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Research Paper

Influence of *in ovo*Administration of Varying Levels of Glucose on the Serum Biochemical Indices and Haematological Parameters of Arbor Acre Broiler Chickens.

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Abstract
In ovo feeding is a nutritional strategy employed during the embryonic stage to optimise growth performance and immune functions for sustainable poultry production. This study was conducted to investigate the effects of in ovoadministration of varying levels of glucose on serum biochemical indices and haematological parameters of Arbor Acre broiler chickens. Two hundred (200) fertile eggs of Arbor Acres strain of broiler chickens were used for the Experiment. Candling was done on the 14th day of incubation and viable eggs were distributed into five(5) treatment groups consisting of the control -T_1 (no in ovo injection), T_2 (Sham group-0.5 ml of sterile water/egg); T_3 (50 mg glucose per egg); T_4 (75 mg glucose per egg); T_5 (100 mg glucose per egg) in a completely randomized design. The in ovoinjection was carried out on 18th day of incubation before being transferred tothe hatcher. The post-hatch chicks were replicated based on the number of chicks hatchedfrom each treatment. Data collected on blood profilewere analysed using SAS (2007).Results showed that serum and haematological indices were significantly ($p<0.05$) influenced, notable among them were: cholesterol, alanine aminotransferase, aspartate amino transferase and packed cell volume, red blood cell.The study concluded that glucose can be injected in ovoat 50-100 mg per egg to prevent adverse effects on broiler chickens.

Keywords: *In ovo feeding, serum biochemical, haematological, aminotransferase, glucose*

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I. Description of Problem

The gastrointestinal tract (GIT) of chicks has a limited capacity to digest nutrients from an external diet rich in carbohydrates and proteins until it fully matures. These nutritional constraints can be addressed through two methods: "Immediate Post-Hatch Feeding," which involves providing food to chicks right after hatching in the hatchery, and "*In ovo* Feeding," which entails administering nutrients directly into the amniotic fluid of late-term embryos [4]. The combination of these two technologies, early feeding and *in ovo* feeding, holds significant promise in mitigating early viability challenges and promoting robust chick development.

Poultry chicks exhibit precocial behavior, meaning they are sufficiently developed and capable of mobility and self-feeding upon hatching. The perinatal period, encompassing the days before and after hatching, is a critical phase for the growth and development of chicks. During this period, the gastrointestinal tract (GIT) undergoes rapid growth, enabling chicks to adapt to external feeding environments by transitioning from utilizing nutrients stored in the egg [5].

Early availability of feed plays a pivotal role in the development of the gastrointestinal tract (GIT), as the GIT undergoes rapid growth during the initial stages of chick development. The postponement of feeding has a detrimental impact on GIT function [6].

The trend of reducing the time to market for broilers each year is further refined through early feeding practices. Research results indicated that immediate access to feed and water significantly enhances broiler

bodyweight [7]. However, practical challenges in providing feed and water at the commercial level often exist. Activities such as transferring chicks from the incubator, gender identification, vaccination, packaging, and transportation can add to the delay in providing access to feed for the newly hatched chicks. An alternative strategy to address this issue is through *in ovo* feeding.

Glucose, a simple sugar with the molecular formula $C_6H_{12}O_6$, is a pivotal component in energy metabolism. It is the most abundant monosaccharide, belonging to the carbohydrate category [8]. Plants and many algae synthesize glucose through photosynthesis, using sunlight energy, water, and carbon dioxide. In plants, it is used to produce cellulose, the most abundant carbohydrate globally [9]. Glucose falls into the category of aldohexoses, characterized by its six carbon atoms and aldehyde group. It can manifest in both open-chain and ring structures.

During the early stages of incubation, carbohydrates serve as the embryo's main energy source, with a shift to protein in mid-incubation, and a reliance on fat during the final weeks. However, as hatching approaches and oxygen becomes limited, fatty acids cannot be oxidized for energy. Instead, glucose stored in glycogen is metabolized anaerobically, along with amino acids and glycerol, through gluconeogenesis. These findings emphasize the critical role of managing energy supply and providing external nutrients to ensure optimal embryo development [10]. Therefore, efforts to enhance the immune system and growth performance of broiler chickens through *in ovo* administration of glucose could reduce susceptibility to diseases, improve production performance, increase farmers' profit margins, and enhance the availability of animal protein for consumers. Given the positive effects of glucose on broiler chicken production, this study explores the inovo administration of glucose to assess its impact on blood profileof Arbor Acre broiler chickens.

II. Materials and Methods.

The hatching aspect of the experiment was carried out at a commercial hatchery in Ibadan and the rearing of the birds took place at the Teaching and Research Farm of Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan. The region is 76 m above the sea level and fall within latitudes 7^0 18`2``N and $7^0$ 18`30``N; and longitude 3^0 22`10``E and $3^0$ 22`41``E. The climate is humid and located in the forest zone of South Western Nigeria. The mean annual precipitation and the temperature are 1,330 mm and 29.3^0 C respectively. It has an average relative humidity of 80% throughout the year (O-ORBDA, 2013).

