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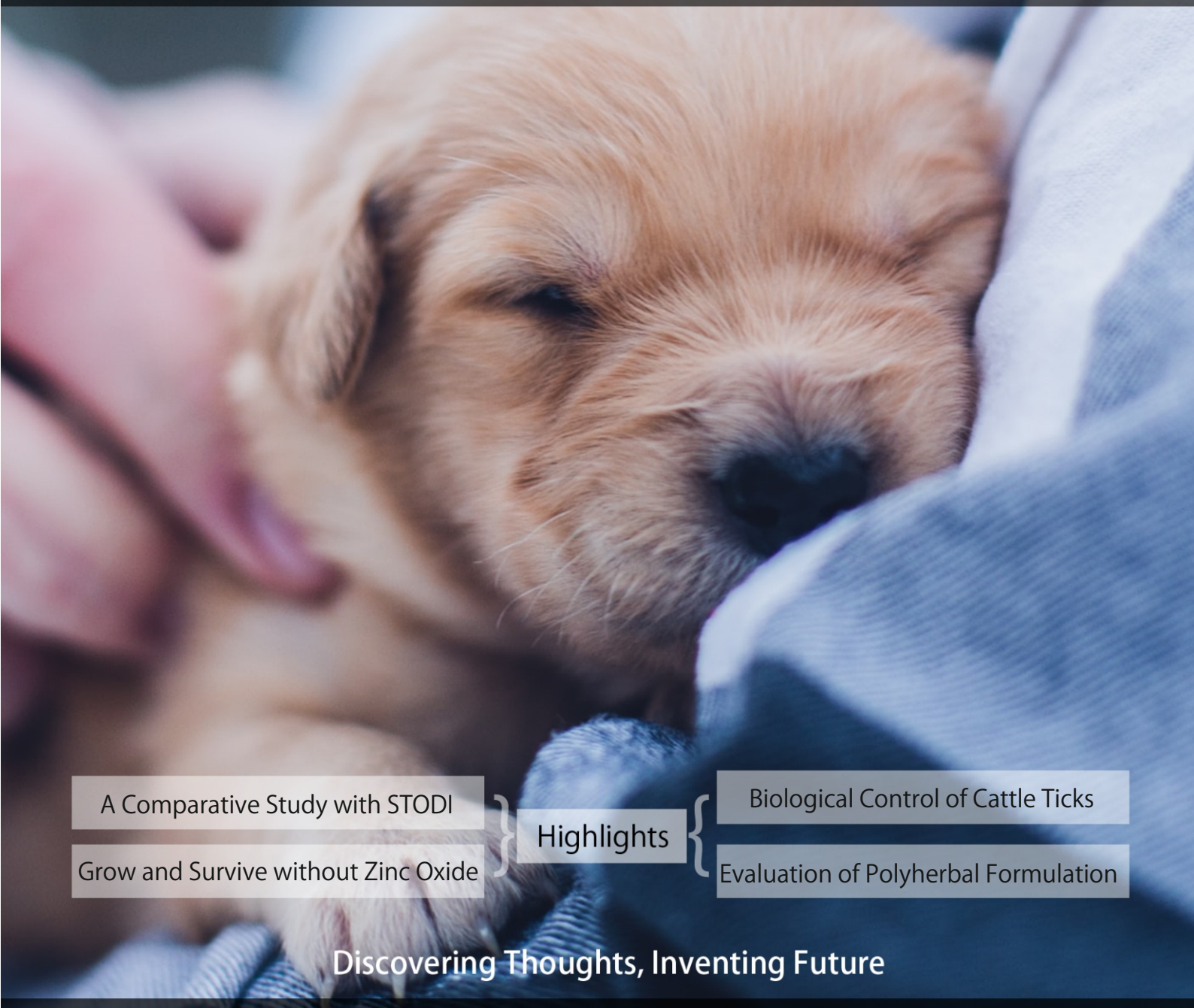

Highlights

A Comparative Study with STODI

Grow and Survive without Zinc Oxide

Biological Control of Cattle Ticks

Evaluation of Polyherbal Formulation

Discovering Thoughts, Inventing Future

VOLUME 22 ISSUE 2 VERSION 1.0

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Evaluation of Polyherbal Formulation at Different Dose Levels on Egg Production and Egg Quality Parameters in White Leghorn Layers Fed with High Energy Diets

By Saravanakumar Marimuthu & Prashanth D'Souza

Abstract- Choline is an essential nutrient in the poultry diet. It plays a vital role in the metabolism and mobilization of accumulated abdominal fat. In layer birds, it helps in improving the rate of egg production, egg quality and prevents fatty liver syndrome. However, synthetic choline has lots of demerits that need to be addressed. To mitigate the drawbacks of choline, the present study was conducted to evaluate the impact of Kolin Plus™, a polyherbal formulation (POH) manufactured by M/s Natural Remedies Pvt Ltd, Bengaluru, India, on the performance of the White Leghorn (WL) layer poultry birds. In the current trial, WL layer hens at the age of 41 to 55 weeks were distributed into 7 (G1 to G7) study groups having 6 replicates (20 birds/replicate) per group. All groups (G2 to G7) except the normal control group (G1) were supplied with high energy diet (HED).

Keywords: *layer poultry birds; choline chloride; polyherbal formulation; egg production.*

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Evaluation of Polyherbal Formulation at Different Dose Levels on Egg Production and Egg Quality Parameters in White Leghorn Layers Fed with High Energy Diets

Effect of Polyherbal Formulation on the Performance of White Leghorn Layers

Saravanakumar Marimuthu [α] & Prashanth D'Souza [σ]

Abstract- Choline is an essential nutrient in the poultry diet. It plays a vital role in the metabolism and mobilization of accumulated abdominal fat. In layer birds, it helps in improving the rate of egg production, egg quality and prevents fatty liver syndrome. However, synthetic choline has lots of demerits that need to be addressed. To mitigate the drawbacks of choline, the present study was conducted to evaluate the impact of Kolin Plus™, a polyherbal formulation (POH) manufactured by M/s Natural Remedies Pvt Ltd, Bengaluru, India, on the performance of the White Leghorn (WL) layer poultry birds. In the current trial, WL layer hens at the age of 41 to 55 weeks were distributed into 7 (G1 to G7) study groups having 6 replicates (20 birds/replicate) per group. All groups (G2 to G7) except the normal control group (G1) were supplied with high energy diet (HED). The layers of G3, G4 and G5 groups were fed with POH added diet at the dose range of 250, 500 and 750 g/ton of feed, respectively. Whereas the birds of G6 and G7 were fed with choline chloride 60% along with HED at a dose level of 500 and 1000 g/ton of feed, respectively. The efficacy of POH was compared with Choline chloride in the study. The layers were assessed for the performance parameters like egg production percentage, feed intake, feed intake per egg and egg quality parameters. The results showed that POH (500 g/ton of feed) supplementation in the layers' feed resulted in better performance among the hens as compared to choline chloride 60% (1000 g/ton) added group. Although, the egg quality data revealed no significant difference between the groups. In conclusion, POH at a concentration of 500 g/ton of feed can replace synthetic choline chloride 60% (1000 g/ton of feed) in layer hens fed with HED.

Keywords: layer poultry birds; choline chloride; polyherbal formulation; egg production.

I. Introduction

Choline is a constituent of the vitamin B complex and an essential nutrient in the poultry diet (Beheshti Moghadam et al., 2021). It is a vital component for building cell structure, metabolism and mobilization of fat. In laying birds, choline plays a crucial role in the removal of needless fat deposited in the hepatic tissue and prevents fatty liver disease (Chaudhari et al., 2017). It helps in synthesizing phosphatidylcholine (PC) that has a significant function in egg yolk formation (Khairani et al., 2016). Additionally, choline serves as a methyl group donor in methionine synthesis and actively influences egg production as well as the performance of the layers (Chaudhari et al., 2017). Choline is necessary for acetylcholine formation which helps in transmitting the nerve impulses (Chaudhari et al., 2017).

The layer birds often suffer from choline deficiency as they are unable to synthesize abundant choline. So layers necessitate choline intake through their diet (Parsons & Leeper, 1984). It was reported that the inclusion of choline in layers' diet enhances their reproductive performance, egg mass, egg weight, egg yield, albumin height, percentage of egg yolk weight, yolk colour and improves the Haugh unit (Chaudhari et al., 2017; Zhai et al., 2013). Thus, it is an indispensable nutrient in improving egg production and the quality of laying birds. The laying hens need an approximate amount of 1300, 900 and 500 mg/kg choline as a dietary supplement from 0 to 6th, 6th to 12th week and 12th week to age of laying eggs respectively (Chaudhari et al., 2017). Each egg comprises 12-13 mg of choline/g of dried whole egg mass (Chaudhari et al., 2017). According to Dänicke *et al.* (2006), the layers require choline at a concentration of 1500 mg/kg of feed for optimum performance. The dietary requisition of choline in layers as recommended by the US National Research Council (NRC) is 1050 mg/kg to get optimum yield (Zhai et al., 2013).

However, synthetic choline has several drawbacks. Choline is usually converted into trimethylamine (TMA) by gut microbiota which is corrosive in nature and causes toxicity (Selvam et al., 2018). The maximum permissible limit of TMA for birds is 200 ppm (Singh Rajesh Kumar, 2019). It triggers malabsorption and respiratory distress resulting in low

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reproductive performance and even death. Choline develops the lump of particles due to the hygroscopic property. Synthetic choline should have a uniform particle size for obtaining the optimum bio-availability (Singh Rajesh Kumar, 2019). Myriads of literatures have reported that the bioactive substances derived from plants may counter the demerits of synthetic choline (Selvam et al., 2018). Therefore, scientists are actively investigating the choline-like nutritional supplement of herbal origin to replace the synthetic choline from the layer's ration.

Kolin Plus™ is a proprietary polyherbal formulation (POH) comprised of *Acacia nilotica (A. nilotica)* and *Curcuma longa (C. longa)* plant parts developed by M/s Natural Remedies Pvt Ltd, Bengaluru, India. The phytoactive compounds present in POH are catechin, curcumin, gallic acid and polyphenols. They are proven to be antioxidant and lipotropic in nature. In the present study, the effect of POH supplementation in layers fed with a high energy diet (HED) has been explored for the rate of egg production and egg quality in comparison with synthetic choline chloride.

II. Materials and Method

a) Feed supplements

All protocols of the *in vivo* experiments were prepared and approved by Animal Ethics Committee of Natural Remedies Private Limited. The POH used as feed supplement in layers' diet, was comprised of powder of phytopharmaceuticals derived from *A. nilotica* and *C. longa* plant parts. HED was provided to the birds of all groups except normal control. HED contains basal diet with additional100 Kcal metabolic energy (ME). Whereas birds of normal control group were fed with basal diet only. The composition of basal diet and HED was mentioned in table 1. Choline chloride 60% was used as synthetic feed supplement in the experiment.

