template DNA and fiveµL of each specific primer were used, reaching a final volume of 30 µL.

DNA amplification: The PCR technique contemplated a stage of DNA denaturation, followed by a stage of alignment of the primers and a final stage of elongation. The semi-nested variant consisted of using a second pair of primers that amplified a fragment of smaller size since it used the amplicon generated by the first pair of primers as the template. The determination of the alignment temperatures for each pair of primers considered the use of a temperature gradient thermal cycler following a standard PCR scheme: initial denaturation at 94°C (two minutes), then 35 cycles: denaturation (94°C, one minute), alignment, elongation (72°C, one minute) and finally an elongation period for five minutes at 72 ° C.

Visualization of the amplified product: It was carried out by electrophoresis in 2% agarose gel (Winkler ®) in Tris-borate buffer (90 Mm Tris-borate, 10 mM EDTA) as solvent. An aliquot of 6 µL of this mixture was deposited in the corresponding well in the gel. Electrophoresis was

carried out at 90 volts for 40 minutes. As a molecular size marker, a standard containing DNA fragments between 100 and 1000 base pairs (bp) (Hyperladder IV, Bioline®) was used. After electrophoresis, the gel was incubated in Ethidium Bromide (0.5 µg / mL) (Fermelo ®) for 35 minutes and then placed in an ultraviolet transilluminator (Transiluminator UVP ®), where it was finally photographed. to obtain record of the results.

Determination of the percentage of nucleotide identity with respect to GenBank®. Five DNA fragments obtained by PCR1 were sent to the company Genytec for the determination of their nucleotide sequence.

Analysis. To obtain a consensus sequence, the 5 sequences obtained were aligned using the Clustal Ω program. The consensus sequence was aligned with one of the official data of the Genbank (access number L13034.1) to obtain the nucleotide identity percentage, which was corroborated using the BLAST *online* program.

Biosafety measures. For this work, the necessary biosafety measures are associated with the manipulation of the *B. bronchiseptica* bacteria, the performance of the PCR and the subsequent visualization of the amplified product. Regarding the former, limited access to facilities was considered, the use of Bunsen burner to delimit a sterile work area, the use of an apron, the use of clean material and the proper disposal of waste. Regarding the implementation of the PCR, a clean and exclusive area for this procedure was delimited, to avoid contamination with genetic material not coming from the sample, and latex gloves were used to carry out the procedures, such as, for example, the use of ethidium bromide, which has mutagenic properties. At the time of using the ultraviolet light transilluminator they were used with UV filter goggles and an acrylic plate placed between the equipment and the one who visualizes the gel. Finally, the gel was incinerated along with the gloves that were used for its handling.

III. Results

Detection of the *fla*A gene by the sn-PCR. Figure 1 shows an Agarose gel subjected to electrophoresis (90 volt, 40 minutes) and incubated in Ethidium Bromide (35 minutes). It displays fragments of DNA of approximate sizes to those expected (362 and 170 bp) by having the size marker between 100 and 1000 bp as reference. The fluorescent bands are clear and unique. No bands are observed in the lanes of the negative or reagent controls.

Determination of the percentage of nucleotide identity with respect to GenBank®. The DNA fragments obtained by PCR for the sample BB3 were sent in fivefold to the company Genytec for the determination of its nucleotide sequence, receiving 5 sequences (Table 3) and obtaining the following consensus sequence from them:

>NTV6
TGAACAAGTTCCAATCGGCCCTGTGTAGCGCCATCGA
CCGCCTGTCGTCGGGTCTGAGCATCAACAGCGCCAA
GGACGACGCGGCCGTCCAGGCCATCGCCTACCGCT
TCACCGCCAACGTCAAGGGCCTGACCAAGGCTGCCC
GCAACGCCAACGACGGCATCTTGATCGCCCAGACGT
CCGAAGGCGCGCTCAACGAAATCAAGAACAACATGC
AGCGCATCCGCGAACTGACAGTTCAGGTCTCCAACG
GCACGTACTCGGCTTCGGACATCGACTCGATCCAGC
AGAAAGTCAACCAGCGCCTGGAAGAAATCAACCCATC
GCCGAGCAGTCCGACTTCAACGGCA

The alignment of this consensus sequence versus the official GenBank data (access number L13034.1) yielded a NIP value of 95% (Table 4).

This NIP value was confirmed in parallel with the value delivered by the BLAST program, which also delivered a value of 95% (Table 5).

IV. Discussion

The diagnosis of *B. bronchiseptica* through bacterial culture involves an incubation period at 37 ° C for 48 hours (Keil and Fenwick, 2000, Couthino *et al* 2009). This time is reduced by using a molecular diagnostic technique such as PCR. However, it is not only in terms of time that PCR represents an advantage over culture, since in terms of sensitivity the PCR technique has yielded positive results in suspicious samples that, when diagnosed by bacteriological culture, have delivered negative results (Canonne *et al*., 2016)

Additionally, and given the range of respiratory charts in which *B. bronchiseptica* participates, implementing a PCR protocol for the diagnosis of this agent is necessary. In fact, during this report, the primers and the proposed protocol were used by professionals of the Microbiology Laboratory to identify microorganisms suspected of corresponding to *B. brochiseptica,* demonstrating the usefulness of the work carried out.

The decision to perform a semi-nested PCR responded to the advantages of this variant over conventional PCR. Although a PCR with only a pair of primers can perfectly serve as an accurate diagnostic method, a nested-PCR increases both the specificity and sensitivity (Mendoza *et al.,* 2001). In fact, the PCR performed corresponds to the semi-nested variant because it was decided to use the primers that the online program suggested as "the best" against the sequence to be identified. In this case the BOR 1R (or BOR 2R) participates in the first reaction with the BOR 1F, and then in the second reaction with the BOR 2F.

Related to the above, after the experience that allowed demonstrating the usefulness of the primers and the protocol, because reactions were performed with other samples suspected of containing *B. bronchiseptica* obtaining positive results. However, what is interesting is that what was described in the preceding lines was observed in practice: there were samples that when confronted with a pair of primers (conventional PCR) gave a negative result, but when the semi-nested PCR was carried out, they turned out to be positive This can be due to an error in the procedure (the non-incorporation of a primer, for example), or to an effect of the number of reaction cycles, since a quantity of DNA not visualized with the first PCR, certainly can be after the second reaction.

The nucleotide identity of the PCR amplicons would correspond to the *fla*A gene of *Bordetella spp.*, according to the of nucleotide identity percentage (NIP = 95%) obtained using the Clustal Ω program (comparing the consensus sequence with one of the data Genbank) or the incorporation of the consensus sequence in the BLAST Program. The above would be validated by obtaining a NIP value≥ 80% (Chopra and Roberts 2001)

Given these results and the extra experiences carried out, it is still pending to continue testing these primers and the protocol in terms of sensitivity. Perhaps making dilutions of a sample and determining to what extent this protocol continues to give a positive result is an experience that could be retaken from here.

The experience of choosing a bacterial agent, investigating its characteristics and investigating what has already been done to generate a diagnosis, is certainly useful to contemplate the theoretical and practical framework of diagnostic work in a laboratory.

Designing primers using the available technological tools and using them in a real experience constitutes a "downhill" enough to know in what terrain the veterinarian themes moves in the diagnostic area today. Having designed a "own method", but that has given more expected results allowing us to identify the proposed agent, is very enriching both as an academic exercise, but also as a concrete tool that, as already mentioned, was already used in an opportunity in the laboratory. In this way, a PCR protocol has been implemented in the Microbiology Laboratory of the Department of Preventive Animal Medicine, in its semi-nested variant that can be used both for the diagnosis of suspicious samples and for future memories of the title of other students.

V. Conclusions

The designed primers allowed to originate unique and clear DNA bands of the expected size in both PCR1 (362 bp) and PCR 2 (170 PB). The presence of these fragments suggests, although it does not guarantee, the presence of the *fla*A gene in the strains. For this reason, nucleotide sequencing is necessary, a procedure that allows us to affirm that the fragment synthesized corresponds to the gene in question. Thus, using the Clustal Ω program, the alignment of the sequenced fragment (consensus sequence obtained from the sample sent in quintuplicate) compared to the sequence provided by GenBank yielded a nucleotide identity percentage of 95.16%. In addition, through the BLAST program, a 95% nucleotide identity percentage was obtained in relation to the flagellin gene, both *B. bronchiseptica* and *B. pertussis.*

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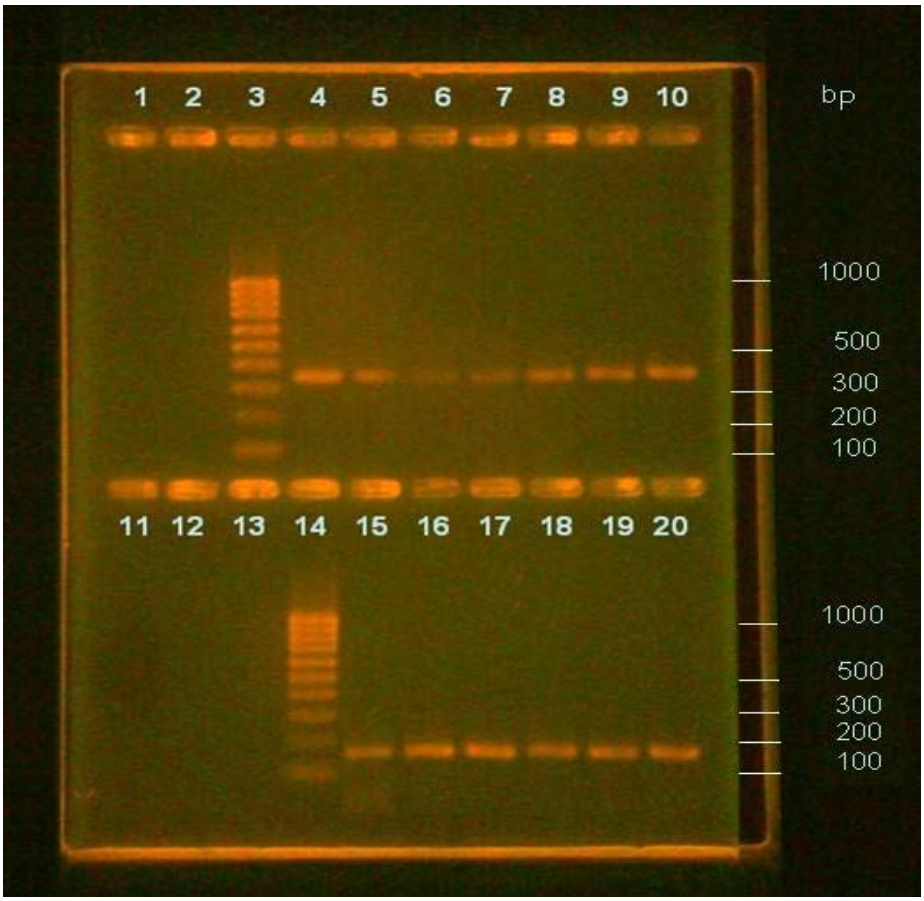