Sourcing of fertile eggs.

Two hundred (200) fertilized eggs were obtained from a 35 weeks old Arbor acre broiler breeders flock. The weight range of the eggs were known (45- 65g) using digital scale and eggs were sorted to remove unsettable eggs (15 eggs), fumigated with Formaldehyde and Potassium Permanganate ($KMnO_4$) and left for about 4hours before setting into the incubator. A total of 185 eggs (92.5%) were set in the incubator after sorting. The eggs were incubated under standard condition of Incubator temperature 37.5ºC +02C and relative humidity of 65% +2%. Turning of the eggs was done automatically.

Experimental Layout

The arrangement of *in ovo* injection of glucose is presented in the experimental layout below. The glucose used were obtained from a reputable chemical store in Ibadan.

Table 1: *In ovo* administration

Treatment groups	***Inovo* administration**
1	Control
2	Sham (0.5ml of sterile water)
3	50mg of Glucose dissolved in 0.5ml sterile water
4	75mg of glucose dissolved in 0.5ml sterile water
5	100mg of glucose dissolved in 0.5ml sterile water

They were all injected at 0.5ml per egg.

Candling and distribution of eggs

The eggs were candled on the 14th day of incubation for fertility/viability test. This is done to ascertain those eggs showing viable embryos under lamina flow. A total of 150 fertile, viable eggs were selected (75% fertility) and allotted into 5 treatments of 30 eggs per treatment of *in ovo* administration.

Procedure of *in ovo*injection of varying levels of Glucose

On the 18th day of the incubation process, injection sites were identified and sterilized with methylated spirits. Subsequently, a pin hole was meticulously made on the surface of the air sac with a specialized pin. Injections were administered into the amniotic cavity at the broad end of the eggs, utilizing 24-gauge hypodermic needles

measuring 19mm in length, according to the method standardized by [11]. The injection point was sealed using melted candle wax and the eggs were relocated to the hatching apparatus.

Post-hatch Chick Management

The Post hatch chick rearing was done at the Teaching and Research farm of Federal College of Animal Health and Production Technology, Moor Plantation, Apata, Ibadan.

A total of 100 chicks were hatched (66.67% hatchability); 25 chicks (83.33 % hatchability) from T1, 24 chicks (80.00% hatchability) from T2, 19 chicks (63.33% hatchability) from T3;

19chicks (63.33% hatchability) from T4; 13 chicks (43.33% hatchability) from T5. The birds were brooded for a period of one-week, same commercial feed and drinking water were provided *ad libitum*. The birds in each treatment were replicated 3 times. The chicks were vaccinated against Newcastle and infectious bursa disease (IBDV) at day 12. The experiment was conducted for a period of 49 days. The experimental design used was completely randomized designs.

Determination of Serum and haematological parameters

Blood samples were obtained on the 21st and 42nd day following hatching from a single chick subject within each experimental replicate, utilizing a needle and syringe inserted into the jugular vein, and directly transferred into sterile bottles containing EDTA (2.5ml each) for subsequent haematological analysis. The haematological parameters determined are: - Packed Cell Volume (PCV); Haemoglobin concentration (Hb), red blood cells and white blood cells. PCV was determined using micro-haematocrit method while haemoglobin concentration was measured spectrophotemetrically by the method of Cyanomethaemoglobin as described by [14], red blood cells and white blood cells were estimated using haemocytometer [15]. White cells differential counts like eosinophils, monocytes, platelets, lymphocytes, basophils and heterocyte were estimated by blood staining techniques. For serum biochemical studies, blood samples were collected into sterile bottles with no anticoagulant. The blood samples were taken to the laboratory for analysis. Serum biochemical parameters determined are: - Total protein (Modified biuret method); Albumin (bromocresol green method); Uric acid (Trinder's enzymatic method); glucose (glucose oxidase method); AST (Reitman and Frankel method) and ALT (Reitman and Frankel method). Serum globulin values were estimated based on the difference between the concentrate of total protein and albumin. The ratio of albumin to globulin (A/C) was also calculated.

Statistical Analysis

The data collected on hatching traits and immune responses were subjected to descriptive statistics using 2013 Microsoft excel package while all other data collected were subjected to Analysis of Variance in a completely Randomized Design.

