



# Impact of *In-Ovo* inoculation of *Aloe Vera* (L.) Burm. F. Medicinal Plant Leaf Extracts on Pre-hatching Growth Responses of Embryo of Broiler Chickens

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## Abstract

*In-Ovo* injection with exogenous materials (Plants leaf extract) used as natural growth promoters, throughout incubation could be a technique to boost up pre-hatching performance of broiler embryo. The objective of the current study was to observe the impact of *in-Ovo* injection of *Aloe vera* leaf extracts on the subsequent pre-hatching growth responses of broiler chickens. One hundred twenty fertile eggs of ISA Brown layer breeders were used in the current study and purchased from District Poultry Farm, Dera Ghazi Khan. The eggs were randomly distributed into four groups. Group 1 (control group, without any injection), group 2 received an injection of 100 ml of 0.9% normal saline, 100 ml of 10 µg/ml of *Aloe vera* was inoculated in eggs of group 3, group 4 was inoculated with 100 ml of 100 µg/ml of *Aloe vera*. The plant leaf extract was injected on the 5<sup>th</sup> day of incubation and embryonic growth parameters were observed after fourteen days of incubation. A highly significant ( $P=0.000$ ) result was observed in body and liver weight, body, beak, fore limb, hind limb, eyes, toes, and shank length. Kidney weight and relative weight of the liver showed significant results ( $P=0.04$ ). Inoculant behavior was examined in other embryogenic attributes such as head diameter, gonads and heart weight, and relative weight of kidney, heart, and gonads. According to the results, it can be concluded that *In-Ovo* inoculation of *Aloe vera* leaf extracts on the 5<sup>th</sup> day of incubation has a positive impact on embryonic growth performance.

**Keywords:** *In-Ovo* Injection; Medicinal Plant (*Aloe vera*); Embryonic Parameters; Broiler Chickens

## 1. Introduction:

The world's human population is increasing from time to time. It is a challenge for scientists to meet

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and fulfil the demand for food. By using plant resources, scientists are trying to provide safe and good food for humans which is free from antibiotic growth promoters. The issue considering antibiotic resistance has created an augmented force to reduce antibiotic use in livestock and poultry production (Anwar *et al.*, 2017). Poultry production remains the widest expansion of all livestock enterprises, it constitutes an important pillar of food security improvement, as well as socio-cultural and economic development in most countries. The business of broiler chicken is a large source of income and quick returns on investment. Poultry production is a source of protein (Cheng *et al.*, 2019).

However, the poultry business in developing countries like Pakistan is facing some challenges. Important challenges included a high increase in prices of feed and feed ingredients and a high feed-to-gain ratio. The use of different growth promoters has resolved these challenges. The most important attempt of them is to use antibiotics in feed. Antibiotics are used as growth promoters in feed and to overcome the effect of diseases. Furthermore, antibiotics are used as a medication to recover bird disease in the water. However, the use of antibiotic growth promoters has some benefits as well as harmful impacts including drug toxicity, residual effects and the development of bacteria resistance (John *et al.*, 2012; Alabi *et al.*, 2017).

*Aloe vera* is an everlasting, drought-resisting, fleshy plant, belonging to the Asphodelaceae family. AV is the oldest plant that applied in the medicine in all over the world. Dry condition is most suitable for the cultivation of AV, such as atmospheric condition of Africa, Asia, Europe, and America. There are more than two hundred fifty species of AV plant. Now-a-days, for commercial uses only 2 species are cultivated. Its flowers are in yellow colour. Maximum height of AV plant is 1.5 to 4 feet and base diameter is 2.5 cm or greater. There are ninety-six percent H<sub>2</sub>O content in AV plant. AV plant is a source of most beneficial compound such as essential oil, essential amino acids, minerals, trace elements, Vit., enzymes and conjugated protein. Two main therapeutic products are produced from AV plant like Aloe Latex or juice. These products are usually in it dried form, is used constipation. Product of AV are available in market in different form, these products are spray, cream, gel, lotion, capsule and liquid (Vogler and Ernst, 1999; Zaghawa, 2004; Jensen *et al.*, 2015).