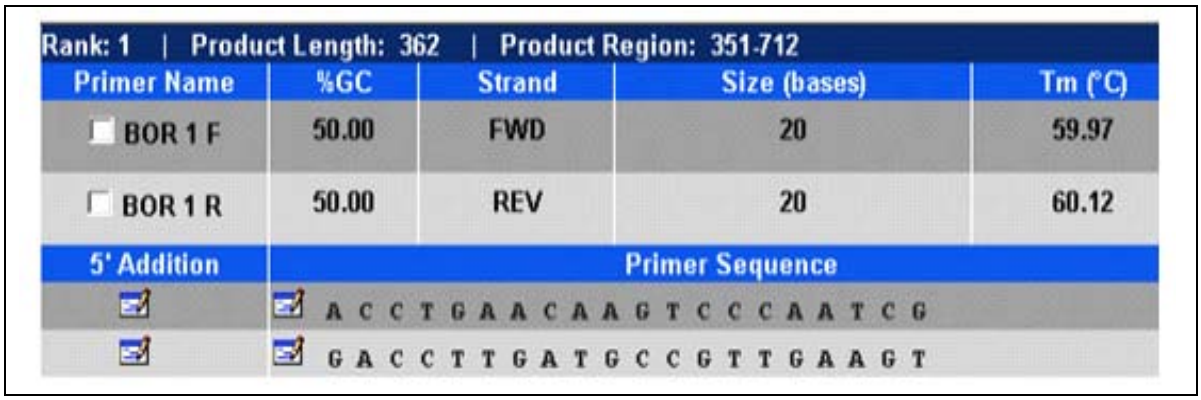
Figure 1: 2% agarose gel electrophoresis

PCR1. Lanes 1, 2: Negative control; lane 3: Marker; lanes 4, 5, 6: DNA from BB1; lanes 7, 8, 9: DNA from BB2; lane 10: DNA from BB3.

PCR2. Lanes 11,12: Negative control; Lane 13: reagent control; Lane 14: Marker; Lane 15,16: DNA from BB1: Lanes17,18: DNA from BB2; Lanes 19,20: DNA from BB3.

Negative control: DNA from *Salmonella* Enteritidis and canine herpes virus. Reagent control: nuclease-free water. MTM: Molecular size marker. 100-1000 bp (Hyperladder IV, Bioline®)

Table 1: Design of **BOR 1F** and **BOR 1R** primers by using Oligoperfect™ *online* program

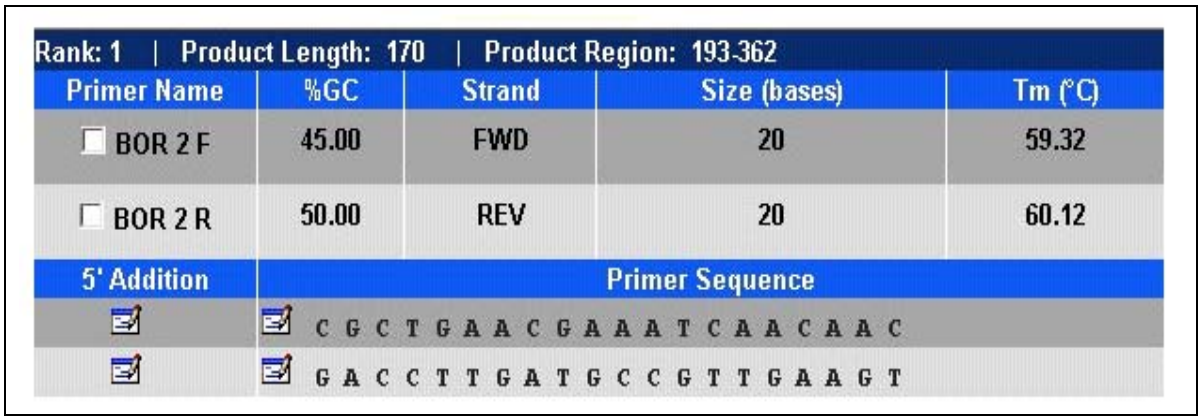


Rank: 1 | Product Length: 362 | Product Region: 351-712

Primer Name	%GC	Strand	Size (bases)	Tm (°C)
BOR 1 F	50.00	FWD	20	59.97
BOR 1 R	50.00	REV	20	60.12

5' Addition	Primer Sequence
	A C C T G A A C A A G T C C C A A T C G
	G A C C T T G A T G C C G T T G A A G T

Table 2: Design of **BOR2F** and **BOR2R** primers by using Oligoperfect™ online program



Rank: 1 | Product Length: 170 | Product Region: 193-362

Primer Name	%GC	Strand	Size (bases)	Tm (°C)
BOR 2 F	45.00	FWD	20	59.32
BOR 2 R	50.00	REV	20	60.12

5' Addition	Primer Sequence
	C G C T G A A C G A A A T C A A C A A C
	G A C C T T G A T G C C G T T G A A G T

Table 3: Obtaining consensus sequence according to Clustal Ω

```
NTV2 ------TGAAAAGTTCCAATCGGCCCTGTGTAGCGCCATCGACCGCCTGTCGTCGGGTTC  54
NTV3 ------TGAACAAGTTCCAATCGGCCCTGTGTAGCGCCATCGACCGCCTGTCGTCGGTTC  54
NTV1 ---------ACAAGTTCCAATCGGCCCTGTGTAGCGCCATCGACCGCCTGTCGTCGGGTC  51
NTV4 -----CTGAACAAGTTCCAATCGACCCTGTGTAGCGCCATCGACCGCCTGTGGTCGGGTC  55
NTV5 TGCACCTGAACAAGTCCCAATCGGCCCTGAGTAGCGCCATCGGGCGCCTGTCGTCGGGTC  60
* **** ******* ***** ************  ******* ***** **

NTV2 TGAGGATCAACAGCGCCAAGGACGACGCGACCGTCCAGGCCATCGCCTACCGCTTCACCG 114
NTV3 TGAGCATCAACAGCGCCAAGGACGACGCGGCCGTACAGGCCATCGCCTACCGCTTCACCG 114
NTV1 TGAGCATCAAGAGCGCCAAGGACAACGCGGCCGTCCAGGCCATCGCCTACCGCTTCACCG 111
NTV4 TGAGCATCAACAGCGCCAAGGACGACGCGGCCGTCCAGGCCAACGCCAACCGCTTCACCG 115
NTV5 TGCGCATCAACAGCGCCAAGGACGACGCGGCCGGCCAGGCCATCGCCAAACGCTTCACGG 120
** * ***** ************ ***** ***  ******* **** * ******** *

NTV2 CCAACGTCAAGGGCCTGACCGAGGCTGCCCGCAACGCCAACGACGGCAACTTGATCGCCC 174
NTV3 CCAACGTCAAGGGCGTGACCAAGGCTGCCCGCAACGCCAACGACGGCATCTTGAACGCCC 174
NTV1 CCAACGTCAAGGGCCTGACCAAGGCTGGCCGCAACGCCAACGACGGCATCTTGATCGCCC 171
NTV4 CCAACGTGAAGGGCCTGACCAAGGCTGCCCGCAACGCCAACGACGGCATCTTGATCGCCC 175
NTV5 CCAACGTCAAGGGCCTGACCCAGGCTGCCCGCAACGCCAACGACGGCATCTCGATCGCCC 180
******** ****** ***** ****** ********************** ** ** *****

NTV2 AGACGTCCGAAGGCGCGCTCAACGAAATCAACAACAAGATGCAGCGCATCCGCGAACTGA 234
NTV3 AGACGTCCGAAGGCGCGCTCAACGAAATCAAGAACAACATGCAGCGCATCCGCGAACTGA 234
NTV1 AGACGTCCGAAGGCGCGCTCAACGAAATCAACAACAACATGCAGGGCATCCGCGAACTGA 231
NTV4 AAACGACCGAAGGCGCGCTCAACGGAATCAACAACAACATGCAGCGCATCCGCGAACTGA 235
NTV5 AGACGACCAAAGGCGGGCTGAACGAAATCAACAACAACCTGCAGCGCATCCGCGAACTGA 240
   ***** ** ****** *** **** ****** *****  ***** ****************
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```
NTV2 CGGTTCAAGTCTCCAACGGCACGTACTCGGCTTCGGACATCGACTCGATCCAGGAGAAAG294
NTV3 CGGTTCAGGTCTCAAACGGCACGTACTCGGCTTCGGACATCGACTCGAGCCAGCAGAAAG 294
NTV1 CAGTTCAGGTCTCCAACGGCACGTACTCGGCTTCGGACATCGACTCGATCCAGCAGAAAG 291
NTV4 CGGTTCAGGTCTCCAACGGCAAGAACTCGGCTTCGGACATGGACTCGATCCAGCAGAAAG 295
NTV5 CGGTTCAGGCCTCCAACGGCACGAACTAGGCTGCGGACATCGACTCGATCCAGCAGGAAG 300
* ***** * *** ******* * *** **** ******* ******* **** ** ***

NTV2 TCAACCAGCGCCTGGAAGAAATCAACATCATCGCCGAGCAGTCCGACTTCAACGGCA-TC 353
NTV3 TCAACCAGCGCCTGGAAGAAATCAACCTCATCACCGAGCAGTCCGACTTCAACGGCA-TC 353
NTV1 TGAACCAGCGCCTGGAAGAAATCAACCTCATCGCCGAGCAGTCCGACTTCAACGGCA-TC 350
NTV4 TCAACCAGCGCCTGGAAGAAATCAACCTCATCGCCGAGCAAACCGACTTCAACGGCAGTC 355
NTV5 TCAACCAGCGCCTGGAAGAAATCAACCGCATCGCCGAGCAGACCGAATTGAGCT------ 354
* ********************** **** ******* **** ** * *
```

Table 4: Alignment of the 351 bp fragment (NTV6) compared to the official sequence of GenBank

```
L13034.1 CATGGCTGCAGTCATCAATACCAACTACTTGTCGCTGGTTGCCCAGAACAACCTGAACAA
NTV6     ----------------------------------------------------TGAACAA
                                                               *******

L13034.1 GTCCCAATCGGCCCTGGGTAGCGCCATCGAGCGCCTGTCGTCGGGTCTGCGCATCAACAG
NTV6     GTTCCAATCGGCCCTGTGTAGCGCCATCGACCGCCTGTCGTCGGGTCTGAGCATCAACAG
         ** ************* ************* ****************** **********

L13034.1 CGCCAAGGACGACGCGGCCGGCCAGGCCATCGCCAACCGCTTCACCGCCAACGTCAAGGG
NTV6     CGCCAAGGACGACGCGGCCGTCCAGGCCATCGCCTACCGCTTCACCGCCAACGTCAAGGG
         ******************** ************* ************************

L13034.1 CCTGACCCAGGCTGCCCGCAACGCCAACGACGGCATCTCGATCGCCCAGACGACCGAAGG
NTV6     CCTGACCAAGGCTGCCCGCAACGCCAACGACGGCATCTTGATCGCCCAGACGTCCGAAGG
         ******* ****************************** ************* *******

L13034.1 CGCGCTGAACGAAATCAACAACAACCTGCAGCGCATCCGCGAACTGACGGTTCAGGCCTC
NTV6     CGCGCTCAACGAAATCAAGAACAACATGCAGCGCATCCGCGAACTGACAGTTCAGGTCTC
         ****** *********** ****** ********************** ******* ***

L13034.1 CAACGGCACGAACTCGGCTTCGGACATCGACTCGATCCAGCAGGAAGTCAACCAGCGCCT
NTV6     CAACGGCACGTACTCGGCTTCGGACATCGACTCGATCCAGCAGAAAGTCAACCAGCGCCT
         ********** ********************************** ***************

L13034.1 GGAAGAAATCAACCGCATCGCCGAGCAGACCGACTTCAACGGCATCAAGGTCCTGAAGTC
NTV6     GGAAGAAATCAACCCATCCGCCGAGCAGTCCGACTTCAACGGCA----------------
         **************    ********** ***************
```

Nucleotide Identity Percentaje (NIP).