Table 1: Feed Composition of the layers

Nutrient	Basal Diet	HED
M.E (Kcal/kg)	2550	2650
CP (%)	13.7	13.7
dLysine, %	0.605	0.590
dMethionine+Cysteine, %	0.500	0.500
dTryptophan, %	0.139	0.137
dThreonine, %	0.512	0.498
dIso-leucine, %	0.512	0.500
dValine, %	0.637	0.603
Calcium, %	3.800	3.800
Sodium, %	0.170	0.170

b) Study design

The trial was conducted at Sri Ramadhoodha Poultry Farm, India. The experimental shed for housing the layer birds was maintained with standard room temperature and humidity as per the guidelines. The study was carried out for a duration of 12 weeks. The White Leghorn (WL) layers at the age of 41 to 55 weeks were distributed into 7 (G1 to G7) study groups as described in table 2. All birds except the normal control group were supplied with HED. The layers of G6 and G7 were fed with choline chloride 60% along with HED at a dose level of 500 and 1000 g/ton of feed, respectively. Whereas the birds of G3, G4 and G5 groups were fed with POH added diet at the dose range of 250, 500 and 750 g/ton of feed, respectively.

Table 2: Experimental design

Group	Dose (g/ton)	No. of birds / Replicate	No. of Replicates / Group	Duration
G1:Normal Control	-	20	6	12 weeks
G2:High Energy Diet Control (HED)	-	20	6	
G3:HED + POH	250	20	6	
G4:HED + POH	500	20	6	
G5:HED + POH	750	20	6	
G6:HED + Choline Chloride 60%	500	20	6	
G7:HED + Choline Chloride 60%	1000	20	6	
POH - Polyherbal formulation; HED - High Energy Diet; HED - Basal Diet + 100 Kcal ME. The extra energy should be provided by increasing the carbohydrate levels in feed				

c) Assessment parameters

The birds were monitored for the parameters like egg production percentage, feed intake and egg quality traits. The weekly experimental data of each group were recorded for a period of 12 weeks starting from 43 to 55 weeks of age of the birds and the mean value was calculated. The average value of 3 weeks *i.e.* week 41 to 43 of age of the birds was considered as baseline and used to compare with the recorded weekly values. The average feed consumption and feed intake per egg were estimated from week 1 to 12 of the entire study period. The weekly egg shell defect percentage for a period of 12 weeks study was evaluated. The egg quality parameters like egg density, Haugh unit (HU), egg shell weight and egg shell thickness were measured and compared between the groups.

d) Statistical analysis

All raw data of the trial were compiled and expressed as mean. The statistical analysis was performed using the one-way analysis of variance (ANOVA). The p value <0.05 was considered as statistically significant.

III. Results

The present study outcomes exhibited the effect POH as feed additive along with HED in the performance of the layer birds of 43 to 55 weeks of age. The baseline value of each parameter was calculated as per the average values from week 41 to 43 of the layers. Then the data were normalized using baseline value, and the POH results were compared with the data obtained from normal diet fed, HED fed, choline chloride 60%, and standard hen day production percentage (HDP).

a) Egg production %

As shown in table 3, the normalized egg production percentage (from week 1 to 6) of the layers fed with POH at a dose level of 500 g/ton of feed was similar as that of choline chloride 60% (1000 g/ton) supplementation. The results of normalized egg production rate of 7^{th} to 12^{th} week of POH supplementation at a dose range of 500 g/ton of feed revealed better performance as compared to normal, HED diet fed, and choline chloride 60% supplemented groups. Although, the baseline egg production was less in POH (500 g/ton) group as compared to standard HDP layers, the mean egg production rate (7-12 weeks) of POH was similar as that of standard value.

Table 3: Average percentage of egg production (EP) of layers

Group	Baseline	Average (1-6)	Diff	Average (7-12)	Diff	Average (1-12)	Diff
G1:Normal Control	94.67	90.74	-3.93	88.03	-6.64	89.38	-5.29
G2:High Energy Diet (HED)	93.83	90.10	-3.73	85.67	-8.16	87.88	-5.95
G3:HED + POH 250g	94.75	89.76	-4.99	85.76	-8.99	87.76	-6.99
G4:HED + POH 500g	93.50	90.52	-2.98	91.24	-2.26	90.88	-2.62
G5:HED + POH 750g	95.96	89.60	-6.36	83.08	-12.89	86.34	-9.62
G6:HED + CCL (60%) 500g	94.63	91.41	-3.22	88.53	-6.10	89.97	-4.66
G7:HED + CCL (60%) 1000g	96.54	93.51	-3.03	89.67	-6.87	91.59	-4.95
BV-300 Standard HDP (%)	94.97	93.65	-1.32	91.85	-3.12	92.75	-2.22

Values were expressed as Mean; POH - Polyherbal formulation; HED - High Energy Diet; CCL – Choline chloride; HDP – Henday egg production

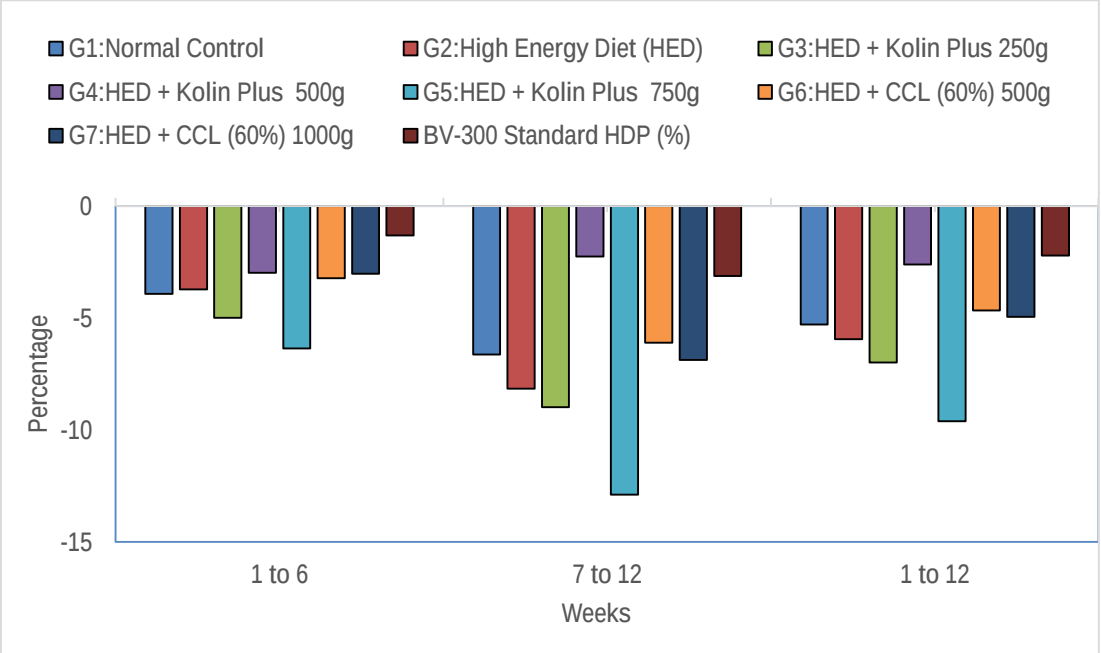

Figure 1: Difference in Egg Production (%) Compared to Baseline

POH - Polyherbal formulation; HED - High Energy Diet; CCL – Choline chloride

The histogram (Figure 1) showed that, the dip in egg production of POH (500g/ton) group in the first 6 weeks was similar as that of choline chloride 60% (1000g/ton) fed birds. The average (7-12 weeks and 1-12 weeks) egg production loss was less in POH (500g/ton) supplemented birds as compared to all other groups.

b) Feed intake

The results presented in table 4, showed that, the normalized (1st to 6th week) total feed consumption of the layer birds of POH (500 g/ton) supplemented group was less when compared to the HED control group. However, the feed intake was higher than choline chloride 60% (1000 g/ton) added group. The data of the normalized feed intake from 7 to 12 weeks revealed that POH (500 g/ton) supplementation resulted in low feed intake as compared to HED fed layers, but higher than choline chloride 60% (1000 g/ton) fed group. Similar result was observed in case of normalized feed intake from 1st to 12th week duration.

Table 4: Average feed intake (g) of the birds

Group	Baseline	Average (1-6)	Diff	Average (7-12)	Diff	Average (1-12)	Diff
G1:Normal Control	108.9	112.8	3.93	112.7	3.75	112.74	3.84
G2:High Energy Diet (HED)	110.9	108.7	-2.20	110.3	-0.65	109.48	-1.43
G3:HED + POH 250g	113.1	109.0	-4.08	109.1	-4.03	109.04	-4.06
G4:HED + POH 500g	113.1	109.1	-3.12	111.0	-2.08	110.50	-2.6
G5:HED + POH 750g	109.7	109.8	0.15	109.4	-0.33	109.61	-0.09
G6:HED + CCL (60%) 500g	110.5	109.4	-1.10	109.0	-1.53	109.18	-1.32
G7:HED + CCL (60%) 1000g	113.6	109.0	-4.60	110.0	-3.65	109.48	-4.12
BV-300 Standard	112.0	112.0	0.00	113.5	1.50	112.75	0.75

Values were expressed as Mean; POH - Polyherbal formulation; HED - High Energy Diet; CCL – Choline chloride

The normalized feed intake per egg in first 6 weeks duration was less in POH (500 g/ton) added group as compared to normal control and HED fed layers. But the feed intake of POH fed layers was observed as higher than choline chloride 60% (1000 g/ton) supplemented group. The data obtained from the layers regarding the normalized feed intake per egg of week 7 to 12, exerted that POH (500 g/ton) supplemented birds consumed less feed as compared to choline chloride 60% (1000g/ton) group. Similar results were observed in case of normalized feed intake per egg from 1st to 12th week of the experiment.

Table 5: Average feed intake (g) per egg of the layers

Group	Baseline	Average (1-6)	Diff.	Average (7-12)	Diff.	Average (1-12)	Diff.
G1:Normal Control	115.1	124.6	9.47	128.3	13.20	126.43	11.33
G2:High Energy Diet (HED)	118.3	120.8	2.53	129.4	11.05	125.09	6.79
G3:HED + POH 250g	119.4	121.7	2.28	127.5	8.08	124.58	5.18
G4:HED + POH 500g	121.0	121.7	0.70	121.9	0.88	121.79	0.79
G5:HED + POH 750g	114.3	122.7	8.43	132.0	17.72	127.38	13.08
G6:HED + CCL (60%) 500g	116.8	119.9	3.10	123.3	6.53	121.62	4.82
G7:HED + CCL (60%) 1000g	117.7	116.7	-1.03	122.8	5.05	119.71	2.01
BV-300 Standard	118.0	119.7	1.67	123.5	5.5	121.58	3.58

Values were expressed as Mean; POH - Polyherbal formulation; HED - High Energy Diet; CCL – Choline chloride

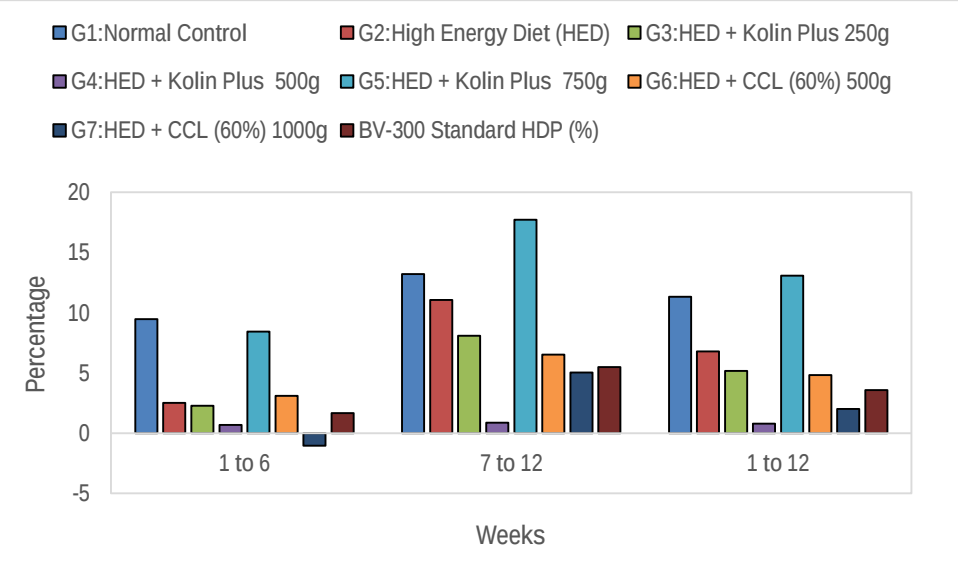

Figure 2: Difference in Feed Intake per Egg (%) compared to baseline

The normalized (1-12 weeks) feed intake per egg of POH (500g/ton) group was better than all the groups including choline chloride 60% (1000g/ton) added diet fed layers.

c) Egg shell defects (ESD) %

As shown in table 6, the average egg shell defects percentage of 1 to 6, 7 to 12 and 1 to 12 weeks of study duration showed values of within the range in all groups in case of before as well as after supplementation.