Additionally, the application of AVG as feed additives enhanced the immunity of broilers and has been suggested as a replacer for antibiotics. The most appropriate feed additive strategy is the oral administration through bird feed, which markedly improves the performances. The ultimate beneficial effects of AVG included in drinking water are not well-

described. Aloe Vera gel could be successfully added to broilers' drinking water with the possibility of enhancing growth performance, immunity, and resistance to pathogenic bacteria (Upadhaya & Kim, 2017; Yadav, 2017; Ebrahim *et al.*, 2020).

The beneficial impacts of AV are inducing the intake of feed, increase amount of digestive enzyme, activities against the microbes. Twenty-eight to fifty-eight days old chicken of Fayoumi consumed the large feed. Diets of broiler containing one to two percent of AVL powder, enhance the BWG (body weight gain) than chickens fed without the AVL powder. Potential impact of AVL powder, increase the feed consumption by the broiler chickens due to change in feed taste, induce the appetite and secretion of digestive enzyme. One to forty days old chick received the feed AVL (0.1%, 0.2% and 0.3%) had significant effect on FCR, large BWG and lower feed cost per kg of body weight as compared to an un-supplemented chicken.

Diet with one, two, five and ten percent of AVL had significant result on larger body weight gain, enhancing the FCR as compared to the chicken fed with un-supplemented feed. BWG of fifty-six days old chicken has been increased significantly, because fed the one percent of AVL feed as compared to birds of control treatment. AV can be mixed with drinking water to enhance the productive responses. It is concluded that addition of 1.5% AV in drinking water for the one to forty-two aged broilers lead to increase the feed consumption ration and body weight gain.

Drinking water contain fifty milligrams per litre AVL methanolic content improved all responses and performance included FCR with no side effect in one to forty-two aged chickens. Scientists of above work illustrated, that BWG of chick enhanced due to intake the diet with AVG (0.5%, 0.75% or 1.0) than the bird of control group. Fifteen millilitres per litre of AVG showed significant performance in FCR and BWG of thirty-five aged birds. Other trails explain, the addition of dry AVG powder (0.25 or 1g/kg) or fresh AVG (0.25 g/kg) in bird's diets from one to thirty-five age enhanced the feed consumption ration.

Best performance in productivity of broiler which fed the AV may be due to the many reasons. 1. the phenol which present in AV has anthraquinones and its derivatives like isobarbaloin, aloe-emodin-9-anthrone and anthrone-C-glycosides. These compounds increase intestinal nutrient absorption and act as antibacterial agents. 2. Enzyme like lipase and amylase can be helped in fats and carbohydrates digestion. 3. Many other substances like many organic acid Vit. Monosaccharides or polysaccharide, and S, F, Zn etc can impact on BWG of birds. 4. Flavonoids of AVG can aid in maintain normal intestine microflora, antagonism, changing in metabolism and enhanced glycogen amount in liver and muscle of birds.

The antibacterial and antioxidant properties of leaves or gel extracts (aqueous, ethanol or acetone) of *aloe vera* have been shown to reduce pathogens and improve intestinal microflora. The enhancement in immunity due to important components like acemannan, can help in betterment of bird's health and responses. Opposite, it has been seen that diet with different AVL percentage of supplement, has different impact on mortality of rates. AV supplemented in diets of birds decreased the mortality rates. In other experiment, it was observed that addition of AVL (one or two percent) in feed of twenty-eight to fifty-eight aged Fayoumi chick declined the mortality and no side effect. Decline in mortality of chicks may have been due to betterment in the immune system in broiler chicken

## 2. Materials and Methods:

### 2.1. Extraction of Leaf Extract of Aloe vera:

Leaf extract of *Aloe vera* (AV) was extracted with slightly modification as previously explained in (Nghonjuyi et al., 2016). Fresh and healthy leaves of *Aloe vera* were collected approximately 29 to 37 cm in length from mature healthy *Aloe vera* plants. The leaves were washed with dH<sub>2</sub>O. The leaves of AV plant

(Arunkumar & Muthuselvam, 2009; Eevuri & Putturu, 2013; Sinurat et al., 2015; Shokrane et al., 2016; N. Yadav et al., 2017; Elwan et al., 2019; Zeeshan et al., 2019).