Seq A	Name	Length	Seq B	Name	Length	Score
1	NTV6	351	2	L13041	1572	95.16

Table 5: Consensus sequence submitted to the BLAST *online* program

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Bordetella bronchiseptica 253 complete genome	549	549	100%	6e-153	95%	HE965806.1
Bordetella bronchiseptica MO149 complete genome	549	549	100%	6e-153	95%	HE965807.1
Bordetella pertussis 18323 complete genome	549	549	100%	6e-153	95%	HE965805.1
Bordetella pertussis CS, complete genome	549	549	100%	6e-153	95%	CP002695.1
Bordetella bronchiseptica strain RB50, complete genome; segment 8/16	549	549	100%	6e-153	95%	BX640444.1
Bordetella parapertussis strain 12822, complete genome; segment 5/14	549	549	100%	6e-153	95%	BX640427.1
Bordetella pertussis strain Tohama I, complete genome; segment 3/12	549	549	100%	6e-153	95%	BX640413.1
Bordetella bronchiseptica flagellin (flaA) gene, complete cds	549	549	100%	6e-153	95%	L13034.1
Bordetella parapertussis Bpp5 complete genome	538	538	100%	1e-149	94%	HE965803.1
Bordetella bronchiseptica strain SB283 flagellin gene, partial cds	536	536	97%	4e-149	95%	AF232941.1
Bordetella bronchiseptica strain SB22 flagellin gene, partial cds	536	536	97%	4e-149	95%	AF232939.1
Bordetella bronchiseptica strain SB521 flagellin gene, partial cds	525	525	97%	9e-146	94%	AF232940.1
Thiobacillus denitrificans ATCC 25259, complete genome	111	111	55%	3e-21	77%	CP000116.1
Chromobacterium violaceum ATCC 12472, complete genome	73.1	73.1	60%	1e-09	73%	AE016825.1

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Perfomance and Carcass Characteristics of Rabbit Fed Graded Levels of Morning Glory Leafs (*Ipomoea Asarifolia* L.)

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Abstract- A study was conducted to evaluate the effects of the inclusion of graded levels of Morning glory *(Ipomoea asarifolia)* leaf meal in the diets of growing rabbits on growth performance, nutrients retention, and carcass characteristics. Four experimental diets were formulated, with inclusion levels of 0, 5, 10 and 15 g/kg of *Ipomoea asarifolia leaf meals as* T_1, T_2, T_3 *and* T_4 respectively. Forty male rabbits used for the experiment were allotted to four treatments groups (1, 2, 3, and 4) with two rabbits per replicate in a completely randomized design. The rabbits were fed with the respective diets for 56 days. Parameters such as fed intake, weight gain, feed conversion ratio (FCR) and mortality rate where monitored. The data generated where subjected to analysis of variance (ANOVA). Where significant difference ($P<0.05$) exists, least significant difference (LSD) was used to compare between the treatment means. The results indicated that growth performance of the animals was not significantly different across the treatments, except for final weight and feed conversion ratio. The effects of diet on carcass characteristic indices showed that *Ipomoea asarifolia* inclusion had no negative effect on the carcass yield. All other parameters differed significantly with exception of stomach, small and large intestine ($p>0.05$).

Keywords: rabbit, performace, carcass, ipomoea asarifolia.

GJSFR-D Classification: FOR Code: 070799

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Perfomance and Carcass Characteristics of Rabbit Fed Graded Levels of Morning Glory Leafs (*Ipomoea Asarifolia* L.)

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Keywords: rabbit, performace, carcass, ipomoea asarifolia.

I. Background To The Study

Rabbit production is important in alleviating animal protein deficiency in Nigeria (Agala and Balogun, 2004). Rabbits have immense potentials and good attributes, which include high growth rate, high efficiency in converting forage to meat, short gestation period, high prolificacy, relatively low cost of production and high nutritional quality of meat which includes low fat and cholesterol levels. Rabbit meat has a high protein level of about 18 % and, its consumption is bereft of cultural and religious biases (Biobaku and Oguntona, 1997; Ndor *et al.,* 2009). Due to these potentials, there is a need to encourage farmers to go into rabbit production to supply animal protein at a cheaper cost.

The plant belongs to the family *Convolvulaceae*, *Ipomoea asarifolia,* also calledmorning gloryis a succulent perennial plant trailing on the ground. *Ipomoea asarifolia* (morning glory). In Nigeria, the traditional names include "Domo" kada, in Hausa, and ''Gboro Ababa, in the Yoruba language) the plat have purple flowers which develop three seeds for sexual propagation, although asexual propagation can also be achieved by stolon. It is found throughout West Africa and is a common weed of hydromorphic soils, low lying and inland valleys, streams and river banks (*Jegede et al.,* 2009). In Nigeria, the leaf of *Ipomoea asarifolia*is not generally consumed by either humans or livestock. It mostly grows like a weed and thus popularly used as compost material, ethno-veterinary and human medication practice and mulch.*Ipomoea asarifolia*is a potential cheap feed ingredient for optimum and sustainable poultry production.

II. Methodology

a) Experimental site

The experiment was carried out at the Livestock Teaching and Research Farm of the Department of Animal Science, Faculty of Agriculture, Usmanu Danfodiyo University, Sokoto, Sokoto state is located in the north-western part of Nigeria between longitudes 4^0 8' and 6^0 54' E and latitudes 12^0 0'N and 13^0 58'N and attain altitude of 350m above sea level (Mamman *et al*., 2000). The state has a semi-arid climate which is characterized by low rainfall, ranging from 500-1300 mm with seasonal variations. Heat is more severe in the state during the months of March and April, but the weather is usually cool in the mornings and hot in the afternoons except during the harmattan period (SSMIYSC, 2010). A minimum temperature of 13^0 C has been recorded in January and a maximum of 44^0 C in

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April (SSDG, 2002). Sokoto has two main seasons, the dry season; which starts from October and last up to April, in some part it may extend to May or June. The wet season begins in most of the state in May or June and lasts up to either September or October (SSMIYSC, 2010).

b) Management of Experimental Animals

Forty male (New Zealand White breed) of 5-6 weeks old rabbits weighing an average of 900 g were sourced from National Veterinary Research Institute, Vom, Nigeria. The animals were housed in cages measuring 35 × 35 × 55 cm (width × length × height). The cages were cleaned. Plastic drinkers and improvised metallic feeding trough were provided in each cage. The drinkers were washed daily. Both feed and water were provided ad-libitum during the experimental period. All the experimental rabbits were identified and allowed two weeks pre-conditioning period to acclimatize. They were medicated against coccidiosis and mange. They were given prophylactic coccidiostat (Ampro-tetracycline), via drinking water as recommended by the manufacturer, the rabbits were dipped with cinatic powder base on the instruction given by manufacturer. The animals were housed in 20 pens containing two rabbits each. The pens are made of concrete floor and zinc roofing. $1M^2$ per rabbit was used, Wayne (2009).The rabbits are fed twice a day (morning and evening. Figure 2shows the photograph of the house containing the cages where the rabbit was reared.

c) Experimental Feed Sources

Four diets were formulated using the following feed ingredients: Maize, fresh *Ipomoea asarifolia* (Morning glory), wheat offal, groundnut cake, groundnut haulms, fish meal, limestone, salt (NaCl) and premix. All the ingredients were purchased from the Sokoto central market, milled and separately bagged for diet formulation. Fresh*Ipomoea asarifolia* leaves were sourced within the main campus of Usmanu Danfodiyo University. The plants were dried under the shade in open-air, milled and kept in air tight containers.

d) Formulation of Experimental Diets

Four experimental diets were formulated and fed as a complete diet (Table 4).*Ipomoea asarifolia* was included at 0, 5, 10,15g /kg inclusion levels. The diets were designated as diet 1, 2, 3, and 4 respectively in the experiment. The composition of the experimental diet and calculated chemical composition are shown in Table 1.

Table 1: Composition of the experiment Diets.

TREATMENTS (supplemented level of *I. Asarifolia g/kg*)

Ingredients	T1 (0)	T2 (5)	T3 (10)	T4 (15)
Maize	34	34	34	34
Wheat offal	17	17	17	17
Groundnut cake	27	27	27	27
Groundnut Haulms	16.50	16.50	16.50	16.50
Fish meal	3	3	3	3
Limestone	2	2	2	2
Salt	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25
Total	100	100	100	100
Supplemented level of *I. Asarifolia* (g/kg)	0	5	10	15
Calculated chemical composition				
Energy (ME/kcal/kg)	2954.29	2954.29	2954.29	2954.29
Crude protein (%)	21.91	21.91	21.91	21.91
Crude fiber (%)	9.50	9.50	9.50	9.50
Ether extract (%)	3.32	3.32	3.32	3.32

e) Experimental Layout

Complete Randomized Design (CRD) was used with four treatments replicated five times with two animals per replicate making a total of forty rabbits.

f) Data Collection

Body weight (g) of each rabbit was taken at the beginning of the experiment (day 0). Subsequently, the measurement was carried out weekly. Feed intake was recorded daily by subtracting the left over from the quantity of feed offered to the animals the previous day. Feed conversion ratio was determined using feed intake and body weight gain. Average daily gain (ADG) was calculated from weight gain and a total number of days of the experimental period.

Feed intake (g/rabbit) =Feed offered (g) - Leftover (g)

Feed conversion ratio (FCR) FCR=DM Intake (g) / live weight gain (g)

Average daily gain (ADG) = (final body weight – initial body weight) / total days of the experiment.

i. *Carcass evaluation and internal organs measurements*

Three rabbits from each treatment were randomly selected. Before slaughter, the rabbits fasted for 12 hours to avoid error due to gut fill. All the rabbits were weighed before and after slaughter. After slaughtering, the tail close to the base was first removed so also the head, feet and the pelt. During evisceration, the internal organs and the gut contents were removed and weighed. The skinless carcass was weighed and expressed as a percentage of live weight. The organs weights were expressed as a percentage of dressed weight. Dressing percentage was as determined as follows:

$$Dressing\ perentage = \frac{Dressed\ weight}{Slaughter\ weight} \times 100$$

Dressing percentage = Dressed carcass weight /Slaughter weight × 100

g) Data Analysis

The data collected from the experiment were subjected to analysis of variance (ANOVA) Significant difference among treatment means were separated by least significant difference (LSD).