Table 6: Average percentage of egg shell defects of the layers

Group	Baseline	Average (1-6)	Average (7-12)	Average (1-12)
G1:Normal Control	0.840	0.570	0.350	0.490
G2:High Energy Diet (HED)	1.420	0.760	0.260	0.580
G3:HED + POH 250g	0.710	0.970	0.490	0.730
G4:HED + POH 500g	0.940	0.490	0.200	0.390
G5:HED + POH 750g	1.350	0.710	0.380	0.60
G6:HED + CCL (60%) 500g	1.670	0.580	0.50	0.630
G7:HED + CCL (60%) 1000g	0.390	1.020	0.840	0.890
Values were expressed as Mean; POH - Polyherbal formulation; HED - High Energy Diet; CCL – Choline chloride				

d) Egg quality

The egg quality parameters like average egg shell density, Haugh unit, egg shell weight and egg shell thickness were assessed for 6 weeks and 12 weeks duration of the study and compared between the groups. There was no significant difference noticed between the groups (table 7 and 8).

Table 7: Average density (g/cm^3) and Haugh Unit (HU) of the eggs

Group	Egg Density (g/cm^3)			HU		
	Baseline	6 weeks	12 weeks	Baseline	6 weeks	12 weeks
G1:Normal Control	1.086	1.077	1.067	72.17	76.83	75.50
G2:High Energy Diet (HED)	1.083	1.072	1.065	73.83	75.83	72.50
G3:HED + POH 250g	1.082	1.077	1.060	73.83	75.50	72.83
G4:HED + POH 500g	1.082	1.078	1.062	76.83	77.67	75.83
G5:HED + POH 750g	1.086	1.076	1.060	75.83	75.67	70.17
G6:HED + CCL (60%) 500g	1.083	1.078	1.059	73.67	73.67	70.00
G7:HED + CCL (60%) 1000g	1.089	1.074	1.066	80.00	63.17	70.50
Values were expressed as Mean; POH - Polyherbal formulation; HED - High Energy Diet; CCL – Choline chloride						

Table 8: Average eggshell weight (g) and egg shell thickness (mm)

Group	Egg Shell Weight (g)			Egg Shell Thickness (mm)		
	Baseline	6 weeks	12 weeks	Baseline	6 weeks	12 weeks
G1:Normal Control	6.169	5.890	5.084	0.413	0.372	0.378
G2:High Energy Diet (HED)	6.009	5.282	5.294	0.401	0.362	0.370
G3:HED + POH 250g	5.735	5.687	5.059	0.402	0.380	0.352
G4:HED + POH 500g	5.796	5.606	4.953	0.414	0.385	0.358
G5:HED + POH 750g	5.838	5.465	5.063	0.401	0.375	0.353
G6:HED + CCL (60%) 500g	5.778	5.589	4.965	0.404	0.380	0.357
G7:HED + CCL (60%) 1000g	5.992	5.431	5.219	0.404	0.370	0.365
Values were expressed as Mean; POH - Polyherbal formulation; HED - High Energy Diet; CCL – Choline chloride						

IV. Discussion

Choline is an essential nutrient for poultry and ubiquitously present in plant as well as animal cells. It is the fundamental component to build the structure of cells. Choline is required for synthesizing phospholipid and phosphatidylcholine that are obligatory for maintaining the integrity of cell membrane (Dänicke et al., 2006). Choline acts as methyl group donor to form methionine which has an important role in protein synthesis (Dänicke et al., 2006). It helps to metabolize fat and mobilize the excess lipid accumulated in the liver of the layers fed with HED (Beheshti Moghadam et al., 2021). In spite of numerous functions of choline, poultry birds cannot synthesize it in sufficient amount (Zhai et al., 2013). Therefore, choline supplementation is necessary in the diet of the birds. Synthetic choline chloride is routinely included as feed additive in layers' diet for a longer period. Although, it has lots of drawbacks like poor absorption rate, hygroscopicity, corrosiveness, non-uniform particle size, residual TMA formation (Selvam et al., 2018). Moreover, TMA concentration exceeding the permissible amount causes

severe respiratory distress leading to fatality among the birds. To get rid of these demerits, scientists are in quest of choline like feed additives of natural origin.

POH is a polyherbal compound developed by M/s Natural Remedies Pvt Ltd, Bengaluru, India. The formulation is comprised of the bioactive constituents of *A. nilotica* and *C. longa* plant parts. Both the plants are well known for their multifarious ethnomedicinal properties. The key chemical constituents present in *A. nilotica* are polyphenols like gallic acid and catechin (Malviya et al., 2011). It is a promising antioxidant and proficient in reducing the oxidative stress (Adewale, 2016). It upsurged the oxidative enzyme activity like catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione-Stransferase (GST) in the liver of N-nitrosodiethylamine induced toxicity in rats (Malviya et al., 2011). Thus, it may act as hepatoprotectant and prevent lipid peroxidation. This statement was in agreement with the investigations done by Narayanan et al (Kannan et al., 2013). The treatment with the plant caused a significant reduction in cholesterol and triglyceride levels in the rodents (Kannan et al., 2013). *C. longa* is the rich source of phytopharmaceutical curcumin which is a natural antioxidant and hepatoprotectant. It scavenges the oxygen free radicals and enhances the activity of antioxidant enzymes. Previous literatures suggested that curcumin efficiently metabolized the dietary fat and accumulated lipid in liver (Labban, 2014; Tranchida et al., 2015). It may alter the fatty changes in liver, biliary hyperplasia and CCl4 induced hepatic injury in rats (Akram et al., 2010). In an experiment, hot water extract of *C. longa* showed a preventive effect against hepatic lipid deposition (Mun et al., 2019). Thus, the choline-like property of POH may exhibit a potential role in fat metabolism and lipotropic effect.

In the present study, POH supplementation in the diet of the layers of 43 to 55 weeks of age showed promising performance among the birds. HED in layer birds results in accumulation of fat in the abdomen and hepatic cells (Shini et al., 2019). However, previous studies also corroborated that herbal choline was a good alternative of synthetic choline chloride and might efficiently metabolize the hepatic fat of the birds (Gangane et al., 2010). Furthermore, it was reported that POH is efficient in fat metabolism and mobilization from hepatocytes to egg cells and improves the production rate. POH at a dose range of 500 g/ton in feed showed better egg production percentage (normalized %) as compared to choline chloride 60%. In addition, the results showed that normalized feed intake per egg percentage, in case of POH (500 g/ton) group was less than choline chloride fed birds. These results revealed that POH might efficiently enhance the performance of the layers. However, the egg quality based on egg density, HU, egg shell weight and egg shell thickness showed no significant differences between the groups. The bioactive compounds present in plant parts of *A. nilotica* and *C. longa* of POH helped in fat metabolism and prevented abdominal fat deposition leading to optimal egg production.

V. Conclusion

In brief, POH at a dose range of 500 g/ton as a natural feed supplement exhibited better performance among the layers of 43 to 55 weeks of age. The cumulative performance parameters viz. percentage of egg production, feed intake (g/egg) were improved following supplementation of POH (500g/ton) as compared to normal control and HED control groups and the performance of POH was similar to CCL 60% (1000g) group. However, no significant change in egg quality parameters was observed between the groups. POH (500 g/ton) exerted choline like characteristics and an ideal replacement of synthetic choline chloride 60% (1000 g/ton) in BV 300 layers fed with HED.

Abbreviations

Phosphatidylcholine (PC); Haugh unit (HU); Trimethylamine (TMA); Polyherbal formulation (POF); High-energy diet (HED); White Leg (WL); Metabolic energy (ME); Difference (diff); One-way analysis of variance (ANOVA); Egg shell defects (ESD); Catalase (CAT); Superoxide dismutase (SOD); Glutathione peroxidase (GPx); Glutathione-S transferase (GST)

Conflict of Interest

None

Acknowledgement

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Association of Cyclopropane Fatty Acid Synthesis with Thermo-Tolerance of Campylobacter Survival

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Abstract- *Aims:* To investigate the possession of the cyclopropane fatty acid synthase *(cfa)* gene by thermo-tolerant and non-thermo-tolerant *Campylobacter* spp. and to examine the presence of the *cfa* gene in *Campylobacter* that survived the scalding stage of poultry processing and to further investigate the *cfa* gene expression at 37ºC in different *C. jejuni* strains that are able or not able to survive at 52ºC.

Methods and Results: The presence of the *cfa* gene in *Campylobacter* strains was determined by PCR. In order to determine the effect of heating on survival, the thermophilic *C. jejuni* were grouped by those collected before and those collected after the scalding stage process both groups being exposed to 52ºC. Quantitative RT- PCR was performed to verify the gene expression level of *cfa* at 37ºC in *C. jejuni* strains able or not able to survive at 52ºC.

Keywords: *campylobacter, cfa gene, heat tolerance, survival, poultry processing.*

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Abstract- ***Aims:*** To investigate the possession of the cyclopropane fatty acid synthase *(cfa)* gene by thermo-tolerant and non-thermo-tolerant *Campylobacter* spp. and to examine the presence of the *cfa* gene in *Campylobacter* that survived the scalding stage of poultry processing and to further investigate the *cfa* gene expression at 37ºC in different *C. jejuni* strains that are able or not able to survive at 52ºC.

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The *cfa* gene was present in thermophilic isolates and absent in non-thermophilic isolates. The *C. jejuni* strains collected after the scalding process surviving 52°C showed to differ significantly in terms of survival P< 0.0420. The *C. jejuni* strains surviving high temperature at 52ºC had higher expression of the *cfa* gene when grown at 37°C and these strains were able to survive elevated temperatures dependent on the level of the *cfa* gene expressed during the stress response.