#### 1.1. Objectives:

- To formulate the method for enhancing the hatching rates of broiler chicken
- To improve the health of chickens at the time of hatching.
- To increase the quality of broiler chickens to fulfil society's demand.

were dissected longitudinally. The fleshy mucilaginous pulp (parenchymatous tissue) was carefully scraped out from the thick layers of epidermis. The three hundred gram of scrape pulp was mixed in seventy percent of methanol solution. The mixture was placed in vibrator (Hy-4 Speed Adjusting Multi-Purpose Vibrator, made in China) at room temperature for 72 hours. After seventy-two hours, the mixture was filtered with Whatman filter paper. The filtrate was evaporated in water bath (Electrical Thermostatic Water Bath, made in China) at 100 °C for 4 hours and 12 g of hydro-alcoholic extract was obtained and shown in Figure 1.



Figure 1: Extraction of leaf extract of *Aloe vera*, figure a, b, c, d, e and f shown the leaf AV, pulp of *Aloe vera*, weighing of leaf pulp, shaking of leaf pulp mixture, heating of mixture of AV (mixed in methanol) on hot plate and filtrate of pulp of AV leaf, respectively.

### 2.2. Experimental Design:

120 fertilized egg of ISA Brown layer-breeders

were purchased from District Poultry Farm, Dera Ghazi Khan. Before incubation of eggs, eggs were washed with 70% ethanol and the air space on eggs

was circled. Circle of air space on eggs helps in injection of plants extract. The average mass of 120 eggs were measured. The eggs of ISA Brown layer-breeders were incubated at standard incubation

conditions including temperature of 37.6°C to 38.6°C, relative humidity of 50% to 61 %. The turning of egg hatching racks was done once an hour and shown in Figure 2.



Figure 2: Incubation of eggs, figure a, b and c elucidate the weighing, circling the air space and incubation of eggs respectively.

### 2.3. Solution Preparation of Plants Extract:

The 0.9% of saline solution was formulated by mixing 4.5 grams of NaCl in 500 ml of dH<sub>2</sub>O. 0.1 gram of leaf extract of Aloe vera mixed in 200 ml of 0.9% of saline solution. This mixture was homogenized with vortex and to obtain 500µg/ml concentrated solution

of medical plant leaves extract.

### 2.4. Grouping of Eggs:

After 4<sup>th</sup> day of incubation, one hundred twenty eggs were divided into four groups and 100µl leaves extract injected in each egg.

Table 1: Different Groups of eggs and treatment effect.

Group No.	Group Name	Concentration of injected solution	Volume of injected solution
Group-1	Negative Control	Eggs without any solution	0 µl



Group-2	Positive Control	Eggs injected with 0.9% saline Solution	100 µl
Group-3	AVT1 ( <i>Aloe Vera</i> , Treatment 1)	Eggs injected with 10µg/ml	100 µl
Group-4	AVT2 ( <i>Aloe Vera</i> , Treatment 2)	Eggs injected with 100µg/ml	100 µl

### 2.5. Growth Responses of Chicken Embryo:

After 14<sup>th</sup> days of incubation, take out the eggs of all groups one by one from incubator and examine the following growth parameters.

- 1) Body weight    2) Body length    3) Length of beak    4) Length of eyes
- 5) Head diameter    6) Shank length    7) Length of hind limb    8) Length of fore limb
- 9) Length of toes    10) Weight of body organs (liver, heart, gonads, and kidneys)    11) Relative weight of

body organs (liver, heart, gonads and Kidneys)    12) Mortality    13) Appearance of feathers and nails.

### 2.6. Statistical Analysis:

The data of all the parameters were analysed by using the Co-statistics.

### 3. Results:

Different embryo growth parameters were measured, and fifteen-day old embryos are shown in Figure 3.



Figure 3: Chick embryos after incubation of fourteen days, figure a, b c, d, group 1 (Negative Control), group 2 (Positive Control), group 3 (AVT1 (*Aloe Vera*, Treatment 1) and group 4 (AVT2 (*Aloe Vera*, Treatment 2).

### 3.1. Body and Organ Weight:

*In-Ovo* injection of AV leaf extract shown highly significant ( $P=0.0000$ ) results in body and liver weight of fifteen-day embryo. The body and liver weight of embryo of *Aloe vera* (AV) treatments (AVT1 and AVT2) enhanced from positive and negative control groups. High body and liver weight of chick embryo were observed in AVT1 and shown in Fig 4.