III. Results

a) Growth performance of rabbit fed graded levels ofIpomoea asarifolia

The growth performance of rabbit feed graded level of *Ipomoea asarifolia* is shown in Table (2).Results indicated a significant difference (P<0.05) in the feed intake, feed conversion ratio final body weight gain and Average Daily Gain. There was no significant difference (P>0.05) in initial body weight of the animals.

Table 2: Growth performance of rabbits fed graded levels of *Ipomoea asarifolia*.

Parameter TREATMENTS (supplemented level of *I. Asarifolia g/kg*)					
	T1 (0)	T2 (5)	T3 (10)	T4 (15)	SEM
Initial body wt (g/rabbit)	924.14	923.57	923.28	923.00	8.09
Final body wt (g/rabbit)	1518.33[a]	1494.33 [a]	1186.69 [b]	1190.67[b]	80.09
Body weight gain (g/rabbit)	594.19[a]	570.76[a]	263.39[b]	267.67[b]	108.27
Feed intake (g/rabbit/day)	136.013[a]	142.623[a]	118.40[b]	108.18[b]	5.76
Feed conversion ratio	12.82[a]	13.99[a]	25.19[b]	22.63[b]	4.67
Average daily gain (g/rabbit)	10.61[a]	10.19[a]	4.70[b]	4.78[b]	1.95
Mortality (%)	0.00 [b]	0.00 [b]	28.57[a]	28.57[a]	5.05

a, b, c, mean values with different superscripts denote significant (p<0.05) difference between mean within the same rows

The initial body weight of the experimental rabbits was similar across treatment. Rabbits fed the control diet and, those fed 5g/kg *IA* had higher feed intake (136 and 142.6g/kg) (P>0.05) that different significantly (P<0.05) from the feed intake of these fed 10 and 15g/kg *IA* (118.40 and 108.18g/kg) (P>0.05). Final body weight of rabbits in the control group and treatment group two was also better (1518.33 and 1494.33g (P>0.05) and significantly higher (P<0.05) than the final body weight of those in treatment groups three and four (1186.7 and 1190.7g) which were similar (P>0.05).

Average body weight gain followed a similar pattern. Rabbits fed the control diet and those fed diet 2 gained more (594.19, and 570.76g) (P>0.05) compared to those fed diet 3 and 4 which had significantly lower (P<0.05) average body weight gain (263.4 and 267.6g) which were also similar to each other, similarly with regards to feed conversion Ratio (FCR). It was better for rabbits fed the control diet (12.52) and those fed 5g/kg *IA* (13.99) and 15g/kg *IA* (25.19) (P>0.05). Rabbit fed 10g/kg *IA* also had similar FCR (22.63) with those fed 15g/ kg *IA* (P>0.05).

The rabbits that consumed the highest level of *IA* (10 and 15g/kg) had the highest mortality rate of 29 %. Those that consumed 0 and 5g/kg *IA* diets survived the experimental period with 0 % mortality. Changes in live weight of rabbits during the eight weeks of the feeding trail is shown in figure 1.

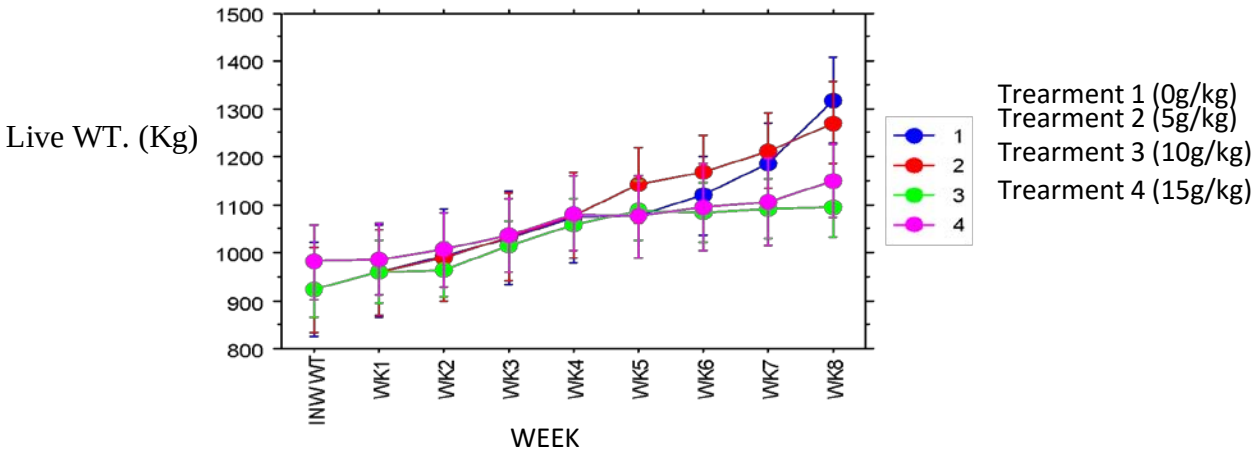

Fig. 1: Live weight changes of Rabbits fed graded levels of *Ipomoea asarifolia.*

b) Carcass Characteristics

The carcass characteristics of rabbit fed graded levels of*Ipomoea asarifolia* meal is shown in Table 3.Results indicated a significantly higher live weight, slaughter weight and dressing percentage (%) for animals fed diets with lower levels of *Ipomoea asarifolia* (Treatment 2) and those fed the control diet (Treatment 1) ($p<0.05$). There was no significant difference in the weight of the kidneys, lungs, heart, small and large intestines ($p>0.05$). Weight of the thighs, shoulder, loin, and the rack were significantly lower for animals fed diets containing higher levels of *Ipomoea asarifolia*($p<0.05$).

Dressing percentage of rabbits fed the control diet and those fed 2 (5g/kg) ware higher and similar to each other (51.52 and 52.37%). Both values were however significantly different from values obtained for rabbits fed diets 3 and 4 (46.22 and 46.30%) which were similar to each other but significantly lower ($P>0.05$) compared to those fed diet 1 and 2.Since the live weight of the rabbits were not similar, there is no basis for statistical comparism. Obviously slaughter and carcass weight must be different.

Table 3: Carcass Characteristics of New-Zealand White rabbit fed graded levels of *Ipomoea asarifolia.*

Parameter	TREATMENTS (supplemented level of *I. Asarifolia g/kg*)				
	T1 (0)	T2 (5)	T3 (10)	T4 (15)	SEM
LW	1518.33^{a}	1494.33^{a}	1186.7^{b}	1190.7^{b}	80.5
SW	1455.66^{a}	1427.66^{a}	1142.66^{ab}	1105.66^{b}	48.38
CW	750.7^{a}	746.7^{a}	530.0^{b}	522.0^{b}	56.0
DP (%)	51.52^{a}	52.37^{a}	46.22^{b}	46.30^{b}	2.51
Kidney (g)	9.00	9.00	8.66	10.66	0.55
Liver (g)	42.0^{a}	47.0^{ab}	38.66^{b}	62.0^{ab}	6.09
Lungs (g)	9.33	10.0	8.88	9.33	1.08
Hear t(g)	3.67	3.67	3.33	3.33	0.33
SI (g)	94.33	82.33	78.33	77.0	9.15
L I (g)	120.0	124.0	120.66	113.0	18.29
Offal (g)	306.0^{ab}	320.0^{a}	256.66^{ab}	248.66^{b}	19.33
Stomach (g)	63.66	63.33	69.00	68.00	16.65
Thigh (g)	216.33^{a}	206.33^{ab}	150.00^{b}	154.00^{b}	18.20
Shoulder (g)	116.00^{ab}	119.00^{a}	85.00^{b}	85.66^{b}	8.43
Loin (g)	98.66^{a}	89.00^{ab}	65.00^{b}	65.66^{b}	7.74
Rack (g)	333.00^{a}	336.33^{a}	255.33^{ab}	250.00ab	25.46

LW=Live weight, SW=Slaughter weight, CW=Carcass weight, DP,= Dressing percentage.SI=Small intestine, I=Large intestine

IV. Discussion

a) Growth performance of rabbits fed graded levels of Ipomoea asarifolia

The significant difference in feed intake, weight gain, average daily gain, and feed conversion ratio is an indication that animals can only utilize *Ipomoea asarifolia* at lower inclusion levels. The average daily weight gain obtained in this study was lower than those reported by Mainasara (2016) but higher than values reported by Wams (2015). The feed intake of the animals further explains the trend of the growth performance. Significant difference in weight gain obtained across the treatments showed poor acceptability of the test ingredient at higher inclusion levels. The performance of rabbits supplemented with *Ipomoea asarifolia* in this study is also comparable to the report of Samokel *et al.* (2006).

b) Carcass Characteristics of rabbits fed Ipomoea asarifolia

The results indicated increased weight of the liver in treatments 3 and 4 showing significant effects of anti-nutritional factors contained in *Ipomoea asarifolia* on the experimental animals the liver grow bigger in order to cope with detoxification of the phyto-Chemicals in the *Ipomoea asarifolia.* The dressing percentage for the rabbits was higher in treatment 1 and 2. The kidney, small and large intestine were all within the range for normal healthy rabbits as evaluated by Mudunuru *et al.,* (2008). The dressing percentage (46.22-52.37 %) reported in this study was lower than 55-67 % reported by Lowa *et al.* (2006).The significantly ($P<0.05$) higher weight of liver and kidney in treatment 4 as observed was due to high content of *Ipomoea asarifolia*. Bone (1979) reported that anti-nutritional factors contained in certain plants because abnormalities as a result of increased metabolic rate of the organs in an attempt to reduce the toxic elements or to convert the anti-nutritional agent to non-toxic metabolism.

V. Conclussion

It was concluded that inclusions of *I pomoea asarifolia* beyond 5g/kg in the diets of rabbit significantly reduced performance.

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Detection of Bla_{TEM} Resistance Gene in Bacteria Described as Nosocomial

By Yáñez, D., Jara, MA. & Navarro C
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Abstract- During the year 1928, Alexander Fleming discovers the penicillin, substance capable of avoiding bacterial growth. Nevertheless, a little time later, bacteria capable of resisting penicillin and new antimicrobials. The above mentioned, still provokes concern in the scientific community, especially in the area of the Public Health, because the indiscriminate use of antimicrobial, subduing, mistakes in the administration frequency and the consumption of small amounts of antimicrobials from food produced by supply animals, are some of the factors that have influenced the appearance of resistant strains, due to the increase in selection pressure on these bacterial populations.

The bacterial aptitude to resist the action of antimicrobials is determined by genes present in the genome of the pathogen, which can be constitutive or skillfully incorporated using different mechanisms.

Considering the described precedents, in this work, by conventional PCR a fragment of the bla_{TEM} gene was detected in three resistant bacteria strains and sequenced, and it was high values of nucleotide identity respect to sequences of GenBank's database, which allowed to obtain three veterinary positive native controls for further investigations.

Keywords: nosocomial infection, antimicrobial resistance, bla_{TEM} gene.

GJSFR-D Classification: FOR Code: 070706

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I. Introduction

Nosocomial Infections. Nosocomial infections correspond to those infections acquired within a hospital ward and whose manifestation, depending on the incubation period, can occur 48-72 hours later, or even once the patient has obtained medical discharge (WHO, 2003). The illnes severity depends on how committed the patient is and the antimicrobial resistance capacity of the causative bacteria.