Conclusions: The *cfa* gene has a direct role for the adaptation of *C. jejuni* during exposure to scalding tank temperatures which are in excess of 50ºC and serves to increase the resistance to the thermal stress in these strains.

Significance and Impact of Study: The *cfa* gene plays a role in the ability of thermophilic Campylobacter spp. to grow at temperatures above 37ºC and therefore tolerate heat stress.

Keywords: campylobacter, cfa gene, heat tolerance, survival, poultry processing.

I. Background

Campylobacter spp. are the leading cause of bacterial foodborne diarrheal disease worldwide (WHO, 2018). Poultry are believed to be the main contributor to human cases of *Campylobacter* spp. with the bacteria being found in both live and slaughtered chickens (Skirrow and Blaser, 2000, Wittenbrink, 2002). The majority of *Campylobacter* spp. associated with chicken carcasses are identified as *C. jejuni* and *C. coli* (PHE, 2015, Wieczorek and Osek, 2015) and these two species are most frequently found in human cases in developed countries. Campylobacter spp. are exposed to many stress factors such as survival in the acidity of the host gut, survival on food and in the environment (Oh et al. 2018). The organism is capable of adapting to these stresses by regulating specific gene expression in response to stress (Murphy et al., 2006), such as *dps, sodB, trxB,* and *ahpC,* oxidative stress defence genes. Gene expression of these are increased through exposure to acid stress (Birk *et al.* 2012). During exposure to cold-shock Campylobacter increases the expression of *sodB* and *Cjo358* stress response genes are increased (Stintzi and Whitworth, 2003). Depending on the stress conditions involved *Campylobacter* has different survival rates (Reid *et al.* 2008).

Many other species of bacteria have the ability to change physiologically during starvation or environmental stress; for example by modifying their membrane lipids in situ by changing the phospholipid unsaturated fatty acid (UFA) to cyclopropane fatty acids (CFA) (Grogan and Cronan, 1997). The presence of cyclopropane ring-containing lipids, especially phospholipids, has been reported for many bacterial species including *Escherichia coli* and *Salmonella typhimurium* and there is a strong correlation between acid survival and chlorosome glycolipid molecules in heat protection (Mizoguchi *et al.* 2013).

Konkel *et al.* (1998) reported that *C. jejuni* preferentially synthesises 24 proteins immediately following heat shock. One of the major heat-shock proteins is DnaJ, which enables *Campylobacter* to colonise chickens while it has been shown that *Campylobacter* DnaJ mutants cannot colonise chickens. This suggests that DnaJ (HSP 40) plays an essential role in *C. jejuni's* thermal tolerance at temperatures above 42ºC (Konkel *et al.* 1998). However, DnaK and DnaJ are both found in non-thermophilic *Campylobacter* and so cannot be a factor in determining thermotolerance (Riedel *et al.* 2020).

The presence of CFAs in bacterial fatty acid membranes has been shown to provide protection against temperature changes and it is therefore likely that lipid composition of membranes changes when the microbial growth temperatures change (Russell *et al.*1995). The aim of this study was to investigate the possession of the cyclopropane fatty acid synthase gene by thermo-tolerant and non-thermo-tolerant

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Campylobacter spp. and to examine the presence of the *cfa* gene in Campylobacter that survived the scalding stage of poultry processing and to further investigate the *cfa* gene expression at 37ºC in different *C. jejuni* strains that are able or not able to survive at 52ºC.

II. Material and Methods

a) Origin of isolates

A total of 60 *C. jejuni* isolates were collected from commercial poultry processing plants in the UK. Samples were taken from carcasses early during the processing cycle, at the post-bleed, immediately after leaving the scalding tank (post-scald) and at the post-chill stages, and 21 isolates (7 from each stage of process) were chosen to be used within the framework of the study. Twenty *Campylobacter* strains for the environmental group were obtained from the laboratory culture collection and original came from different sources (water, sheep, soil). All *Campylobacter* isolates had been identified to the species level using conventional multiplex PCR (Lund et al. 2004).

b) Bacterial strains and culture conditions

The *C. jejuni* isolates were recovered from frozen storage by direct plating onto blood agar (BA; Oxoid Ltd.). Plates were incubated at 37ºC for 48 h in a MACS-MG 1000 anaerobic cabinet (MAC-Cabinet; Don Whitley Scientific, Shipley, UK) with a microaerobic gas mixture consisting of 10% CO_2, 5% O_2 and 2% H_2, balanced in N_2. These cultures were used for Deoxyribonucleic acid (DNA) extraction.

c) DNA Extraction

The DNA from the samples was extracted using the QIAamp® DNA extraction Kit (Qiagen, Crawley UK) according to manufacturer's instructions. Eluted DNA was stored at -20ºC until used.

d) PCR for detection of the cfa gene in Campylobacter isolates

DNA was extracted from *C.jejuni* isolates in order to examine the presence or absence of the *cfa* gene, PCR primers specific for this gene were designed based on the gene sequence information in the Campy database which was determined by conventional PCR using custom-designed primers (Table 1). Twenty-five µl PCR reaction mix were prepared as follows: 12.5 µl GoTag mastermix (Promega, UK), 1 µl forward primer (1:10 dilution), 1 µl reverse primer (1:10 dilution), 9.5 µl nuclease-free water and 1 µl DNA. The cycling conditions were as follows: 95ºC for 10 min and then 40 cycles of 95ºC for 60 seconds, 50ºC for 60 seconds, and 72ºC for 90 seconds. Samples were incubated at 72ºC for 5 min and held at 4ºC until processed. A 10 µl aliquot was taken from the amplified PCR products and analysed by gel electrophoresis at 100 V for 90 min using 1X TBE (0.89 M Tris borate, 0.02 M EDTA) running buffer on 2% agarose gels (Sigma-Aldrich). Gels were stained with 10 mg/ml ethidium bromide solution (Sigma-Aldrich) and visualised on a UV gel documentation system. A DNA-molecular ladder (50-bp and 100-bp ladder) (Hyper ladder, Bioline, UK) was included in each gel so that the size of products could be determined. Presence of a band of the appropriate length was taken to be a positive result. *C. fetus* (NCTC 10842) was used as a negative control and *C. jejuni* (NCTC 11168) was used as a positive control.

e) Purification of PCR products for sequencing

The PCR products were purified using the QIAquick® PCR purification Kit (Qiagen) according to the manufacturer's instructions. A 15ul aliquot of purified DNA with a concentration of 1 ng/ µl (150- 300 bp) was mixed with 2 µl of 10 pmol/ µl (10 µM) sequencing primer (cfa forward) in a microcentrifuge tube (Eurofins). Purified PCR products were sent to Eurofins Genomics, MWG Operon (Ebersberg, Germany) for sequencing. Sequence data was assembled with Multiple Sequence Alignment (Clustral Omega) and CLC Sequence Viewer 6.7.1 (CLC bio, Aarhus, Denmark) was used to align the sequences.

f) Heat tolerance

In order to determine the effect of heating on survival, 71 *C. jejuni* strains were divided into two groups: pre and post scald, 36 and 35 isolates were collected from each group respectively. As described by Hughes et al (2009) with slight modification, the isolates were recovered from frozen storage as described above. To determine survival at 52ºC, isolates were sub cultured in 50 ml Mueller Hinton broth (Oxoid), in a Bijoux and a 10µl loopful of bacteria was added and mixed thoroughly for each sample. These were incubated microaerobically at 37ºC for 48 h using the MAC-Cabinet. Following incubation 50 µl from each sample was pipetted into a 300µl microcentrifuge tube which was placed in a water bath at 52ºC for 30 mins. Serial dilutions were made in microtitre plates by pipetting 180 µl of PBS (Oxoid) into the wells of rows two to eight and 40 µl of samples into the wells of row one. Twenty µl of samples from row one was removed and mixed into the next row. This was continued across the plate. To check for the presence of bacteria in the wells 20 µl samples from each well were pipetted onto BA plates and incubated under microaerobic conditions at 37ºC for 48 h as described above. The number of bacteria that survived was determined using the following formula.

Number of bacteria= N x D x 50 x^{d}10, in which: N= number of bacteria per spot, D and d = dilution number

g) Gene expression analysis

Twenty-four different *C. jejuni* strains were recovered from frozen storage as described above. Following recovery, they were plated onto BA and

incubated at 37ºC for 48 h in a microaerobic atmosphere using the Campygen gas generating system (Oxoid). A 10µl loopful of culture was inoculated into 7 ml Mueller Hinton broth (Oxoid) in 7 ml Bijoux and incubated under microaerobic conditions at 37ºC for 48 hours as described previously. Prior to extraction a 100µl aliquot of culture broth was pipetted into MH broth in a 25 cm^2 tissue culture flask at 37ºC for 24 hours. Following incubation 2 ml from each culture was pipetted into 2.0 ml microcentrifuge tubes. The tubes were centrifuged at 8000 g for 10 minutes. To the resulting pellet, 800 µl of tri reagent (Sigma) was added and the tubes left at ambient temperature for 10 minutes. This was followed by the addition of 200 µl chloroform (Sigma) and the tubes were then centrifuged for 10 min at 13000 g. The upper aqueous layer was removed while avoiding the interphase and this was transferred to a clean 2.0 ml microcentrifuge tube (Fisher Scientific). A Qiagen® RNeasy Mini kit was used for RNA extraction following manufacturer's instructions. The extracted RNA was frozen immediately at -80 ºC for further analysis.

h) Real Time -Polymerase Chain Reaction

Quantitative RT- PCR was performed to verify the gene expression level of *cfa* at 37ºC in *C. jejuni* strains able or not able to survive at 52ºC. The following primer sequences were used for detection of *cfa* and *16S* rRNA genes in *C. jejuni* isolates: The forward cfa-RT 5' ACTATGAGCTATTCTTGCGCT 3' (21) reserve cfa-RT 5' AACCCCAGCCACCAACCTATA 3' (20), the forward 16S rRNA CCAGCAGCCGCGGTAAT (17) and the 16S rRNA GCCCTTTACGCCCAGTGAT (19) using the QuantiTect SYBR Green RT-PCR kit (QIAGEN) according to the manufacturer's recommendations. The comparative threshold (Ct) value corresponds to the PCR cycle at which the first detectable increase in fluorescence associated with the exponential growth of PCR products occurs, using comparative threshold cycle ($\Delta\Delta C_T$) (Livak and Schmittgen, 2001). The relative expression of each gene was determined three times in each of three experimental RNA samples, normalised to the *16S* rRNA reference gene and expressed as fold difference in quantity of cDNA molecules present in *C. jejuni* that could survive (+ve) at 52ºC to that present in *C. jejuni* that could not survive (-ve) at 52ºC.

i) Statistical analysis used in this study

For determination of *P*-value in heat tolerance experiments.

Fisher's analysis, a 2x2 contingency table and one-tailed *P*-value was used. *P* values were considered to be significant <0.05.

P-values for the gene expression work were determined using SPSS, Mann-Whitney U test (*P* value <0.05 was considered to be significant) using Graph Pad Prism (V.6.0) software package for the graph Mac (Graphpad, San Diego, USA).