The weight of gonads and heart was non-significant and shown in table 2 and 3. The body organ weight (gonads and heart) groups of *Aloe vera* (AVT1 and AVT2) increased from both control groups. The weight of kidney was significant ( $P=0.0235$ ) and maximum increase observed in AVT1 and represented in figure 4. The ANOVA analysis noted in Table 3.

Table 2: Role of *In-Ovo* injection administration of *Aloe vera* on body and body length of fifteen-day incubation chick embryo.

S. No.	Attributes	Groups			
		Group 1	Group 2	Group 3	Group 4

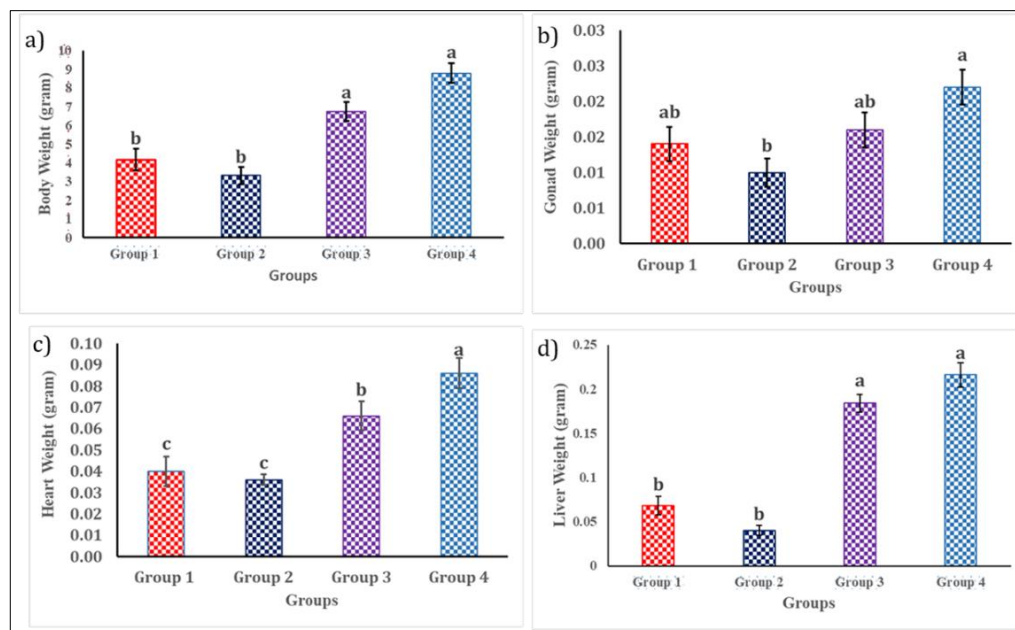
1	BW (gram)	4.17±0.58	3.32±0.45	6.74±0.50	8.80±0.53
2	GW (gram)	0.01±0.00	0.01±0.00	0.02±0.00	0.02±0.00
3	HW (gram)	0.04±0.01	0.04±0.00	0.07±0.01	0.12±0.01
4	LW (gram)	0.07±0.01	0.04±0.01	0.18±0.03	0.19±0.01
5	KW (gram)	0.04±0.01	0.03±0.00	0.07±0.02	0.05±0.01
6	RWG (gram)	0.38±0.11	0.33±0.06	0.19±0.02	0.25±0.04
7	RWH (gram)	0.94±0.06	1.21±0.27	0.80±0.07	1.37±0.17
8	RWL (gram)	1.61±0.12	1.26±0.16	2.29±0.11	2.20±0.18
9	RWK (gram)	0.99±0.10	0.90±0.08	0.89±0.03	0.62±0.08
10	BL (cm)	4.54±0.48	4.22±0.23	6.54±0.13	6.60±0.21
11	BEL (cm)	0.44±0.05	0.38±0.06	0.76±0.06	1.12±0.07
12	FLL (cm)	1.40±0.14	1.44±0.09	2.30±0.17	2.18±0.12
13	HLL (cm)	2.40±0.27	2.00±0.23	3.70±0.04	4.40±0.15
14	HD (cm)	1.16±0.10	1.22±0.09	1.38±0.06	1.40±0.09
15	EL (cm)	0.70±0.09	0.86±0.02	1.00±0.05	1.14±0.08
16	TL (cm)	0.58±0.02	0.52±0.07	0.98±0.05	1.12±0.05
17	SL (cm)	0.58±0.06	0.52±0.09	1.06±0.05	1.02±0.06
18	RM	9%	10%	2%	5%
19	AN	No	No	Yes	Yes
20	AF	No	No	Yes	Yes