III. Results

Table 1 reveals that the cholesterol, Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) mean values were significantly different ($p<0.05$) across the treatments. Birds on T2 exhibited the highest mean value in cholesterol (158.86mg/dl) statistically different ($p<0.05$), followed by those that were in control T1 (131.35mg/dl), T4 (124.97mg/dl), T3 (119.99mg/dl) and T5 (109.91mg/dl) respectively that were similar ($p>0.05$). Also, AST and ALT mean values varied significantly ($p<0.05$) across the treatments with T5 (15.30 IU/L) having the highest value and T2 (7.42 IU/L) been the lowest in AST. Birds in T4 had the highest value (12.92 IU/L) in ALT and the lowest value (6.88 IU/L) in T2 and T1 (7.40 IU/L) that were similar ($p>0.05$). There were no significant differences ($p>0.05$) in the total protein, albumin, globulin, and creatinine values across the treatment groups.

Table 1: Effect of *in-ovo* administration of varying levels of glucose on the serum biochemical indices of Arbor Acre broiler chickens at week 3.

Parameters	T1	T2	T3	T4	T5	Pvalue
GLU(mg/dl)	226.85±11.68	210.03±4.33	179.17±14.15	218.98±13.29	228.09±11.09	0.069
TP(g/dl)	2.43±0.11	2.55±0.12	2.66±0.20	2.42±0.08	2.53±0.17	0.749
ALB(g/dl)	1.69±0.13	1.61±0.07	1.55±0.13	1.79±0.01	1.77±0.05	0.338
GLO(g/dl)	0.74±0.22	0.74±0.13	1.11±0.12	0.63±0.08	0.76±0.20	0.291
ALB/GLO	2.28	1.71	1.40	2.84	2.33	-
CRT(mg/dl)	0.43±0.12	0.51±0.08	0.52±0.09	0.48±0.25	0.68±0.05	0.752
CHOL(mg/dl)	131.35±7.71^{b}	158.86±7.96^{a}	111.99±5.12^{b}	124.97±1.54^{b}	108.91±1.23^{b}	0.016
AST(IU/L)	7.96±2.39	7.42±1.70	12.02±2.83	9.58±0.60	15.30±0.84	0.074
ALT(IU/L)	7.40±0.98^{b}	6.88±0.18^{b}	9.76±0.46^{ab}	12.92±1.65^{a}	10.61±1.59^{ab}	0.022

Table 2 indicates that there were significant differences ($p<0.05$) in Packed cell volume (PCV), Red blood cell (RBC), White blood cell (WBC), platelets, lymphocytes and heterophil values across the treatment groups. However, there were no significant difference in ($p>0.05$) in Hb, monocyte, eosinophil and basophil across the treatment means. In PCV, the highest value of (29.33) in T1 was observed followed by T2 (28.67), T4 (27.67) and T3 (26.33) which were significantly ($p<0.05$) higher than T5 (24.33). In RBC, T2 (3.38), T1 (3.35), T4 (3.32) and T3 (3.00) were statistically ($p<0.05$) similar but significantly ($p<0.05$) higher and different from T5 (2.50). The WBC followed the same trend and in RBC. The platelet value in T1 shows significant difference ($p<0.05$) and higher value (16.16) in T1 than all other treatments. T4, T5, T3 and T2 were statistically ($p<0.05$) the same. In heterophil, T5 had the highest value (36.67) that is significantly different ($p<0.05$) from treatment 3 (30.67) and T4 (27.67) that are statistically similar ($p<0.05$) while the lowest values were obtained in T2 (25.67) and T1 (25.00). In lymphocyte, T1, T2 and T4 are statistically similar but were significantly ($p<0.05$) different from T3 (60.67) and T5 (56.67).

Table 2: Effect of *in-ovo* administration of varying levels of glucose on the haematological indices of Arbor Acre Broiler chickens at week 3.

Parameters	T1	T2	T3	T4	T5	Pvalue	Range
PCV (%)	29.33±1.20^{a}	28.67±0.67^{a}	26.33±0.86^{ab}	27.67±0.33^{a}	24.33±1.20^{b}	0.022	22-50
Hb(g/dl)	9.63±0.47	9.03±0.28	8.77±0.12	20.33±5.78	20.50±6.01	0.090	7-18
RBC(x10^{6}/UL)	3.35±0.03^{a}	3.38±0.02^{a}	3.00±0.13^{a}	3.32±0.04^{a}	2.50±0.32^{b}	0.013	2.5-3.5
WBC(x10^{6}/UL)	17.08±2.50^{a}	17.27±3.28^{a}	15.63±8.11^{a}	17.25±3.01^{a}	14.23±3.19^{b}	0.003	12-35
PLT(x10^{4})	16.16±72.18^{a}	12.46±59.25^{b}	13.20±72.34^{b}	13.23±34.86^{b}	13.23±68.87^{b}	0.016	
LYM(%)	66.33±1.76^{a}	66.33±0.88^{a}	60.67±0.88^{b}	65.33±1.33^{a}	56.67±1.20^{c}	0.001	42-85
HET(%)	25.00±1.53^{c}	25.67±0.88^{c}	30.67±1.45^{b}	27.67±0.33^{b}	36.67±1.33^{a}	0.001	10-53
HET/LYM	0.38	0.38	0.51	0.42	0.65	--	0.22-1.7
MON(%)	4.00±0.58	3.33±0.88	4.00±0.58	3.00±0.58	3.00±0.58	0.668	0.05-5
EOS(%)	3.33±0.67	4.67±1.20	4.33±1.45	3.67±1.20	3.67±0.67	0.900	0.0-1.75
BAS(%)	0.33±0.33	0.00	0.33±0.33	0.33±0.33	0.00	0.737	0.0-2.8