III. Results

a) Presence of Campylobacter cyclopropane fatty acid synthase gene

The presence of the *cfa* gene in *Campylobacter* strains grouped by source was determined by PCR (Table 1). The *cfa* gene was present in all *C. jejuni* strains isolated from the poultry abattoir (Table 2), whereas, in the non-abattoir associated strains, the cfa gene was absent in *C. fetus, C. helveticus, C. sputorum* and *C. fecalis* (Table 2). The strains isolated from abattoirs originally came from chickens and were adapted to 42ºC (chicken body temperature). In strains isolated from environmental sources (non-chicken), where the temperature was below 42ºC the gene was absent (Table 2).

b) Heat tolerance survival

Thirty-six samples of *C. jejuni* collected before and 35 collected after the scalding stage process 'both groups were exposed to 52ºC' showed to differ significantly in terms of survival $P< 0.0420$ (Table 3), with the proportion of strains collected after the scalding process surviving 52ºC being roughly twice as high as before the scalding process.

c) The cfa gene expression

Two groups of *C. jejuni* isolated from chicken abattoirs, previously identified to contain a *cfa* gene using PCR, were exposed to 52ºC in a waterbath. The results obtained by QRT-PCR analysis using the *cfa* as the gene targets showed that expression of *cfa* mRNA relative to *16S* mRNA differed. When cultured at 37ºC, *C. jejuni* strains turned out to have the ability to survive at 52ºC and had a significantly higher expression of the *cfa* gene compared to *C. jejuni* strains that appeared not to survive at 52 ºC (Figure 1).

IV. Discussion

The ability of *C. jejuni* to tolerate conditions found during processing can be considered an important factor associated with their survival (Oh *et al.* 2018). To understand the mechanisms involved, *Campylobacter* spp. isolates from the environment and *C. jejuni* from various location in the chicken slaughter line were compared for their ability to produce the *cfa* gene, which has been shown previously to be associated with increased survival at elevated temperature (Grogan and Cronan, 1997, Zhang and Rock, 2008). In the present study, *C. jejuni* strains isolated from processing plants are more likely to possess the *cfa* gene, whereas the *cfa* gene was not present in *C. fetus, C. helveticus* or *C. sputorum* isolated from non- abattoir sources (Table 2). The source was seen to have a great influence on the presence of the

cfa gene and the presence of this gene could explain thermophilic survival temperatures above 37°C (Table 2). The upregulation of the *cfa* gene was seen when the bacterial cell started to enter the stationary phase in E. coli (Chang et al. 2000b) and under thermal resistance they modified the profile of their phospholipid fatty membrane (Annous *et al.*1999; Zhang and Rock, 2008). The biosynthesis of CFAs effected stability and integrity of the cell membrane at high temperatures (Guzzo, 2011; Dufourcet al. 1984). This change supports the role of CFAs in the stress tolerance which is encoded by the *cfa* gene and enables cells to physiologically adapt to the condition of heat stress (Guzzo, 2011). Interestingly, all isolates obtained from the processing plants had the *cfa* gene present (Table 2), suggesting that Campylobacter strains colonising birds were able to withstand the challenge of the heat during processing plant regardless of *cfa* gene synthase.

During slaughter, Campylobacter spp. in and on chicken carcasses are subjected to temperatures higher than 50°C (Osiriphun *et al.* 2012) and this is a form of stress because under normal conditions Campylobacter does not grow at temperatures higher than 42°C (Park, 2002). To mimic circumstances of heat tolerance survival and maintenance of the survival response in *C. jejuni* strains were examined for survival of 52°C (Table 3). This experiment demonstrated a higher capacity of post scald *C. jejuni* strains to adapt to and survive heat (Table 3). A temperature of 52°C was selected as the whole chicken carcass is subjected to a scald tank with a water temperature of approximately 52 to 55°C to remove the feathers (Lehner *et al.,* 2014).In the present study the differences in survival between isolates collected pre and post scald stage were significant ($P < 0.05$). A large proportion of the *C. jejuni* strains from the post scald stage can survive higher temperatures compared to those from the pre scald stage (Table 3). The ability to adapt to higher temperatures correlates with the upregulation of specific genes, including *groEL* and *rpoD* that enhance survival of heat-stress (Klancniket al. 2008). The heat-stress response mechanisms of *C. jejuni* resulted in changes in morphology and protein profile when exposed to 48°C and to 55°C for a short time and their culturability and viability correlated with an altered protein profile and decreased virulence properties (Klancnik *et al.* 2014).

Heat stress is a key feature of poultry processing and only the bacteria that survive these abattoir stresses can reach human hosts. Since high temperature causes a physical change in the composition of the bacterial membrane lipids (Zhang and Rock, 2008), it has been demonstrated that the *cfa* gene has evolved responsively to this change (Chang *et al.* 2000). In the present study the thermophilic *C. jejuni* strains that survived at 52°C and cultured at 37°C were significantly more likely to increase the level of the relative *cfa*/rRNAgene expression compared to the control strain of *C. fetus* (Figure 1). This suggests that the *cfa* gene alters the fatty acid composition significantly in order to stabilise the membrane (Dufourc *et al.* 1984, Chang and Cronan, 1999). *C. fetus* does not have the *cfa* gene (Table 2) so is not able to grow at temperatures higher than 37°C. This confirms the involvement of *cfa* in cyclopropane fatty acids biosynthesis. The findings presented here show that there is significant variation in the relative *cfa*/rRNA levels expressed between strains surviving at 52°C and strains unable to survive at this temperature when both groups are cultured at 37°C (Figure 1). *C. jejuni* strains surviving at 52°C expressed the *cfa* gene and are adapted to survive when cultured at 37 0C as evidenced by its increased response characteristics of altered CFA during temperature growth. Similar studies observed that the *cfa* gene was regulated under stress conditions in other pathogens. For example, E. coli expressed the *cfa* gene under acid adaptation (Grandvalet *et al.* 2008) and *S. typhimurium* induced the expression of the *cfa* gene under acid stress (Kim et al. 2005).

The proportion of *cfa* mRNA transcripts increases with the increasing amount of the CFA in the bacterial membrane (Chang and Cronan, 1999). In the present study different expression was seen with *C. jejuni* strains that were able to survive at 52°C, where some strains had a low level of expression of mRNA in the *cfa* gene (less than 0.2 relative *cfa*/ *16S* rRNA levels of gene expression) (Figure 1). This suggests that not all strains express *cfa* to the same extent. In previous studies the low level of CFA synthesis was linked to the level of conversion of UFAs to CFAs and to the substrate specificity of the CFA synthase (Grandvalet *et al.* 2008).

Although the two major shock proteins DnaK and DnaJ are found in non-thermophilic *Campylobacter,* their role is small and limited. Some non-thermophilic *Campylobacter* are unable to colonise the chicken gut (Kempf *et al.* 2006) as the chicken's body temperature is 41.7 ºC. Results suggest that the *cfa* gene may in some way play a role in the ability of *C. jejuni* to colonise chickens and/or to persist in the chicken gut. This to suggest that the lack of the *cfa* gene could be a factor in determining thermo-tolerance. Thermo-tolerant bacteria are able to colonise chickens, as the presence of the gene will allow cells to adapt to survive above 37 ºC. Campylobacter isolates that can colonise birds were able to withstand the challenge of the processing plant regardless of *cfa* gene synthase (Table 2). Absence of the *cfa* gene was associated with the inability of the organism to colonise birds (Hermans *et al.* 2011).

This study also found that the source of Campylobacter species and the presence of the *cfa* gene could explain the difference in ability of strains to tolerate high temperatures. These data support the role of the *cfa* gene in the thermophilic pathogen for promoting its survival as reported in other studies (Chen *et al.* 2014; Chang *et al.* 2000).

Temperatures above 42^{0}C may not be optimal for *Campylobacter* spp. to grow (Stintzi, 2003), but *C. jejuni* strains are adapting their survival at 52^{0}C by expressing the *cfa* gene. The expression level of *cfa* synthase involved in levels of different heat stress response mechanisms was described by Klancnik *et al.* (2006) with *C. jejuni* having elevated level of gene products in response to heat stresses. The change of the fatty acid membrane composition depends on the biosynthetic reactions that use modified lipid acyl components (Russell, 2002). The expression of the *cfa* synthase gene is reported to be important in the survival of *S. typhimurium* during the stationary phase, as this bacterium expresses high levels of this gene when entering this stage and the expression of *cfa* mutants was absent (Kim *et al.* 2005).

The present study shows that there is a degree of variation seen in the relative *cfa*/rRNA levels expressed in the strains surviving at 52^{0}C (Figure 1). Some *C. jejuni* strains have a higher *cfa* gene expression than other strains also surviving at 52^{0}C and this indicates that these strains may alter their fatty acid composition in a different way during survival to stabilise their membrane and to cope with higher temperatures. This finding is supported by Hughes et al. (2009) who found that *C. jejuni* altered different fatty acids due to temperature challenge. The difference in the *cfa* gene expression levels has also been observed in several strains of *E. coli* when the bacteria is induced to express the *cfa* gene and the subsequent synthesis of CFA in cell membrane phospholipids that increases to protect the cells from death (Chang and Cronan, 1999). As shown in Figure 1 some *C. jejuni* strains surviving at 52^{0}C show lower levels of mRNA expression at 37^{0}C and this may account for a lack of the major change in fatty acid composition, but these strains are still able to express the *cfa* gene which is required in order to alter their fatty acid composition to tolerate high temperatures. The findings indicate that the *cfa* synthesis gene was present in abattoir strains but not in the non-poultry abattoir group, suggesting that *cfa* contributes to the ability of Campylobacter to grow at temperatures above 37^{0}C. Strains of *C. jejuni* that survived passage through the abattoir scald tank were more likely to be able to survive at elevated temperatures than pre-scald strains.

This study shows that the change in gene expression induced by *C. jejuni* survival at 52 0C is evidence to suggest that the expression of the *cfa* gene is an important factor for survival at elevated temperatures and allows the consequential survival of *Campylobacter* spp. throughout the poultry processing chain which ultimately leads to infection in humans (Skarp *et al.* 2016).

In conclusion, these results provide evidence that thermophilic *C. jejuni* cells induced the *cfa* gene that is important for acquisition of heat resistance. A link of this thermo-tolerance to the *cfa* gene expression associated synthesis of high levels of cyclic fatty acids impacts the survival during the scalding stage of poultry processing. Understanding the mechanism of Campylobacter during poultry abattoir processing may help in improving control measures to reduce the burden of Campylobacter and implementing strategies to prevent disease.

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Table 1: Primers used in this study for detection of the *cfa* gene in *Campylobacter* isolates

The number of bases of each primer is indicated in brackets after the sequence.

Target gene	Primers		Sequence (5'-3')
Cfa	*cfa1*	Forward	GTWTTTTGGGATMAAGAAG (19)
		Reverse	TCCAACAACCTTAACTCC (18)
Cfa	*cfa2*	Reverse	GCAGARGCACAAGAACGAAG (20)
		Forward	GGGGTTGGCTTTCTATTATG (20)
Cfa	*Cfa3*	Forward	TTGATTTAAAAGAAGGAGAAAAGC (24)
		Reverse	CATGTTCAAACATACCCACAGA (22)

Table 2: Presence of the *cfa* gene in *C. jejuni* isolates from the poultry abattoirs and non-abattoir*. The PCR was used to detect the gene, using a specific primer sequence. Twenty samples from pre and post scald were chosen to represent all PFGE profiles.