**BW**= Body weight, **GW**= Gonads weight, **HW**= Heart weight, **LW**= Liver weight, **KW**= Kidney weight, **RWG**= Relative weight of gonads, **RWH**= Relative weight of heart, **RWL**=Relative weight of liver, **RWK**= Relative weight of kidney, **BL**= Body length, **BEL**= Beak length, **FLL**= Fore limbs length, **HLL**= Hind limbs length, **EL**, Eyes length, **TL**, Toes length, **SL**= Shank length, **RM**= Rate of mortality, **AN**= Appearance of nails. **AF**= Appearance of feathers.

### 3.2. Relative Weight of Body Organs:

ANOVA analysis of relative body organ weight (RBOW) displayed in table 2 and 3, the result of RBOW (liver, gonads, heart, and kidney) was non-significant except the relative weight of liver ( $P=0.0464$ ). The relative weight of gonads (RWG) and kidneys declined

in treatments of AV as compared to the control groups. AVT2 boosted the RWH of fifteen-day embryo. RWL escalated in AVT1 and AVT2 groups than both control groups. Graph representation of RBOW was shown in Figure 5.



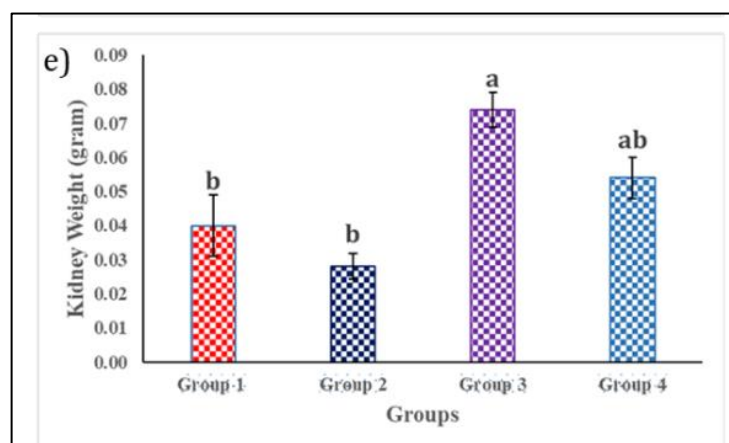


Figure 4: Body and body organs weight. Fig. a, b, c d and e illustrate the weight of body, gonads, heart, liver, and kidney respectively.

### 3.3. Length of Body and Body Part:

Length of body and body parts were analysed through the co-statistics software and recorded in table 3. Analysis illustrates the positive impact of In-Ovo inoculation of AV after the fourth day of incubation of egg. The result of body and body parts (beak, eye, fore limb, hind limb, shank, and toes) length was highly significant ( $P=0.0000$ ) and non-significant ( $P=0.05$ ) in

head diameter. In-Ovo dietary supplement of AV leaf extract raised the length of body and its parts of chick embryo as compared with the control groups. High enhancement of body and its parts (eye head diameter, beak, hind limb, and toes) length of fifteen-day chick embryo was recoded in AVT2 group. Maximum increased in fore limb and shank length of embryo observed in AVT1. The graphical representation, mean and standard error of body and its parts were shown in Figure 7 and Table 2.