Nosocomial infections constitute an great risk of hospitalization and cannot be prevented always (Weese, 2010, Gaschen, 2008), presenting more frequently in intensive care units. This happens for several reasons: 1) the duration of the stay is usually longer than in other sectors of hospitals; 2) invasive medical processes such as the introduction of urinary catheters, allow the entry of opportunistic organisms; 3) the patients present immunocompromise or strong stress due to the disease they present or to the actual treatment and 4) the excessive use of antimicrobials allows the constant selection of resistant bacteria (Steele, 2009).

Public Health. The real impact of nosocomial infections in veterinary hospitals is not known, since the existing information, although, has constantly been increasing, remains very restricted to a small number of outbreaks and cases observed in reference hospitals (Weese, 2010). In human hospitals the incidence of nosocomial infections is lower than that observed due to the fact that patients spend less time in the Intensive Care Unit in relation to the human being, there is a lower prevalence of immunosuppressive diseases and there is a lack of infrastructure that allows a stay from the patients (Gaschen, 2010).

The main problems associated with nosocomial infections in dogs and cats are the transmission of multi resistant bacteria from patient to patient by the hands of hospital workers, transmission of infectious diseases from patient to patient and of zoonoses that could affect workers and even the owners of the patients (Gaschen, 2010).

Bacterial Nosocomiales Agents: Multiresistant bacteria commonly isolated in veterinary hospitals of small animals *include E. coli*, *Enterobacter* spp., *Enterococcus* spp., as well as several species of *Staphylococcus* and *Clostridium* spp. In Europe, *Acinetobacter baumannii* identified as resistant and causing nosocomial infections both in units dedicated to the care of small animals and in equine in a veterinary teaching hospital (Gaschen, 2010). Three of these bacteria have generated increasing concern as Vancomycin-resistant Enterococci (VRE), multiresistant E. coli and methicillin-resistant Staphylococcus aureus (MRSA) have appeared. The latter has been a problem for many years for human medicine, but today it generates great concern in the veterinary medical community, while multiresistant E. coli and MRSA are associated with nosocomial infections in hospitals of small and older animals, VRE are scarcely described as a nosocomial infectious agent in small animals (Steele, 2009).

Antimicrobial Resistance. Antimicrobial resistance is understood as the mechanism by which bacteria can reduce the action of antimicrobial agents (Fernández *et al.,* 2003). When the concentrations that the antimicrobial can reach in the organism do not exceed the minimum inhibitory substantially and, during prolonged times, although linked to the type of agent in question, the bacterium has all the possibilities to

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survive and is defined as resistant. In contrast, when the opposite occurs, the bacterium is defined as susceptible (Errecalde, 2004). This resistance can be a characteristic of an organism (intrinsic) or acquired by mutation or incorporation of genetic material (extrachromosomal DNA) or transposons (chromosomal or integrated to plasmids). The most frequent mechanisms of gene exchange between bacteria are conjugation, transduction, and transformation. The variations of these processes are developed through genetic engineering. An example of this is electrotransformation (Danchin *et al.,* 2002; Cabrera *et al.,* 2007). Depending on the antibiotic and the bacterial species, existe four types of resistance mechanisms: a) enzymatic inactivation of the antibiotic, b) modification of the site white; c) changes in the permeability of the bacterial membrane due to the substitution of outer membrane proteins (porins) when modifying its internal caliber; and d) expulsion of the antibiotic due to the overproduction of efflux pumps that prevents access of the antibiotic to the target site in the bacteria. The first mechanism described is one of the most common biochemical processes that provide resistance (Danchin *et al.,* 2002; Garza et al., 2009).

The Β-Lactams. Under this denomination, a continually growing number of antimicrobials is grouped, whose origin goes back to 1928 when Alexander Fleming discovered a substance capable of inhibiting the growth of *Staphylococcus aureus*. The β-lactams form the group of antimicrobials currently used in clinical practice and include penicillins, cephalosporins, carbapenems and beta-lactamase inhibitors, among others (Mediavilla and García-Lobo, 2004.). Structurally they have in common the β-lactam ring, associated with a thiazolidine ring, forming the 6-amino penicillanic acid responsible for the biological activity and a side chain changes that gives the antimicrobial and pharmacokinetic characteristics to the different antimicrobial derivatives (Mediavilla and García-Lobo, 2004). These chemical modifications reducing the capacity of antimicrobial resistance. The β-lactams inhibit the synthesis of the bacterial wall, acting in the final stage of peptidoglycan formation, called transpeptidation. This group of antimicrobials presents an analogy with an amino acid precursor of the chain of peptidoglycan, D-alanyl-D-alanine. Therefore they are capable of competing for the active site of an enzyme called "Penicillin-binding Protein" (PLP), which participates in the cross-linking of peptidoglycan. In this way the β-lactams are covalently bound to the active site of the PLP, which produces the irreversible inactivation of the enzyme, stopping the peptidoglycan formation and producing the osmotic lysis of the bacterial cell (Mediavilla and García-Lobo, 2004.). The introduction of a methoxyphenyl group to the side chain of the penicillins gave rise to methicillin, which is resistant to the enzymatic inactivation produced by the beta-lactamases of *Staphylococcus aureus*. By adding an amino group to the side chain of the Benzylpenicillin, the Aminopenicillins were created to broaden the spectrum of action of penicillins. Thus, ampicillin is effective against Gram-negative bacteria such as *Escherichia coli* and *Haemophilus influenzae* (Mediavilla and García-Lobo, 2004).

Bacterial Resistance To Β-Lactams. The most important mechanism of β-lactam resistance is the production of β-lactamases, which correspond to enzymes that hydrolyze the β-lactam ring, thereby becoming biologically inactive compounds. These enzymes are synthesized by Gram-positive and Gram-negative bacteria (Mediavilla and García-Lobo, 2004) and at present hundreds of β-lactamases are known, there are four types of them: 1) class A, represented by TEM-type β-lactamases; 2) class B, which correspond to rare metalloenzymes; 3) class C, represented by cephalosporinases of enterobacteria; 4) class D, represented by cloxacillinases.

The β-lactamases are encoded by the blaTEM gene. The term TEM is coined thanks to Temoniera, a patient from whom the first bacterium with characteristics of β-lactam resistance was isolated (Mediavilla and García-Lobo, 2004).

Molecular Genetics Techniques. The study and monitoring of these antimicrobial resistance genes have achieved thanks to the implementation of molecular genetic techniques, which allow detecting areas of interest in the genome of a microorganism.

The polymerase chain reaction (PCR) is one of these molecular methods, which consists in the in vitro synthesis of a white DNA sequence in a repetitive manner, thanks to the use of primers or primers (highly specific oligonucleotide sequence) They recognize small sequences that flank the segment of the genome to be amplified. This are achieved using Taq polymerase, an enzyme from the bacterium *Thermophilus aquaticus* that can incorporate free nucleotides at the 3'-end of the splitter, generating copies of the white sequence exponentially, at short intervals of time and high temperatures. It is characterized by its thermostability, high processivity (adds many nucleotides before being discarded) and high fidelity (Mullis and Faloona, 1987).

The PCR technique is consist in the consecutive repetition of three steps, which together make up a cycle. First, the target DNA is denatured, forming simple strands. This process is carried out at temperatures that vary between 90 and 96 ° C. The second step is the hybridization or annealing of primers to simple strands. The temperature of this stage is specific to each pair of primers. The third and final stage is the synthesis of DNA thanks to the action of a thermostable polymerase. The result is two double strands of DNA, formed by the original strand, and the new strand formed, which will be the target strand or template of the next cycle. This

process is repeat between 20 and 40 times (Mullis and Faloona, 1987). At the end of the series of cycles millions of copies of DNA zone will be generate, which are visualized as bands when performing electrophoresis, in which the DNA is separate according to its molecular weight and its negative electric charge. The DNA molecules are deposite in a polyacrylamide or agarose gel immersed in a buffer solution, which is subject to an electric field. The concentration of the gel determines the density of the solution, and therefore, the speed with which the molecules of interest move. Indeed, the smaller the size of the products of the reaction, the more concentrated the gel must be use; thus, diagnostic PCR generally uses 2% agarose concentrations (Mullis and Faloona, 1987; Danchin and Yuen, 2002; Pennington, 2002.). Thus, the objective of this report was to detect the bla_{TEM} gene involved in ampicillin resistance in bacteria described as nosocomial, through the polymerase chain reaction (PCR), subsequently from the obtained sequences; it was established the percentage of nucleotide identity concerning to official GenBank ® data. This will make an approximation of the situation presented by these genes in the hospital facilities of the University of Chile and a first approach of the subject in veterinary medicine within the country, to develop epidemiological studies to establish the behavior of this gene in the future.

II. Material and Methods

Samples. Three bacterial strains resistant to Ampicillin were studied, according to the Kirby-Bauer plate diffusion method, obtained in previous work (Jara *et al.,* 2009): *Pantoea agglomerans*, *Enterococcus faecium* and *Staphylococcus intermedius* (Table 1).

Table 1: Bacterial species suspected of carrying the blaTEM gene. According to Gram stain and antimicrobial susceptibility to Ampicillin

	Bacterias Gram (-)			Bacterias Gram (+)	
#	Especie	A	#	Especie	A
360	*Pantoea agglomerans*	R	034	*Enterococcus faecium*	R
A: Ampicilina: R: resistente.			P4	*Staphylococcus intermedius*	R

Bacterial DNA obtaining. The extraction of bacterial DNA was carried out using a commercial kit for extraction and purification (Genomic DNA Purification kit, Fermentas®), from cultures of 106 CFU / ml. Briefly, to 200 µL of bacterial culture, 400 µL of lysis solution was added, incubated for five minutes at 65 ° C, homogenizing manually every 1.5 minutes. Immediately, 600 µL of chloroform was added by gently mixing and inverting five times. Then it was centrifuged at 10,000 rpm for two minutes (Heraus Sepatech Biofuge®). After centrifugation, the upper phase is collected in an Eppendorf tube, and 800 µL of precipitation solution was added, mixed gently and centrifuged at 10,000 rpm for two minutes. The obtained pellet is resuspended by the addition of 100 µL of 1.2 M sodium chloride solution. To this mixture, 300 µL of cold ethanol is added and kept at -20 ° C for ten minutes. Then, it was centrifuged at 10,000 rpm for four minutes; the supernatant are removed and resuspended in 100 µL of nuclease-free water (Winkler®). Finally, this DNA was used immediately to perform the PCR test.

Detection of the blaTEM gene using the PCR technique. A 96-well Apollo thermocycler (CLP, USA) was used to carry out the polymerase chain reaction and a protocol that included the temperatures, the estimated time for each stage and the number of applicable cycles (Tenover *et al.,* 1994).

Partidores. The primers were used: 5'-TGGGTGCACGAGTGGGTTAC -3 'and 5'- TTATCC GCCTCCATCCAGTC -3' (Tenover *et al.,* 1994).