Samples	Pre and Post Scald stage	PFGE profile	Sources*	*Cfa* presence
*C. jejuni*3A3/2	Pre	C7	Abattoir	YES
*C. jejuni*2A5/2	Pre	C6	Abattoir	YES
*C. jejuni*1A4/2	Pre	C23	Abattoir	YES
*C. jejuni*1B3/1	Post	C2	Abattoir	YES
*C. jejuni*1A3/2	Pre	C1	Abattoir	YES
*C. jejuni*1B1/1	Post	C4	Abattoir	YES
*C. jejuni*1A2/3	Pre	C4	Abattoir	YES
*C. jejuni*2A4/2	Pre	C3	Abattoir	YES
*C. jejuni*1A1/3	Pre	C7	Abattoir	YES
*C. jejuni*1A1/4	Pre	C3	Abattoir	YES
*C. jejuni*1A5/2	Pre	C27	Abattoir	YES
*C. jejuni*3B4/2	Post	C6	Abattoir	YES
*C. jejuni*2B2/3	Post	C7	Abattoir	YES
*C. jejuni*2B5/1	Post	C7	Abattoir	YES

*C. jejuni*1A2/3	Pre	C4	Abattoir	YES
*C. jejuni*1A3/3	Pre	C12	Abattoir	YES
*C. jejuni*3A4/2	Pre	C5	Abattoir	YES
*C. jejuni*1B5/1	Post	C23	Abattoir	YES
*C. jejuni*3B2/3	Post	C5	Abattoir	YES
*C. jejuni*3B3/2	Post	C7	Abattoir	YES
C.sputorumss.sputorum NCTC 11528	-	-	Non-abattoir	NO
C. sputorum ss. fecalis NCTC 11367	-	-	Non-abattoir	NO
C. jejuni ss doylei NCTC 11951	-	-	Non-abattoir	NO
C. sputorum ss. fecalis NCTC 11415	-	-	Non-abattoir	NO
C. helveticus NCTC 12470	-	-	Non-abattoir	NO
C. fetus ss fetus NCTC 10842	-	-	Non-abattoir	NO

**Abattoir samples: thermophilic C. jejuni; Non-abattoir: other Campylobacter species*

Table 3: Heat survival of *C. jejuni* strains at 52 ºC from abattoirs isolated before and after exposure to the scald tank. Fisher's exact test was used to determine the differences between the heat tolerance groups. *P*-values less than 0.05 were considered significant.

	N. of sample	+ve survive 52 ºC	-ve survive 52 ºC	P value
Pre-scald	36	7	29	0.0420
Post-scald	35	15	20	

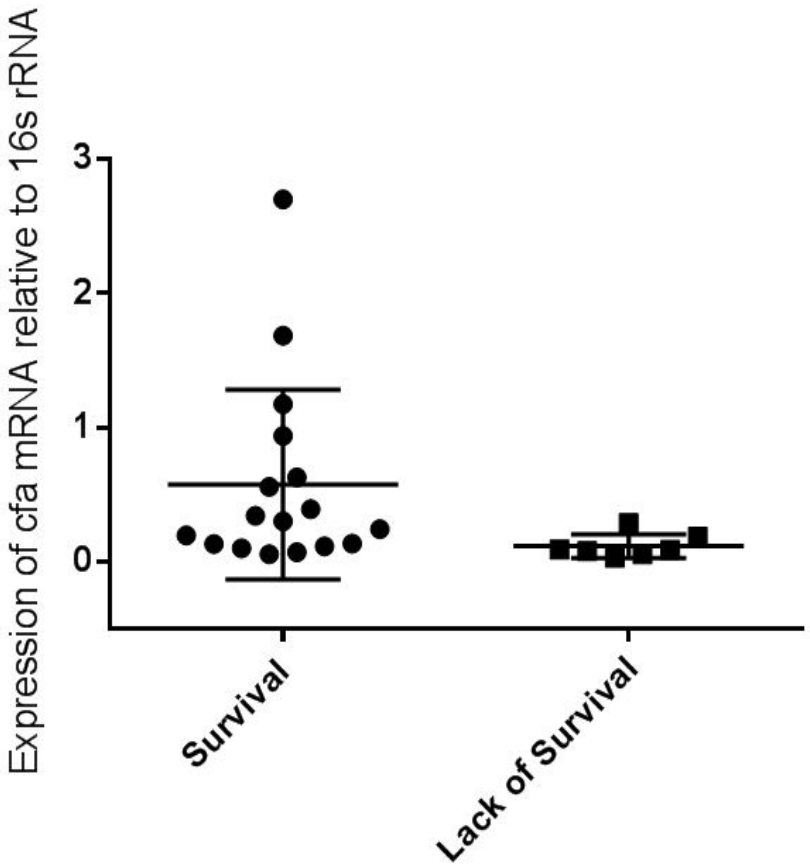

Figure 1: Relative *cfa*/ *16S* rRNA levels of gene expression at 37 ºC after *C. jejuni* strains were exposed to 52 ºC, showing either survival (+ve) or lack of survival (-ve) at this temperature. Both groups were assessed by real-time PCR (control strain of *C. fetus* which lacks the *cfa* gene). Twenty-four different *Campylobacter* spp. isolates were used. Error bars show the standard deviations, experiments were carried out in triplicate, using Mann-Whitney U test (*P* value <0.05)

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How to Grow and Survive without Zinc Oxide in Pig Production? A Comparative Study with STODI®

By Saravanakumar Marimuthu & Prashanth D'Souza

Abstract- During weaning, piglets are exposed to a range of stressors, all of which cause gastrointestinal and immune system issues. Post-Weaning Diarrhoea (PWD) is a multifactorial illness characterized by the frequent secretion of watery faeces, which can cause stunt growth, morbidity, and even death. STODI® is the most incredible option for ZnO to reduce PWD and increase pig growth performance in the first few weeks after weaning. STODI® is a polyherbal combination including *Acacia nilotica, Andrographis paniculata, Holarrhena antidysenterica, Punica granatum, and Terminalia bellirica*. This can lower the incidence of diarrhoea and promote the growth of the piglet.

Keywords: piglet, post-weaning diarrhoea, STODI® and ZnO.

GJMR-G Classification: DDC Code: 636.400954 LCC Code: SF396.I4

Strictly as per the compliance and regulations of:

How to Grow and Survive without Zinc Oxide in Pig Production? A Comparative Study with STODI®

Saravanakumar Marimuthu [α] & Prashanth D'Souza [σ]

Abstract- **During weaning, piglets are exposed to a range of stressors, all of which cause gastrointestinal and immune system issues. Post-Weaning Diarrhoea (PWD) is a multifactorial illness characterized by the frequent secretion of watery faeces, which can cause stunt growth, morbidity, and even death. STODI® is the most incredible option for ZnO to reduce PWD and increase pig growth performance in the first few weeks after weaning. STODI® is a polyherbal combination including *Acacia nilotica, Andrographis paniculata, Holarrhena antidysenterica, Punica granatum, and Terminalia bellirica*. This can lower the incidence of diarrhoea and promote the growth of the piglet.**

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I. Introduction

Piglets are exposed to various stress factors during weaning, all of which lead to gastrointestinal and immune system problems. Nutritional, environmental, physiological, and health aspects are examples of these factors. The early post-weaning stage is often marked by poor growth performance.

Post-weaning diarrhoea (PWD) is a multifactorial condition characterized by frequent discharge of watery faeces that can cause a growth check, morbidity and mortality (Madec *et al*., 2002). To reduce PWD and increase pig growth performance in the first few weeks after weaning, pig breeders have traditionally depended on antibiotic growth promoters in pre-starting and starter feeds. This was standard practice until antibiotics as various government agencies outlawed growth promoters. Following the restriction, pig breeders were forced to look for other options to preserve good gut health and avoid post-weaning performance declines. As a result, large amounts of ZnO in piglet meals (2,000 ppm or greater) became more popular, and the swine industry accepted it as standard practice. It was demonstrated to be an effective and reasonably cost strategy for avoiding and controlling post-weaning diarrhoea, improving growth performance, feed intake, and digestion in piglet.

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Though there are various advantages to utilizing ZnO in piglet diets, recent publications have focused on its environmental impact and role in antimicrobial resistance spread. However, it is hardly surprising that regulations governing its usage are evolving and that the European Union voted to prohibit the use of therapeutic levels of ZnO in June 2022. It's a need of time to find an alternative to ZnO. STODI® is a proprietary polyherbal formulation developed by Natural Remedies Pvt. Ltd. contains a blend *of Acacia nilotica, Andrographis paniculata*, *Holarrhena antidysenterica, Punica granatum* and *Terminalia bellirica*. This can potentially reduce the incidence of diarrhoea and improve piglet immunity by augmenting gut integrity, normalizes gut peristalsis, anti-inflammatory effects on the intestinal lumen and enhancing the gut microbiome. Hence, it can be used to replace the ZnO with additional benefits to the pig production. Thus, the current study aims to test the effect of STODI® on the development and growth of piglets by preventing the post-weaning diarrhoea.

II. Research Methodology

The present study was conducted in South Lleida, Spain on 26 days old 262 (nos.) Dan Bred Hybrid piglet for seven weeks. Two hundred and sixty two piglets were divided into control (129) and STODI® (133) supplemented groups based on body weight. The two groups were housed in two sheds with identical environmental conditions and installations. The sheds were divided into five pens, with approximately 25 piglets each. The trial protocol was prepared as per the guidelines laid by the Institutional Animal Ethics Committee (Natural Remedies Private Limited).

During the pre-starter phase (26-36 days), the control group was fed with ZnO (3100 ppm), and the treatment group were fed with STODI® (4 kg/ton of feed). In starter phase 1 (37-51 days), the control group were fed with 300mg Amoxicillin, and the treatment group were fed with 2 kg STODI®/ton of feed and 300mg Amoxicillin. In starter phase 2 (52-61 days), the control group was fed without any supplementation and the treatment group with 2 kg STODI®/ton of feed. The general running and routine of the farm were not altered. Initial body weight was recorded at starting of the trial,

followed by after the pre-starter phase (Day 36), at the end of starter phase 1 (Day 51) and at the transition exit (after the end of starter phase 2). The faecal score was not taken for each piglet but for each pen. Morbidity and mortality were also observed during the trial.

III. Results and Discussion

Body weight, average daily gain and the faecal score of piglets of both the groups, are given in table 1, after replacing ZnO with STODI® having similar body weight gain and faecal score. Morbidity and mortality were found to be zero percent during the trial. It may be due to the active compound present in STODI®.