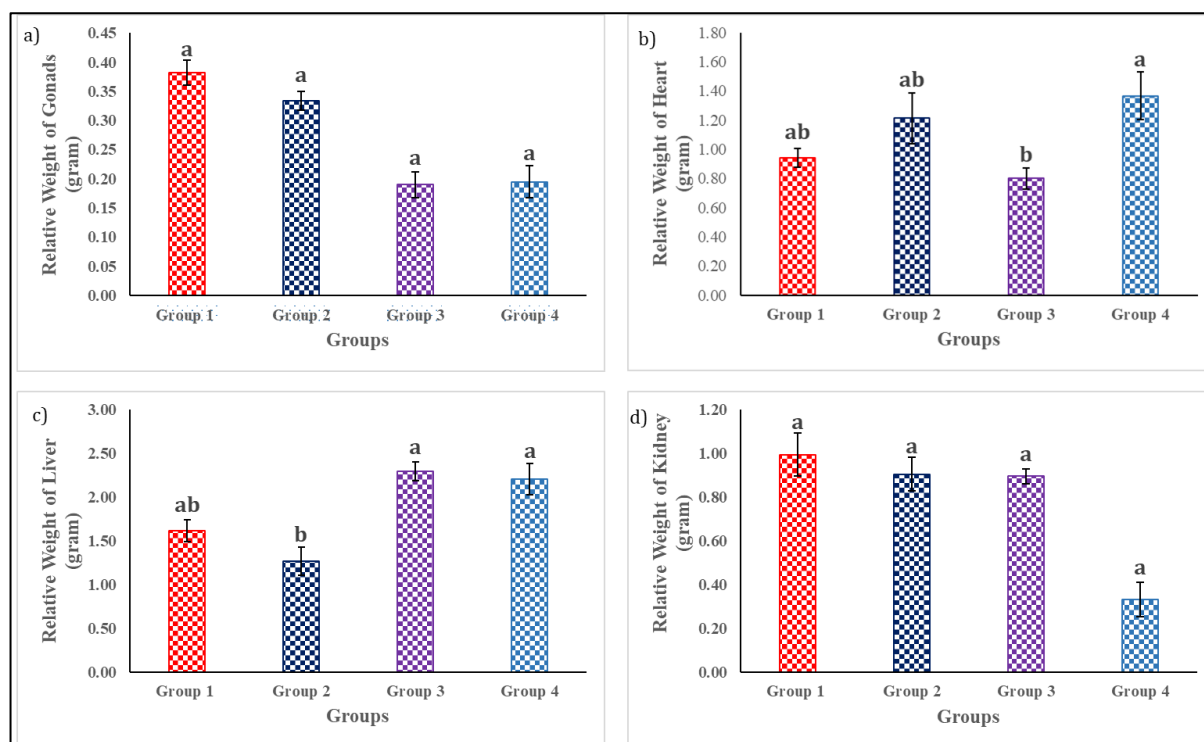


Figure 5: Influence of medicinal plant leaf extract on body relative organ. Figure a, b, c and d demonstrate the RWG, RWH, RWL and RWK respectively of embryo of eggs treated with AVTI, AVTII, 0.9% normal saline and without injection.

Table 3: F-values derived from ANOVA for broiler chick embryo growth responses.

S. No.	Attributes	F-ratio	P-value	LSD <sub>0.05</sub>
1	BW (gram)	33.77	0.0000***	1.44
2	GW (gram)	2.167	0.1319ns	0.0085
3	HW (gram)	1.07	0.3897ns	3.261
4	LW (gram)	21.718	0.0000***	0.0507
5	KW (gram)	4.156	0.0235*	0.0291
6	RWG (gram)	1.5931	0.2302ns	0.203
7	RWH (gram)	2.46	0.100ns	0.495
8	RWL (gram)	3.327	0.0464*	0.805
9	RWK (gram)	1.01	0.4142ns	0.49
10	BL (cm)	19.66	0.0000***	0.88
11	BEL (cm)	21.01	0.0000***	0.228
12	FLL (cm)	10.895	0.0004***	0.463
13	HLL (cm)	39.056	0.0000***	0.553
14	HD (cm)	1.84	0.1892ns	0.261
15	EL (cm)	12.614	0.0002***	0.168
16	TL (cm)	29.65	0.0000***	0.159
17	SL (cm)	19.26	0.0000***	0.1942

F= F-ratio was obtained from ANOVA Tables, LSD=Least significant difference at P=0.05

### 3.4. Rate Mortality:

High rate of mortality was observed in positive control. Injection of AV leaf extract at 5th day

of incubation in eggs, the embryo mortality rate in AVT1 and AVT2 treated eggs was decreased as compared the control groups. Lowest mortality was measured in the embryo of eggs that injected with AVT1. Embryo mortality graph was shown in figure 6.