Mix the reaction to perform the PCR test. A 2X PCR Master Mix kit (Fermentas®), containing the thermostable polymerase, the deoxynucleotide triphosphates (dNTPs), the reaction buffer and MgCl2, was used. In a 0.2 mL Eppendorf tube, 15 µL of Master Mix, five µL of each of the primers and five µL of the DNA sample were added, obtaining a total volume of 30 µL. DNA amplification. The DNA amplification contemplates an initial denaturation at 94°C for one minute and then 30 cycles (94°C for two minutes, 57°C for one minute and 72°C for two minutes). Finally, a final extension are made at 72°C for ten minutes. The expected band size was 526 base pairs (Tenover *et al.,* 1994).

Visualization of the amplified products. It was performed by electrophoresis in 2% agarose gel (Winkler®) in TAE buffer (Fermentas ®). The PCR product was mixed with one µL of the commercial loading product, 6X Mass Ruler Loading Dye Solution (Fermentas ®), which it has glycerol to give density to the sample and bromophenol blue to verify the progress of the migration of the DNA bands. An aliquot of 6 µL of this mixture are deposit in the respective well of the gel.

Electrophoresis was carried out at 89 V for ninety minutes. As a molecular size marker, a standard containing DNA fragments between 100 and 2000 bp (DNA ladder (Fermentas ®)) was used. The gel was immersed in ethidium bromide (0.5 µg/mL) (Fermelo ®), and visualized in a transilluminator of ultraviolet light (Transilluminator UVP ®) to be finally photographed.

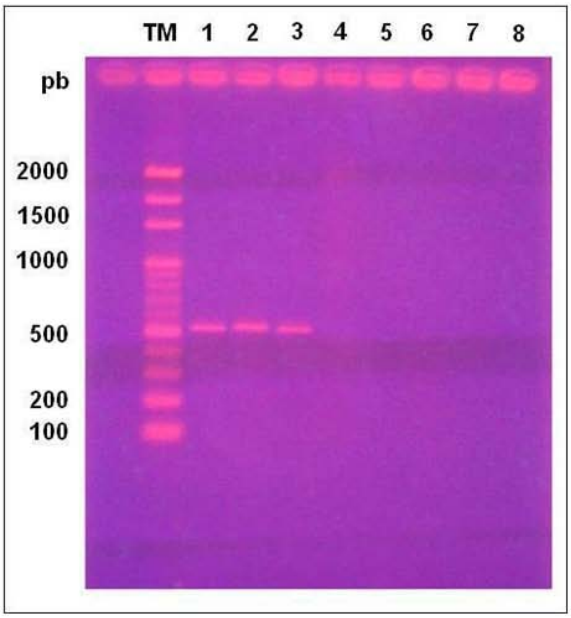
Biosafety measures. The laboratory work was carried out according to the biosafety levels established for the microbiology and animal virology laboratories, such as the use of clean material, correct waste disposal and the use of a closed apron and gloves in practical work. The process of visualization of the amplified product involves the use of ethidium bromide and a transilluminator of UV light, so when the gel was visualized an acrylic plate and glasses with UV filter were used. Subsequently, the elimination of the gel contemplated its incineration since ethidium bromide has -among others- mutagenic properties.

Sequencing. The DNA fragments were sent to the Sequencing Center of the GENYTEC company according to their requirements.

Analysis. From the sequences delivered by GENYTEC, a consensus sequence are obtained for each bacterium studied. Subsequently, the open-access online program called Clustal Ω was used to align the consensus sequences obtained together with the variants of the blaTEM gene fragment (GenBank accession number AF188199.1, and FJ668751.1 corresponding to *E. coli*; GU734697.1, GU734696.1, and GU734695.1 obtained from strains of *K. pneumoniae*;) and thus the percentage of nucleotide identity could be established (Thompson *et al.,* 1994,Genbank, 2018). Also, a multiple alignments was made between all the variants mentioned for the blaTEM gene and thus observe the percentage of nucleotide identity between them.

III. Results

Detection of the blaTEM gene in bacteria described as nosocomial. When carrying out the PCR according to the protocol established (Steele, 2009) in genetic material extracted from bacteria described as nosocomial, the expected white sequence are obtained. The bands obtained presented a molecular size of around 500 base pairs, and it was possible to visualize them clearly (Figure 1).



Lane	Content
TM	*Hyperladder II*. 100-2000 pb
1	DNA *P. agglomerans*
2	DNA *E. faecium*
3	DNA *S. intermedius*
4	DNA Feline herpes virus
5	DNA Canine herpes virus
6	cDNA Canine distemper virus
7	H_2O
8	Empty

Figure 1: Detection of blaTEM gene in three bacterial strains described as nosocomial

Sequencing and determination of nucleotide identity percentage (NIP) The sequences of the three amplified samples (in duplicate) were obtained satisfactorily (Annex 1). Multiple alignments using the Clustal Ω program of each consensus sequence and the official data for the bla$_{TEM}$ gene (GenBank accession number AF188199.1) indicated a high percentage of identity (≥93%) (Table 2). Also, it was observed that all variants of the blaTEM gene compared in this study, showed PIN close to 100%, which allows to assert that we are facing a highly conserved gene and therefore we can work with any of them in future studies.

Table 2: Nucleotidic Identity Percentage (NIP)

Percent Identify Matrix-Created by Clustal2.1

1: S.indermedius	100.00	89.02	91.40	86.52	86.52	86.30	86.52	86.52	86.52	86.52	92.56
2: E.faecium	89.02	100.00	90.02	90.93	90.93	90.47	90.47	90.47	90.47	90.47	90.47
3: P.agglomerans	91.40	90.02	100.00	96.53	96.93	96.30	96.53	96.53	96.53	96.53	96.53
4: A4359287.1	86.52	90. 93	96.53	100.00	99.90	97.89	99.52	99.52	99.53	99.53	99.54
5: af427133.1	86.52	90.93	96.53	99.90	100.00	97.99	99.52	99.52	99.53	99.53	99.54
6: Af093512.1	86.30	90.47	96.30	97.89	97.99	100.00	99.84	99.84	99.84	99.84	99.77
7: Gu734696.1	86.52	90.47	96.53	99.53	99.53	99.84	100.00	100.00	100.00	100.00	100.00
8: Gu734697.1	86.52	90.47	96.53	99.52	99.52	99.84	100.00	100.00	100.00	100.00	100.00
9: Gu734695.1	86.52	90.47	96.53	99.53	99.53	99.84	100.00	100.00	100.00	100.00	100.00
10: FJ668751.0	86.52	90.47	96.53	99.42	99.53	99.65	100.00	100.00	100.00	100.00	100.00
11: Af188199.1	92.56	90.47	96.53	99.54	99.54	99.77	100.00	100.00	100.00	100.00	100.00

IV. Discussion

Recognizing that antimicrobial resistance constitutes a threat to public health, since resistant agents increase the morbidity and mortality rates, increasing the patient's stay in a care center, the determination of resistance genes could guide the use of different therapeutic alternatives (Deutscher and Friedman, 2010). In this aspect, the results obtained in this study allow us to make some reflections on the resistance observed by the three bacterial strains against the beta-lactam used: First, the methods used: conventional PCR, electrophoresis in 2% agarose gel and visualization by ultraviolet light, allow obtaining a DNA fragment of around 500 bp, which is the first indication of the presence of the gene blaTEM, as previously mentioned in the literature (Arros, 2010; Tenover *et al.,* 1994). Thus, it will not be necessary to design other primers through any of the computer programs (*in silico* design) nor does it make necessary to make changes in the PCR protocol used (Tenover *et al.,* 1994). Secondly, by sequencing of the amplicons independently and obtaining the consensus sequence for each of them, the Clustal Ω program delivered valuable information both between the consensus sequences, as well as between each consensus sequence and the official data (GenBank: accession number AF188199.1): a PIN value greater than or equal to 93%. The latter is consistent with the fact that the blaTEM gene is a highly conserved gene in nature, reaching PIN values close to 100% when comparing various official GenBank data. This would make it possible to ensure that each amplicon - independent of origin - are a constitutive part of the blaTEM gene, each constituting in itself a native positive control for the detection of this gene in bacteria described as nosocomial, validated both by its size (~500 bp) as per its nucleotide sequence. Finally, in a future study it will be possible to have both a native control for nosocomial bacteria both Gram positive and Gram negative, which satisfies one of the primary objectives of this title memory. In the case of gram-positive environmental bacteria described as nosocomial, *Enterococcus faecium* (NIP = 96%) may be used and in the case of Gram-negative bacteria: *Pantoea agglomerans* (NIP = 96%).

V. Conclusions

In this title report, the bla_{TEM} gene was detected from bacteria described as nosocomial, isolated from veterinary hospital facilities. This was demonstrated both by the presence of a DNA band of around 500 bp and by the nucleotide sequencing of the obtained amplicon. In addition, the obtained amplicons can be used as positive native controls of veterinary origin, for the first time in Chile.

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ANEXX 1: Sequences for Clustal Omega analysis

a) Consensus Sequences by Genytec

>*P.agglomerans* (consenso 1)

CCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGATCTGCTATCGGGCGCGGTATTATAACAGTATT
GACGCCAGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCAT
CTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACAACTGACAA
CGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAG
CTGAATGAAGCTATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAAGGGCAACAACGTTGCGCAAA
CTATTAACTGGCGAACTACTTGC

>*E.faecium* (Consenso 2)
CCTTGAGAGTTTTCGCCCCGAAAAACGTTTTCATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTAACCAAATATTGA
CGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCT
TACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCATAACCATGAGTGATAACACTGCGGCCAACTTAAAAAAGACAACG
ATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCAGTAACTCGCCTTGATCGTTGGGAACCGGTTTCTG
AATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAA
CTACTTAC

>*S.intermedius* (Consenso 3)
CCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCAATGATGAGCAGTTTTAAATTTCTGCTATCTGGCGCGGTATTATCGCGTATTGA
TTCTGGGCAAGAGCAACTCGGTCCCCGCATACACTATTCTCAGAATGACTTGCTTGAGTACTACCAGTCACAGAAAAGCATCTTA
CGGATCGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATCACACTGCGGCCAACTTACTTCTCACAACGAT
CGGAGGACCGAACGAGCTAACCGCTTTTTTGCACAACATGCGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGTTTCTGA
ATGAAGCCATACCAAACGCCGAGCCTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCTAACTATTAACTGGCGAA
CTACTTAC

b) Oficial sequences by Genbank

>GU734697.1 >GU734696.1 >GU734695.1 >FJ668751.1 >AF188199.1 >AY359287.1 >AF427133.1
>AF093512.1

ANEXX 2: Clustal Aligment

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S. intermedius      CGCCCCGAAGA-ACGTTTTCAATGATGAGCAGTTTTAAATTTCTGCTATCTGGCGCGGTA   72
E. faecium          CGCCCCGAAAAA--CGTTTTCATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTA   71
P. agglomerans      CGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGATCTGCTATCGGGCGCGGTA   73
AY359287.1          CGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGTGCGGTA   419
AF427133.1          CGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGTGCGGTA   419
AF093512.1          CGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGTGCGGTA   419
GU734696.1          CGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGTGCGGTA   176
GU734697.1          CGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGTGCGGTA   180
GU734695.1          CGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGTGCGGTA   180
FJ668751.1          CGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGTGCGGTA   233
AF188199.1          CGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGTGCGGTA   73
                    ********* *   **  ********** ******* ******** ** ******