The mixture of polyherbal plants and their active compounds and properties are discussed below. Antimutagenic (Aqil *et al.,* 2008), antibacterial (Aqil & Ahmad, 2007) and immunomodulatory (Atal *et al.,* 1986) characteristics have been identified for *Holarrhena antidysenterica*. It has both gut stimulatory and inhibitory components, which acts through histaminergic and Ca^{++} antagonist pathways, explaining the plant's folkloric use in gastrointestinal motility problems such as constipation, colic, and diarrhoea. Extracts of the bark, leaves, immature fruit and fruit rind of pomegranate (*Punica granatum L.*) have been given to halt diarrhoea, dysentery and haemorrhages (Agunu *et al.,* 2011). *Acacia spp*. is a versatile tree that exhibits antiplasmodial activity (El-Tahir *et al.*, 1999) and has been used to cure various ailments, including diarrhoea, dysentery, and bleeding piles (Rahaman, 2010). *Andrographis paniculata* is an important medicinal plant and is widely used around the world. It has been reported to have a broad range of pharmacological effects, including antidiarrheal (Gupta *et al*., 1990) and immunostimulatory (Iruretagoyena *et al*., 2005). *T. bellirica* as a whole or its specific components possess ethnomedicinal attributes and are used in various herbal formulations. It is used as an astringent, laxative, anthelmintic (Kumar and Khurana, 2018) and antidiarrheal agent (Singh, 2011). The above properties present in STODI® (a mixture of polyherbal plants) may help to improve the growth performance and body weight gain of piglets by preventing PWD.

Table 1: Effects of STODI® supplementation on Body Weight and Faecal Scoring

Parameters	Control	STODI®
Initial Body Weight	6.19	6.35
BW after end of pre-starter phase (Day 36)	9.58	9.83
BW after end of starter phase 1 (Day 51)	14.36	14.89
BW after end of starter phase 2 (Day 61)	20.84	20.83
Average Daily Gain (g)	419	414
Faecal Score	1.18	1

IV. Conclusion

The current study demonstrates that STODI® can substitute Zinc Oxide without impairing the growth performance of piglets with an improved faeces score.

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Biological Control of Cattle Ticks through Native Entomopathogenic Nematodes *(Steinernema Carpocapsae)*

By S. Kachhawaha, Ajay Kumar Maru & A.U. Siddiqui

Abstract- Entomopathogenic nematodes have been successfully used as biological control agents for insects of economically important crops. In the present study, the bioefficacy of two different strains of entomopathogenic nematodes, *Steinernema carpocapsae* STSLU and *S. carpocapsae* STUDR against two different cattle hard ticks, *Rhipicephalus microplus* and *Hyalomma savignyi* was evaluated based on percentage mortality under laboratory conditions. The adult female of cattle ticks were inoculated with infective juveniles (IJs) of the strains *S. carpocapsae* at different inoculum levels. All the treatments were replicated four times at 20° C in a B.O.D. incubator. The percentage mortality of the cattle ticks was determined every 24 hours up to 120 hours from the time of inoculation.

Keywords: ticks, biological control, epns, entomopathogenic nematodes, steinernema carpocapsae, rhipicephalus microplus, hyalomma savignyi.

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Biological Control of Cattle Ticks through Native Entomopathogenic Nematodes *(Steinernema Carpocapsae)*

S. Kachhawaha [α], Ajay Kumar Maru [σ] & A.U. Siddiqui [ρ]

Abstract- Entomopathogenic nematodes have been successfully used as biological control agents for insects of economically important crops. In the present study, the bioefficacy of two different strains of entomopathogenic nematodes, *Steinernema carpocapsae* STSLU and *S. carpocapsae* STUDR against two different cattle hard ticks, *Rhipicephalus microplus* and *Hyalomma savignyi* was evaluated based on percentage mortality under laboratory conditions. The adult female of cattle ticks were inoculated with infective juveniles (IJs) of the strains *S. carpocapsae* at different inoculum levels. All the treatments were replicated four times at 20º C in a B.O.D. incubator. The percentage mortality of the cattle ticks was determined every 24 hours up to 120 hours from the time of inoculation. The experimental results showed the cattle tick *R.microplus* was more susceptible to both strains than *H. Savignyi.* Whereas the *S. carpocapsae* STSLU was more efficient than *S. carpocapsae* STUDR and which cause 100 and 97.5 % mortality of *R. microplus* and *H. savignyi*, respectively at a concentration of 250 IJs/Petri dish after 120 hours of inoculation. The entomopathogenic nematode can be cultured easily in an artificial medium and highly reproductive efficiency, broad host range and longer storage ability, easily apply and safe for the host. Both the tested strains were promising to control *R. Microplus* and *H. savignyi.* This may be evaluated further in field conditions in different seasons and temperatures. Future research may be directed towards emerging technologies of ticks control without acaricide uses.

Keywords: ticks, biological control, epns, entomopathogenic nematodes, steinernema carpocapsae, rhipicephalus microplus, hyalomma savignyi.

I. Introduction

Ticks can be found on many hosts, including cattle, buffalo, horses, donkeys, goats, sheep, deer, pigs, dogs, and wild animals. Ticks are one of the leading monetary menaces to the cattle industry worldwide, affecting productivity, health and welfare. They are obligate blood-feeding ectoparasites that infest 80 percent of the cattle worldwide (Grisi *et al*., 2014). Livestock are the major source of livelihood but due to unhygienic in a herd and open grazing the chances of ectoparasite in livestock will be more common and causing heavy blood losses, irritation, hide damage and weight losses resulting in lower productivity (Kaur *et al*., 2016). Loss of appetite in heavily tick-infested cattle was found responsible for 65 % of the bodyweight reduction (Seebeck, 1971). These ectoparasites are among the most critical health problems like babesiosis, theileriosis, anaplasmosis and anemia (Kocan *et al*., 1998). Ticks are highly responsible for economic losses worldwide, putting food safety at risk (Fernanedz-salas *et al*., 2017). In India, almost all the livestock species suffer from tick infestations India alone the cost of ticks and ticks born diseases (TTBDs) in animals has been estimated direct loss of more than 2000 crore per annum (Ghosh et al., 2007). According to the FAO (2004), 80 % of the world`s cattle population is exposed to ticks infestation and has estimated the impact of 7.3 US S/head/year. In addition to directly affecting their hosts, ticks are also the most important group of parasitic arthropods as vectors of pathogens that affect domestic animals and wildlife (Perez de Leon *et al*., 2020). Tick-borne pathogens are the foremost reason for transboundary livestock diseases, listed as notifiable by the World Organization for animal health (Esteve-Gasent *et al*., 2020). The TTBDs have been recognized as a major cause of production loss predominantly in tropical and subtropical countries of the world (De Castro, 1997; Parthiban *et al*., 2010; Lurthu *et al*., 2012; Arunkumar and Nagarajan, 2013; Mondal *et al*., 2013). Since the beginning of 20th centuary investigators have documented numerous potential tick bio-control agents including pathogens, parasitoids and predictors of ticks (Samish & Alexseev, 2001). Entomopathogenic nematodes (EPNs) are parasites of insects. These are characterized by carrying specific symbiotic bacteria of the genus *Xenorhabdus* or *Photorhabdus* in their intestine (Boemare *et al*. 1993). Symbiotic bacteria play an important role in the pathogenicity of the nematodes bacteria complex to insect host and the subsequent reproduction of the nematodes in the host (Akhurst and Boemare 1990). EPNs are currently used as biopesticides to control several important insect pests worldwide (Shapiro Ilan *et al*., 2002).

EPNs are associated with symbiotic bacteria therefore they are extraordinary lethal to many important

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soil insect pests. Biological control of insect pests using EPNs has gained importance in current years. Because they are highly virulent and killing their host within 24 to 48 hrs. They can be cultured easily *in vivo* as well as *in vitro* (on artificial diet), longer storage ability, have a high reproductive potential, broad host range, and can easily be applied in soil and foliage without adverse effects on non-target organisms (Georgis *et al*., 1991). They are safe for plant and animal health. Recently, it has been demonstrated that the entomopathogenic nematode, *Steinernema carpocapsae* has the potential to use as a biological control agent against cattle tick, *Rhipicephalus microplus* and *Hyalomma savignyi*, which is considered to be the most important tick parasite of livestock in the world (Monteiro *et al*., 2010). The major objective of the present investigation was to determine the effects of *Steinernema carpocapsae* on mortality of *R. microplus* and *H. Savignyi* at different levels of inoculums under laboratory conditions for effective bio-control of cattle ticks.

II. Materials and Methods

The bio-efficacy test of indigenous EPNs strains of *Steinernema carpocapsae* STSLU and *S. carpocapsae* STUDR were conducted on important cattle tick, *Rhipicephalus microplus* and *Hyalomma savignyi* under laboratory conditions. Total sterilized 24 Petri plats were used for this experiment. The 25 cattle ticks were placed on Whatman filter paper no. 1 in each Petri plate and inoculated infective juveniles (IJs) from both the strains of *S. carpocapsae* at different inoculum levels *viz.*, 50, 100, 150, 200 and 250 IJs/ Petri plate. All the treatments were replicated four times and placed at 20° C under B.O.D. incubator condition. The observations were taken on per cent mortality of cattle ticks after every day up to 5 days from the time of inoculation.

III. Results

The experiment was conducted for evaluating the potential of the entomopathogenic nematodes (EPNs) indigenous strains *S. carpocapsa*e against cattle ticks at different inoculum levels under laboratory conditions. The bio-efficacy was tested based on percent mortality of the cattle ticks *R. microplus* and *H. savignyi* were found susceptible against both the strains of *S. carpocapsa*e STUDP-1 and STSLU under laboratory conditions. The maximum mortality of *R. microplus* was recorded 100 per cent with *S. Carpocapsae* STSLU followed by 97.5 with *S. carpocapsae* STUDP-1 @ 250 IJs per tick after 120 hrs (Table 1). Whereas the maximum per cent mortality of *H. Savignyi* was 97.5 per cent with *S. Carpocapsae* STSLU followed by 92.5 with *S. carpocapsa*e STUDP-1 @ 250 IJs per tick after 120 hrs (Table 2).

IV. Discussion

Tick mortality caused by EPNs seems to be due to the rapid proliferation of the nematode symbiotic bacteria within the ticks, since the nematodes do not go through their natural cycle within ticks and most infective juveniles die shortly after entry (Hassanain *et al*. 1999). In vitro experiments demonstrated that tick hemolymph hinders the growth of EPNs (Zangi, 2003). Similar studies in this regard were made by Kocan *et al.* (1998) who also reported that infective juveniles (IJs) of different EPNs strains (*Steinernema glaseri, S. riobravus, S. carpocapsae, S. feltiae* and *Heterorhabiditis bacteriophora*) appeared to be the most effective in killing ticks and invaded and killed 30 to 100% of replete females. Samish *et al.* (2000) reported that the mortality of *Rhipicephalus bursa*, and *Rhipicephalus sanguineus* adult ticks were recorded after 0.3 to 8.0 days of their exposure in Petri dishes to 5 entomopathogenic nematode strains. Maru *et al.* (2011) also recorded a cent per cent mortality of *R. microplus* was observed at 500 *S. carpocapsae* IJs/Petri plate after the fourth day of inoculation. Similar studies were made by Samish *et al.* (1999) that the Mexican strain of *Steinernema carpocapsae* was most efficient, inducing 100% tick mortality at a concentration of 50 nematodes per square centimeter to our study 97.5 % mortality of ticks through EPN.