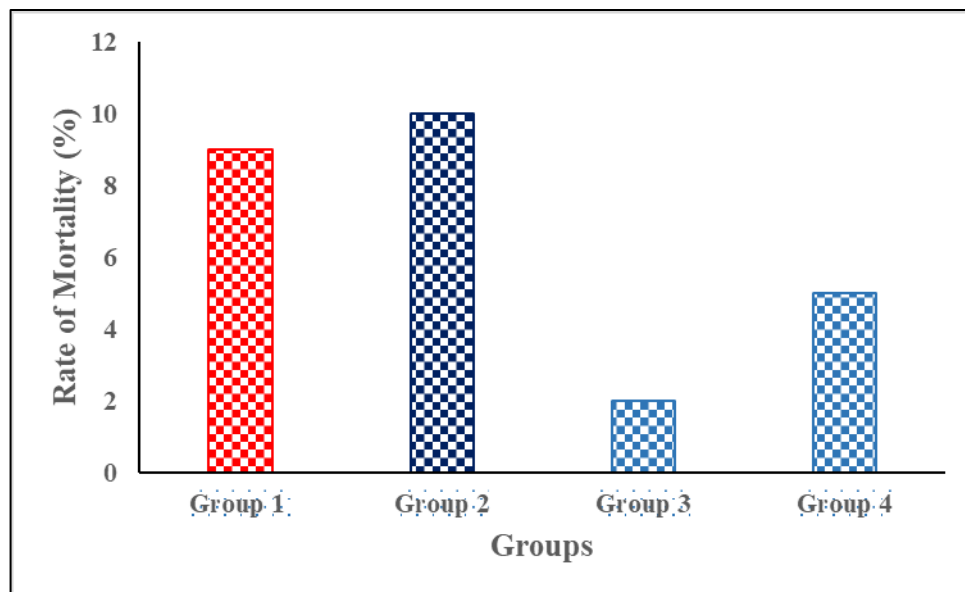


Figure 6: Influence of *In-Ovo* inoculation of medicinal plant leaf extract at 5<sup>th</sup> day incubation on mortality of 15<sup>th</sup> day of embryo.

### 3.5. Appearance of Nails and Feathers:

*In-Ovo* injection administration of Aloe vera at five-day of incubation on appearance of nails and

feathers of fifteen-day incubation embryo showed the positive impact. Nails and feather appeared on the embryo took out from eggs that injected with AVT1 and AVT2 at 5th incubation.