S. intermedius      TTATC-GCGTATTGATTCTGGGCAAGAGCAACTCGGTCCCCGCATACACTATTCTCAGAA   131
E. faecium          TTAACCAAATATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAA   131
P. agglomerans      TTATAACAGTATTGACGCCAGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAA   133
AY359287.1          TTATC-CCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAA   478
AF427133.1          TTATC-CCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAA   478
AF093512.1          TTATC-CCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAA   478
GU734696.1          TTATC-CCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAA   235
GU734697.1          TTATC-CCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAA   239
GU734695.1          TTATC-CCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAA   239
FJ668751.1          TTATC-CCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAA   292
AF188199.1          TTATC-CCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAA   132
                    ***    * **** * ****************** **********************

S. intermedius      TGACTTGCTTGAGTACTA-CCAGTCACAGAAAAGCATCTTACGGATCGCATGACAGTAAG   190
E. faecium          TGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAG   191
P. agglomerans      TGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAG   193
AY359287.1          TGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAG   538
AF427133.1          TGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAG   538
AF093512.1          TGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAG   538
GU734696.1          TGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAG   295
GU734697.1          TGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAG   299
GU734695.1          TGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAG   299
FJ668751.1          TGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAG   352
AF188199.1          TGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAG   192
                    ******* ********* ************************** *************

S. intermedius      AGAATTATGCAGTGCTGCCATAACCATGAGTGATCACACTGCGGCCAACTTACTTCTCAC   250
E. faecium          AGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTAAAAAAGAC   251
P. agglomerans      AGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACAACTGAC   253
AY359287.1          AGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGAC   598
AF427133.1          AGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGAC   598
AF093512.1          AGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCTGCCAACTTACTTCTGAC   598
GU734696.1          AGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCTGCCAACTTACTTCTGAC   355
GU734697.1          AGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCTGCCAACTTACTTCTGAC   359
GU734695.1          AGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCTGCCAACTTACTTCTGAC   359
FJ668751.1          AGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCTGCCAACTTACTTCTGAC   412
AF188199.1          AGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCTGCCAACTTACTTCTGAC   252
                    ********************************** ******* *********    **

S. intermedius      AACGATCGGAGGACCGAACGAGCTAACCGCTTTTTTGCACAACATGCGGGATCATGTAAC   310
E. faecium          AACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCAGTAACT   311
P. agglomerans      AACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAAC   313
AY359287.1          AACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAAC   658
AF427133.1          AACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAAC   658
AF093512.1          AACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAAC   658
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GU734696.1        AACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAAC  415
GU734697.1        AACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAAC  419
GU734695.1        AACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAAC  419
FJ668751.1       AACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAAC  472
AF188199.1       AACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAAC  312
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S. intermedius    TCGCCTTGATCGTTGGGAACCGGTTTCTGAATGAAGCCATACCAAACGCCGAGCCTGACA  370
E. faecium       CGCCTTGATCGTTGG-GAACCGGTTTCTGAATGAAGCCATACCAAACGACGAGCGTGACA  370
P. agglomerans    TCGCCTTGATCGTTG-GGAACCGGAGCTGAATGAAGCTATACCAAACGACGAGCGTGACA  372
AY359287.1       CCGCCTTGATCGTTG-GGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACA  717
AF427133.1       CCGCCTTGATCGTTG-GGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACA  717
AF093512.1       TCGCCTTGATAGTTG-GGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACA  717
GU734696.1        TCGCCTTGATCGTTG-GGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACA  474
GU734697.1        TCGCCTTGATCGTTG-GGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACA  478
GU734695.1        TCGCCTTGATCGTTG-GGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACA  478
FJ668751.1       TCGCCTTGATCGTTG-GGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACA  531
AF188199.1       TCGCCTTGATCGTTG-GGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACA  371
          * *   * * * * *  *********** ********** ***** *****

S. intermedius    CCACGATGCCTGTAGCAATGGCAACAACGTTGCGCTAACTATTAACTGGCGAACTACTTA  430
E. faecium       CCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTA  430
P. agglomerans    CCACGATGCCTGTAGCAAGGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTG  432
AY359287.1       CCACGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTA  777
AF427133.1       CCACGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTA  777
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GU734696.1        CCACGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTA  534
GU734697.1        CCACGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTA  538
GU734695.1        CCACGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTA  538
FJ668751.1       CCACGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTA  591
AF188199.1       CCACGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTA  431
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A Study on Proline and Glycine Betaine Contents as Salinity and Drought Tolerant Indicators in Solanum Lycopersicum L. (Cultivar: Roma)

By Ja'afar, U., Aliero, A.A. & Yahaya, T.O.
Federal University

Abstract- In this research, accumulation of free proline and Glycine betaine contents was investigated in Solanum lycopersicum L. in response to environmental stress (drought and salinity). After salt and drought treatments in the field the plant samples both stressed and unstressed were used to evaluate the level of free proline and Glycine betaine contents. Proline and Glycine betaine content increase significantly ($P<0.05$) with increasing salt concentration in a concentration dependent manner. However, the result did not differ significantly among drought induced plants. The low level of proline and Glycine betaine contents produce by the plant induced with salinity and drought is a possible indicator of water deficit and salt tolerant in Solanum lycopersicum (cultivar: Roma).

Keywords: *proline, glycine betaine, solanum lycopersicum, drought, salinity.*

GJSFR-D Classification: *FOR Code: 070199*

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A Study on Proline and Glycine Betaine Contents as Salinity and Drought Tolerant Indicators in Solanum Lycopersicum L. (Cultivar: Roma)

Ja'afar, U. [α], Aliero, A.A. [σ] & Yahaya, T.O. [ρ]

Abstract- In this research, accumulation of free proline and Glycine betaine contents was investigated in *Solanum lycopersicum* L. in response to environmental stress (drought and salinity). After salt and drought treatments in the field the plant samples both stressed and unstressed were used to evaluate the level of free proline and Glycine betaine contents. Proline and Glycine betaine content increase significantly ($P<0.05$) with increasing salt concentration in a concentration dependent manner. However, the result did not differ significantly among drought induced plants. The low level of proline and Glycine betaine contents produce by the plant induced with salinity and drought is a possible indicator of water deficit and salt tolerant in Solanum lycopersicum (cultivar: Roma).

Keywords: *proline, glycine betaine, solanum lycopersicum, drought, salinity.*

I. Introduction

Osmolyte accumulation (OA) is frequently cited as a key putative mechanism for increasing yields of crops subjected to drought and salinity conditions (Levitt 1980). The hypothesis is that OA results in a number of benefits that sustain cell and tissue activity under water-deficit conditions. It has been proposed as an effective tolerance mechanism for water deficits and salinity, which could be enhanced in crops by traditional plant breeding, marker-assisted selection or genetic engineering, to generate drought-tolerant crops (Shibli *et al*., 2007). Under conditions where water deficits and salinity threaten crop survival, yields are so low that even large fractional yield gains offer little practical benefit to growers. Indeed, the often-cited benefit of turgor maintenance in cells is likely to result in crop behaviour that is exactly opposite to what is beneficial to crops (Shibli *et al*., 2007). The one clear mechanism identified in this review for beneficial yield responses to OA is in the maintenance of root development in order to reach water that may be available deeper in the soil profile (Zhu 2001).

Glycine betaine (GB) and proline are two major organic osmolytes that accumulate in a variety of plant species in response to environmental stresses such as drought, salinity, extreme temperatures, UV radiation and heavy metals. Although their actual roles in plant osmo tolerance remain controversial, both compounds are thought to have positive effects on enzyme and membrane integrity along with adaptive roles in mediating osmotic adjustment in plants grown under stress conditions (Ashraf and Foolad 2005).

Pepper (*Capsicum spp.*) are tropical woody vines and herbaceous plant having aromatic herbage minute flower spikellets Members of genus Capsicum are shrubs, perennials or annual herbs. The roots are often rhizomatous and leaves can be either simple with entire margin and are positioned at the base of or along the stem and alternate, opposite or whorled in arrangement (Singh *et al.,* 2012). Vegetable crops are mainly produced from irrigated agriculture and the process of soil salinization is dramatically exacerbated and accelerated by crop irrigation which imports large quantity of salts that were not there before. The majority of crop plant are relatively salt sensitive and unable to tolerate high level of salinity (Zhang *et al.,* 2014).

II. Materials And Methods

The field experiment was conducted in botanical garden Department of Biological Sciences Usmanu Danfodiyo University, Sokoto Nigeria and the laboratory experiment was carried out in the Biology laboratory Department of Biology Federal University Birnin Kebbi, Nigeria.

a) Plant Growth Condition

The seeds of *Capsicum annuum* were collected and surface sterilized in 5% sodium hypochlorite and washed with distilled water. The seeds were sown in the nursery bed in green house, and then uniformly germinated seedlings were 14 days old were selected and transferred to polygene bags containing the mixture of sand and organic manure (3:1) adopting the method of Gumi *et al*. (2013)

b) Solution Formation and Salt stress Inducement

Sodium chloride (NaCl) was weighed and dissolves in water to make 30, 60 and 90 mM concentrations of salt which were used to water the plants. The seedlings were divided in to 8 groups, first represent control, second, third and fourth received 30, 60 and 90 mM of NaCl treatments. The fifth represent control watered twice daily (morning and evening), sixth

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watered once in a day, seventh watered once in two days and eighth watered once in three days (Gumi *et al*., 2013). Each treatment was replicated three times and each replicate consist of three plant and the plant were subjected to the treatment for 27 days.

c) Determination of Free Proline Content

Aliquots of ground plants material is heated for 20 min in pure ethanol as well as in water. The resulting mixture was left overnight at 4°C, and centrifuged at 14000 rpm (5 min). The cold extraction procedure was repeated on the pellet and supernatants pooled and used for the analysis (Carillo *et al*., 2008). In 1.5 ml screw-cap tubes, 1000 μl of reaction mix was pipette with 500 μl ethanolic extract. Proline standard completed with up to 400μl of ethanol: water (40:60 v/v). The sealed tubes, were mixed and heated at 95°C in water bath for 20 min and centrifuge (1 min, 10000 rpm), contents were transferred to a 1.5 ml cuvette tubes and read at 520 nm using spectrophotometer. Free proline content was calculated using the following equation.

$$proline = (Absextract - blank) \div slope(Vol.extract \div Vol.aliquot) \times 1 \div FW$$

Where: Abs extract is the absorbance determined with the extract, Blank (expressed as absorbance) and slope (expressed as absorbance·nmol-1), Volume extract is the total volume of the extract, Vol aliquot is the volume used in the assay, FW (expressed in mg) is the amount of plant material extracted. In plant tissues, proline typically ranges from 0.5 (unstressed) 1 to 50 (stressed) μmol.g^{-1}.

d) Determination of Glycine Betaine Content

Glycine betaine content was determined by frozen the plant material in liquid nitrogen immediately after harvesting, grinded and the pestles were pre chilled in liquid nitrogen. The frozen samples were placed in the mortar and pulverized to a fine powder.