V. Conclusion

The development of anti-tick biological control agents is still in its babyhood. Furthermore, the various steps required for commercialization of these products (production, storage and delivery) and education of consumers (storage, application and evaluation of results) are still in the future. Ticks infestation is a significant cause of economic losses to the dairy industry all over the world. At present, acaricides are mostly used for tick's control. To the extent possible, dairy farmers and veterinarians should make use of an integrated tick control strategy based on the utilization of biological control methods, breeding for tick resistance breeds etc. Nematodes are potentially used tools for ticks control because engorged ticks are susceptible to EPNs. However, the use of nematodes may be limited to defined ecological niches because their pathogenicity is reduced by low humidity or temperature and differences in the susceptibility among the various tick stage and species. Ticks have numerous natural enemies but Entomopathogenic have only a limited pragmatic role in tick's control. At present TTBDs control is mainly affected by the widespread use of acaricides like organophosphates, carbamates, pyrethroids, BHC/cyclodines, amidines, macrocyclic lactones and benzoyl phenyl ureas leading to various problems such as resistance, residues, environment pollution and high cost. These factors reinforce the need for alternative

approaches to control ticks infestations. Several plants and herbs have been shown to possess anti-tick insecticidal, growth-inhibiting, antimolting and repellent activities. A number of reports are available on the use of vaccines for tick control on the horizon effect of different extracts of plant material on tick species. Due to severe problems associated with the continuous use of acaricides on animals, integrated ticks management is recommended. Increasing public health concern over tick-born diseases demands the strategic control of ticks on animals that transmit diseases to human beings. The development of improved formulations is also important. Finally, in-depth studies are needed to elucidate the interaction between nematodes and ticks under field conditions.

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Competing Interests

The author declares that he has no competing interests.

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Table 1: Bioefficacy of *S.carpocapsae* STUDP-1 and *S. Carpocapsae* STSLU against *R. microplus* Data in parenthesis are angular transformed values

No. of IJs/ insect	EPNs	Percent mortality at different time intervals (hrs.)				
		24	48	78	96	120
50	*S.carpocapsae* STUDP-1	10.0 (18.44)	25.0 (30.00)	37.5 (37.76)	60.0 (50.77)	72.5 (58.37)
	S.carpocapsae STSLU	12.5 (20.70)	27.5 (31.63)	47.5 (43.57)	65.0 (53.73)	75.0 (60.00)
100	*S.carpocapsae* STUDP-1	22.5 (28.32)	40.0 (39.23)	52.5 (46.43)	70.0 (56.79)	85.0 (67.21)
	S.carpocapsae STSLU	25.0 (30.00)	45.0 (42.10)	67.5 (55.24)	75.0 (60.00)	85.0 (67.21)
150	*S.carpocapsae* STUDP-1	35.0 (36.27)	50.0 (45.00)	67.5 (55.24)	82.5 (65.27)	92.5 (74.11)
	S.carpocapsae STSLU	42.5 (40.69)	55.0 (47.87)	75.0 (60.00)	85.0 (67.21)	92.5 (74.11)
200	*S.carpocapsae* STUDP-1	52.5 (46.43)	65.0 (53.73)	75.0 (60.00)	92.5 (74.11)	95.0 (77.08)
	S.carpocapsae STSLU	55.0 (47.87)	75.0 (60.00)	85.0 (67.21)	92.5 (74.11)	97.5 (80.90)
250	*S.carpocapsae* STUDP-1	67.5 (55.24)	77.5 (61.68)	85.0 (67.21)	95.0 (77.08)	97.5 (80.90)
	S.carpocapsae STSLU	65.0 (53.73)	82.5 (65.27)	90.0 (71.56)	97.5 (80.90)	100.0 (90.00)
Control	Water	0.0	0.0	0.0	0.0	0.0
SEm±		0.637	1.302	2.709	2.806	2.443
CD (0.05%)		1.920	3.924	8.166	8.457	7.363
CV (%)		16.98	9.41	10.53	8.44	6.37

Table 2: Bioefficacy of *S. carpocapsae* STUDP-1 and *S. carpocapsae* STSLU against *H. savignyi*

No. of IJs/ insect	EPNs	Percent mortality at different time intervals (hrs.)				
		24	48	78	96	120
50	*S. carpocapsae* STUDP-1	5.0 (4.05)	12.5 (20.70)	17.5 (24.73)	32.5 (34.76)	57.5 (49.31)
	S. carpocapsae STSLU	5.0 (4.05)	12.5 (20.70)	27.5 (31.63)	47.5 (43.57)	67.5 (55.24)
100	*S. carpocapsae* STUDP-1	12.5 (20.70)	25.0 (30.00)	32.5 (34.76)	52.5 (46.43)	70.0 (56.79)
	S. carpocapsae STSLU	15.0 (22.79)	25.0 (30.00)	47.5 (43.57)	65.0 (53.73)	75.0 (60.00)
150	*S. carpocapsae* STUDP-1	25.0 (30.00)	42.5 (40.69)	55.0 (47.87)	67.5 (55.24)	80.0 (63.44)
	S. carpocapsae STSLU	30.0 (33.21)	47.5 (43.57)	57.5 (49.31)	75.0 (60.00)	85.0 (67.21)
200	*S. carpocapsae* STUDP-1	37.5 (37.76)	55.0 (47.87)	65.0 (53.73)	80.0 (63.44)	87.5 (69.30)
	S. carpocapsae STSLU	42.5 (40.69)	65.0 (53.73)	75.0 (60.00)	85.0 (67.21)	92.5 (74.11)
250	*S. carpocapsae* STUDP-1	45.0 (42.13)	62.5 (52.24)	77.5 (61.66)	90.0 (71.56)	92.5 (74.11)
	S. carpocapsae STSLU	57.5 (49.31)	72.5 (58.37)	82.5 (65.27)	90.0 (71.56)	97.5 (80.90)
Control	Water	0.0	0.0	0.0	0.0	0.0
SEm±		0.636	1.311	2.739	2.856	2.453
CD (0.05%)		1.909	3.933	8.217	8.567	7.359
CV (%)		16.87	9.29	10.57	8.47	6.36

Data in parenthesis are angular transformed values

Fig. 1: Photomicrograph of *Rhipicephalus microplus* parasitized by *Steinernema carpocapsae*

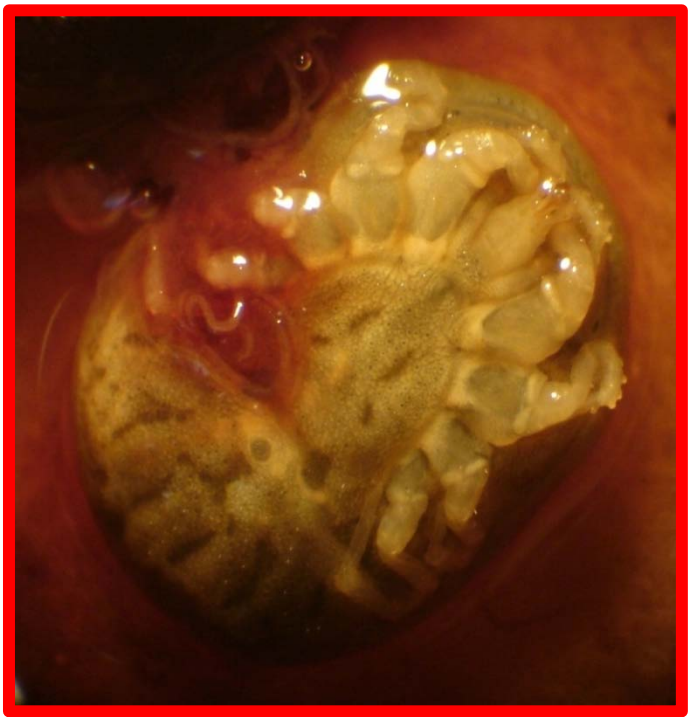

Fig. 2: Photomicrograph of *Hyalomma savignyi* parasitized by *Steinernema carpocapsae*

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All the Fellow members of FMRC get a badge of "Leading Member of Global Journals" on the Research Community that distinguishes them from others. Additionally, the profile is also partially maintained by our team for better visibility and citation. All fellows get a dedicated page on the website with their biography.

Career | Credibility | Reputation

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Career | Credibility | Reputation

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Career | Credibility | Financial

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Early invitations to all the symposiums, seminars, conferences

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All members get access to 5 selected scientific museums and observatories across the globe. All researches published with Global Journals will be kept under deep archival facilities across regions for future protections and disaster recovery. They get 10 GB free secure cloud access for storing research files.

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ASSOCIATE OF MEDICAL RESEARCH COUNCIL is the membership of Global Journals awarded to individuals that the Open Association of Research Society judges to have made a 'substantial contribution to the improvement of computer science, technology, and electronics engineering.

The primary objective is to recognize the leaders in research and scientific fields of the current era with a global perspective and to create a channel between them and other researchers for better exposure and knowledge sharing. Members are most eminent scientists, engineers, and technologists from all across the world. Associate membership can later be promoted to Fellow Membership. Associates are elected for life through a peer review process on the basis of excellence in the respective domain. There is no limit on the number of new nominations made in any year. Each year, the Open Association of Research Society elect up to 12 new Associate Members.

To the institution

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Global Journals sends a letter of appreciation of author to the Dean or CEO of the University or Company of which author is a part, signed by editor in chief or chief author.

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Certificate, LoR and Laser-Momento

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Career | Credibility | Exclusive | Reputation

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Recognition on the Platform

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Career | Credibility | Reputation

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Associates can publish articles (limited) without any fees. Also, they can earn up to 30-40% of sales proceeds from the sale of reference/review books/literature/publishing of research paper

Exclusive | Financial

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Get a remuneration of 15% of author fees

Associate members are eligible to join as a paid peer reviewer at Global Journals Incorporation (USA) and can get a remuneration of 15% of author fees, taken from the author of a respective paper.

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Associate	Fellow	Research Group	Basic
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Certificate, LoR and Momento **2** discounted publishing/year **Gradation** of Research **10** research contacts/day **1 GB** Cloud Storage **GJ** Community Access	**Certificate,** LoR and Momento **Unlimited** discounted publishing/year **Gradation** of Research **Unlimited** research contacts/day **5 GB** Cloud Storage **Online Presense** Assistance **GJ** Community Access	**Certificates,** LoRs and Momentos **Unlimited** free publishing/year **Gradation** of Research **Unlimited** research contacts/day **Unlimited** Cloud Storage **Online Presense** Assistance **GJ** Community Access	**GJ** Community Access

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

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:

1. Authors must go through the complete author guideline and understand and *agree to Global Journals' ethics and code of conduct,* along with author responsibilities.
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.
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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

It is required for authors to declare all financial, institutional, and personal relationships with other individuals and organizations that could influence (bias) their research.

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Plagiarism is not acceptable in Global Journals submissions at all.

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- Writings
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- Graphic representations
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- Any other original work

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2. Drafting the paper and revising it critically regarding important academic content.
3. Final approval of the version of the paper to be published.

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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.

Declaration of funding sources

Global Journals is in partnership with various universities, laboratories, and other institutions worldwide in the research domain. Authors are requested to disclose their source of funding during every stage of their research, such as making analysis, performing laboratory operations, computing data, and using institutional resources, from writing an article to its submission. This will also help authors to get reimbursements by requesting an open access publication letter from Global Journals and submitting to the respective funding source.

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.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

Tips for writing a good quality Medical 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 medical research 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:

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- 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.

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

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