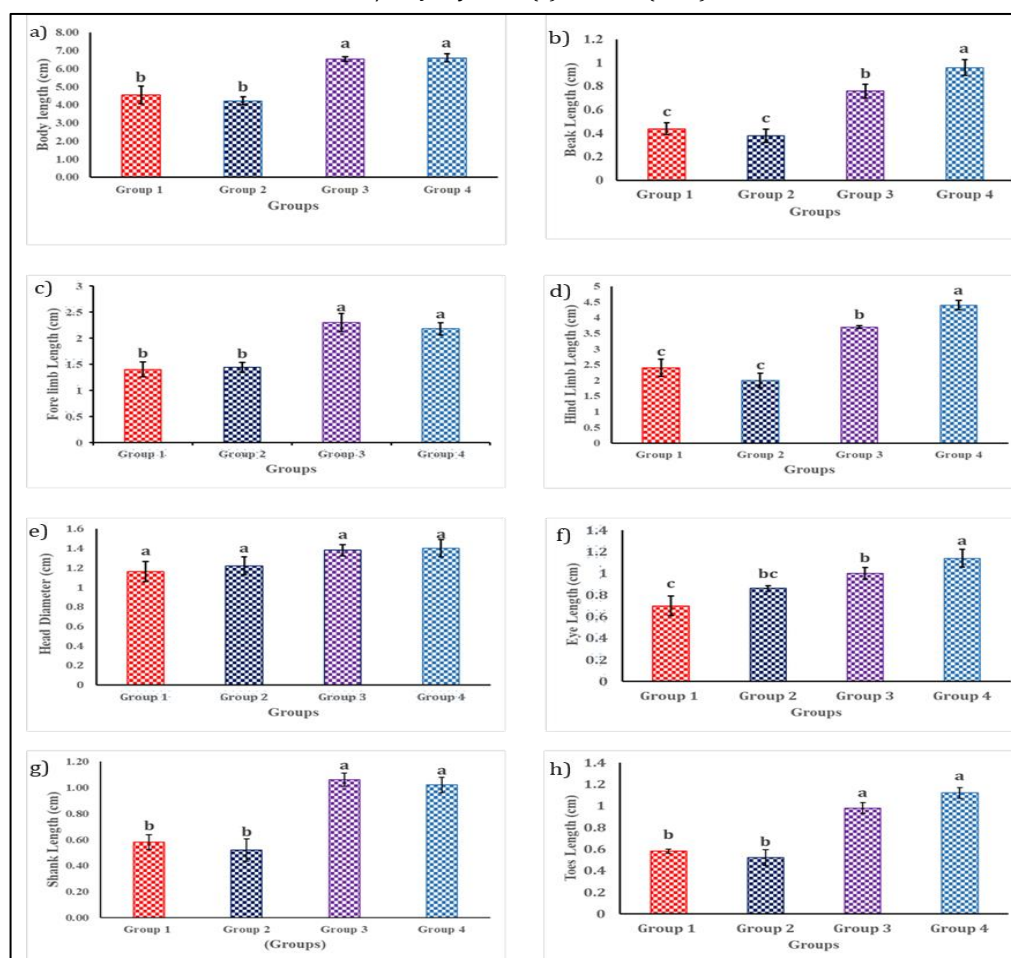


Figure 7: Role of *In-Ovo* injection administration of *Aloe vera* on body and body length of fifteen-day incubation chick embryo. Figure a, b, c, d, e, f, g and h illustrate the body, beak, fore limb, hind limb, head diameter, eyes, shank and toes length respectively of embryo of eggs treated with AVTI, AVTII, 0.9% normal saline and without injection.

#### 4. Discussion:

Current studies explain the impact of *Aloe vera* leaf extract on performances of 15-day incubation chick embryos. The plants leaf extracts have positive effect on the body length, head diameter, eye length, foot length, shank length, beak length, fore and hind limb length. The body length, head diameter, eye length, foot length, shank length, beak length, fore and hind limb length enhanced in all groups (AVTI and AVTII) as compared to the positive and negative controls groups (Derakhshanfar *et al.*, 2019). Length of body and body parts showed the highly significant ( $p \leq 0.01$ ) result due to AVLE has antimicrobial, antibiotic, immunological and growth and development properties and many vitamins. The study of (Ali *et al.*, 2019) describe the effect of in-Ovo injection of ALFATOXIN B1, the body length, head diameter, eye length, foot length, shank length, feather length and hind limb length were decreased. Another study (Afzal, 2019) illustrates the in-Ovo supplement

of ethanol on development of embryos, mean body length and mean hind limb length declined in ethanol treated groups than the embryo chick of control groups.

Body weight and body organ weight (heart, gonads, kidney, and liver) increased in chick embryos of eggs injected with 10µg/ml of AV and 100µg/ml of AV. Highly significant ( $p \leq 0.01$ ) result determined in body and body organ weight (liver) due to AVLE has antimicrobial, antibiotic, immunological and growth and development properties and many vitamins. Relative weight of body organ (gonads and kidneys) declined in AV treated groups. Relative weight of heart of AVTI group embryos is smaller than control groups and enhanced in embryos of AVTII group as compared to control. Increased in RWBO (liver) observed in all groups of both experiments. RWBO (kidney) is smaller than positive and negative controls in 10µg/ml and 100µg/ml AV. Current study showed better result as compared to the study of (Derakhshanfar *et al.*, 2019).

## 5. Conclusion:

It was concluded that in ovo injection of Aloe vera leaf extract has differential effects on embryo of broiler chicken. This extract improved the embryonic growth performance especially 100µg/ml (AVT2) of aloe vera leaf extract showed positive impact on pre hatching embryo i.e., weight and length of body parts while 10µg/ml (AVT1) extract also showed improvement in performance of broiler chickens. AVT1 reduces mortality rate as well in comparison of other groups. In future, in ovo injection of various herbal plant extracts can be performed for further investigation.

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