The powder was transferred/weighted to several pre cooled 1.5 mL tubes (eppis) and stored at –80 °C. The samples (40-50 mg FW) were suspended in 1 ml of MilliQ grades water, subjected to a freeze thaw cycle by freezing in liquid nitrogen and thawing at 40°C for 20 minutes, and left overnight at 4°C. Samples were then centrifuged at 14000 g, 4°C for 5 minutes. The clear supernatants were separated from the pellets. The eluted GB (retention time 4-5 min) was detected by measuring the absorbance at 200 nm using a diode-array spectrophotometer (model 7310) and quantified by a comparison of peak surface areas with those obtained with pure GB standard solutions in the range 0.05-4 mM (Carillo *et al*., 2008).

GB content was calculated as follows:

$$GB \quad content = Absorbance \quad peak \quad area \quad exact \div slope \times$$
$$Vol. \quad exact \div Vol.aliquot \times concentration \quad factor$$

Where: Absorbance Peak Area extract is the peak area (absorbance at 200 nm) determined with the extract, slope (expressed as absorbance·nmol -1) is determined by linear regression, Vol. extract is the total volume of the extract, Vol. aliquot is the volume of extract injected onto the HPLC column and FW (expressed in mg) is the amount of plant material extracted.

e) Statistical Analysis

The results were expressed as mean of three replicates and the data obtained were subjected to one way analysis of variance test. The difference between the mean were determined by least significant difference using MINITAB statistical software.

III. Results

The result on the effects of salinity and drought on Proline and Glycine Betaine Content in *Capsicum annuum* were presented in figure 1 and 2.

a) Effects of salinity on proline and Glycine betaine content of Solanum lycopersicum

The free proline and glycine betaine content significantly ($P<0.05$) affected by salt stress episodes in a concentration dependent manner. Control recorded the lowest contents of both proline and Glycine betaine content. However, the lowest contents were observed in plant treated with 90mM of salt (Figure 1). Mean comparison shows significant difference ($p<0.05$) between the control group and salt treated groups.

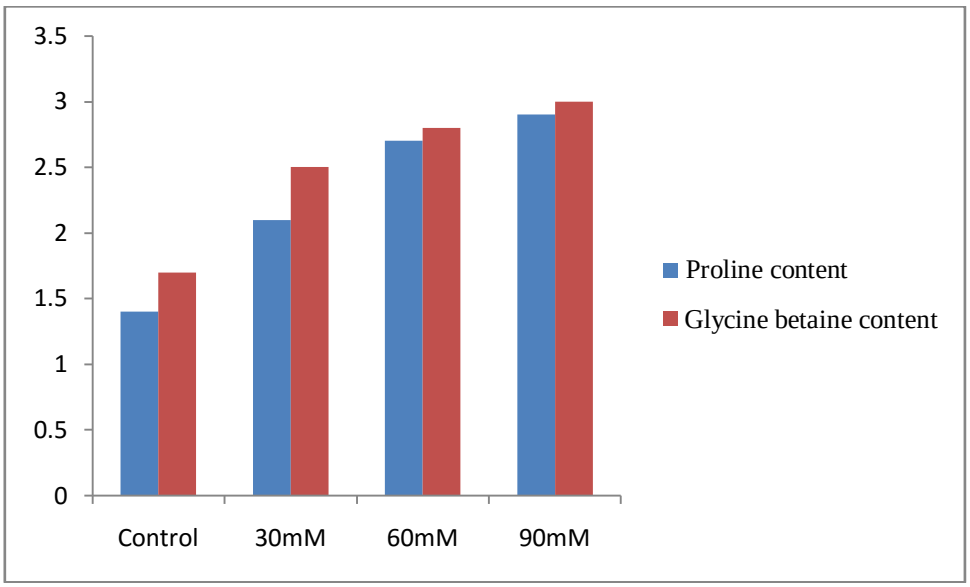

Figure 1: Effects of salinity on free proline and glycine betaine content of *Solanum lycopersicum*

b) *Effects of Drought on proline and Glycine betaine content of Solanum lycopersicum*

Proline and glycine betaine content significantly ($P<0.05$) affected by drought in *Capsicum annuum.* Control recorded the lowest contents of 1.4 and 1.7μMol^{-1} respectively followed by Plants watered once in a day, the lowest contents of 2.9 and 3 μMol^{-1} were recorded in plants watered once in three days. Means comparison did not shows significant difference ($P>0.05$) between the drought induced plants. However, the result shows a significant difference between the control and stress induced plants.

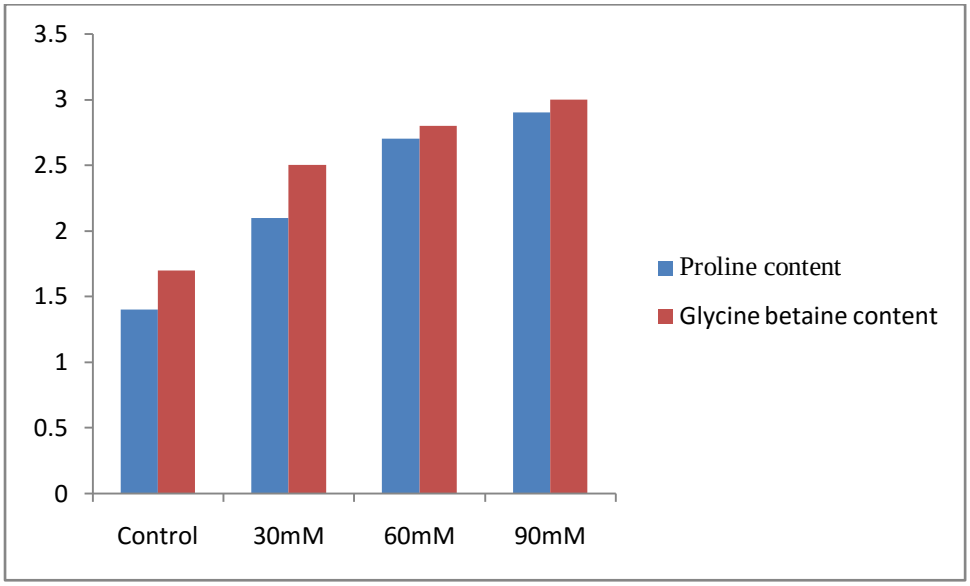

Figure 2: Effects of drought on free proline and glycine betaine content of *Solanum lycopersicum*

IV. Discussion

In this research, the contents of free proline and glycine betaine increase with increasing salt concentration. Similarly, highest proline and glycine betaine contents were recorded in plants watered once in three days, the contents decrease in plant watered once in two days. The accumulation of proline and glycine betaine is often proposed as a solution to overcoming negative effects of water deficit and salinity in crop production. This has been proposed as an adaptive mechanism of salt and drought tolerance (Gumi *et al*., 2013). In this study, the content of proline and glycine betaine did not differ significantly among the drought induced plants, but differ from the control. This explain that higher proline and glycine betaine content

accumulated in stressed plants. This findings agreed with the finding of Gumi *et al*. 2013, Ja'afar *et al*. 2018 and Asraf and Haris, 2004. Carillo *et al*. reported that proline typically ranges from 0.5 is regarded as unstressed and 1 to 50 is regarded as stressed. According to IPGRI descriptors of tomato, Plants which produce GB typically ranges from 0.2-1 is regarded as unstressed and GB content between 6-13 is considered as stressed. This infers that *Solanum lycopersicum* (cultivar: Roma) is a salt and drought tolerant cultivar to some extent.

V. Conclusion

From these findings, Solanum lycopersicum produced low proline and Glycine betaine content under salinity and drought conditions. This could be possible indicator of salinity and drought tolerance in the cultivar studied.

Acknowledgment

Our appreciation and gratitude to Dr. A.M. Gumi of the Department of Biological Sciences Usmanu Danfodiyo University Sokoto for his support in analysis and discussing the finding of this research.

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- In case, the chairperson needs to be replaced then consent of 2/3rd board members are required and they are also required to jointly pass the resolution copy of which should be sent to us. In such case, it will be compulsory to obtain our approval before replacement.
- In case of "Difference of Opinion [if any]" among the Board members, our decision will be final and binding to everyone.

"

Preferred Author Guidelines

We accept the manuscript submissions in any standard (generic) format.

We typeset manuscripts using advanced typesetting tools like Adobe In Design, CorelDraw, TeXnicCenter, and TeXStudio. We usually recommend authors submit their research using any standard format they are comfortable with, and let Global Journals do the rest.

Alternatively, you can download our basic template from https://globaljournals.org/Template.zip

Authors should submit their complete paper/article, including text illustrations, graphics, conclusions, artwork, and tables. Authors who are not able to submit manuscript using the form above can email the manuscript department at submit@globaljournals.org or get in touch with chiefeditor@globaljournals.org if they wish to send the abstract before submission.

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Authors must ensure the information provided during the submission of a paper is authentic. Please go through the following checklist before submitting:

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2. Authors must accept the privacy policy, terms, and conditions of Global Journals.
3. Ensure corresponding author's email address and postal address are accurate and reachable.
4. Manuscript to be submitted must include keywords, an abstract, a paper title, co-author(s') names and details (email address, name, phone number, and institution), figures and illustrations in vector format including appropriate captions, tables, including titles and footnotes, a conclusion, results, acknowledgments and references.
5. Authors should submit paper in a ZIP archive if any supplementary files are required along with the paper.
6. Proper permissions must be acquired for the use of any copyrighted material.
7. Manuscript submitted *must not have been submitted or published elsewhere* and all authors must be aware of the submission.

Declaration of Conflicts of Interest

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- Writings
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The corresponding author should mention the name and complete details of all co-authors during submission and in manuscript. We support addition, rearrangement, manipulation, and deletions in authors list till the early view publication of the journal. We expect that corresponding author will notify all co-authors of submission. We follow COPE guidelines for changes in authorship.

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Unless specified in the notification, the Editorial Board's decision on publication of the paper is final and cannot be appealed before making the major change in the manuscript.

Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

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Preparing your Manuscript

Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.

Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11'", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.

Format Structure

It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

All manuscripts submitted to Global Journals should include:

Title

The title page must carry an informative 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) where the work was carried out.

Author details

The full postal address of any related author(s) must be specified.

Abstract

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

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

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning 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 a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

Abbreviations

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends

Tables: Tables should be 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. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Eletronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). 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.

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Tips for Writing a Good Quality Science Frontier Research Paper

Techniques for writing a good quality Science Frontier Research paper:

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. 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.

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

4. Use of computer is recommended: As you are doing research in the field of science frontier then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.

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

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

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. 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 for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

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

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

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

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon 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 for 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 of 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 criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to 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 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 avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

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

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite 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 approaches 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. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a 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 limit the initial two items to no more than one line each.

Reason for writing the article—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 for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity 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 of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets 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 for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory 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 soundly written procedures segment allows a capable scientist to replicate 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 to give the least amount of information that would permit another capable scientist to replicate 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 section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show 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:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the 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—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and 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 as 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. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give 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 manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit 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 section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an 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 implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully 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 the prospect, and let it drop at that. Make a decision as to whether each premise is supported or 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 an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of 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 other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

The Administration Rules

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

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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
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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
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References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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