*Range value: Talebi*et al*,.2005

a,b,cTreatments with similar superscript in the row are not significantly different ($p>0.05$) from each other at 5%

PCV (Packed cell volume), Hb (Haemoglobin), RBC (Red blood cell), WBC (White blood cell), PLT (Platelet), LYM (Lymphocyte), HET (Herophil), MON (Monocyte), EOS (Eosinophil), BAS (Basophil)

Table 3 reveals that ALT mean values were significantly different ($p<0.05$) across the treatments. Birds in T2 had the highest mean value of 8.61 IU/L of ALT while T5 had the smallest mean value of 4.29IU/L of ALT. T2 and T3 are significantly ($P<0.05$) higher than T5, T1 and T4. However, glucose, total protein, albumin, globulin, cholesterol, AST and creatinine values were not significantly different ($p>0.05$) from each other across the treatments.

Table 3: Effect of *in-ovo* administration of varying levels of glucose on the serum biochemical indices of Arbor Acre broiler chickens at week 6

Parameters	**T1**	**T2**	**T3**	**T4**	**T5**	**Pvalue**
GLU(mg/dl)	214.83±26.69	238.28±13.43	235.41±27.87	243.84±10.27	269.29±12.87	0.458
TP(g/dl)	3.51±0.25	3.30±0.33	4.48±0.66	3.94±0.21	3.86±0.18	0.272
ALB(g/dl)	1.85±0.04	2.01±0.16	1.79±0.15	1.93±0.05	2.03±0.14	0.587
GLO(g/dl)	1.66±0.29	1.30±0.18	2.69±065	2.01±0.17	1.83±0.15	0.135
Alb/Glo ratio	1.11	1.54	0.67	0.96	1.11	-
CRT(mg/dl)	0.59±0.06	0.61±0.03	0.62±0.08	0.69±0.00	0.75±0.03	0.206
CHOL(mg/dl)	135.36±8.71	116.20±11.83	123.01±0.18	130.95±10.99	106.13±15.43	0.375
AST(IU/L)	81.10±7.91	94.59±2.23	99.30±6.71	106.77±5.76	105.15±5.77	0.100
ALT(IU/L)	4.53±0.68^{b}	8.61±1.98^{a}	8.43±1.43^{a}	4.87±0.48^{ab}	4.29±0.21^{b}	0.048

Table 4 shows that only platelet was statistically significant ($p<0.05$) across the treatment groups. T2 produced the highest mean of 13.03±32.82x10^4, followed by T4 (12.52±49.10), T5 (12.37±43.33) which are similar statistically while the lowest value was obtained in T1 (11.03±48.41x10^4) which are statistically similar ($p<0.05$) with T3 (11.50±30.55) and T5 (12.37±43.33).

Table 4: Effect of *in-ovo* administration of varying levels of glucose on the haematological indices of Arbor Acre Broiler chickens at week 6.

Parameters	T1	T2	T3	T4	T5	Pvalue
PCV (%)	29.00±1.00	33.00±3.61	29.33±0.67	30.67±0.88	28.67±2.96	0.637
Hb(g/dl)	9.40±0.38	10.80±1.25	9.37±0.34	9.73±0.37	9.07±0.93	0.550
RBC($x10^6$/UL)	3.33±0.05	3.44±0.13	3.32±0.06	3.34±0.07	2.88±0.52	0.513
WBC($x10^6$/UL)	15.32±71.64	16.93±87.67	15.65±43.10	17.15±27.54	16.43±16.27	0.577
PLT($x10^4$)	$11.03±48.41^{c}$	$13.03±32.82^{a}$	$11.50±30.55^{bc}$	$12.53±49.10^{ab}$	$12.37±43.33^{abc}$	0.040
LYM(%)	65.33±1.45	67.33±2.03	61.67±3.18	69.00±1.73	63.33±2.03	0.194
HET(%)	28.00±3.08	26.00±2.00	30.00±3.51	23.33±0.88	29.33±1.86	0.218
HET/LYM	0.49	0.39	0.49	0.34	0.46	-
MON(%)	3.00±0.58	2.67±0.33	3.33±0.88	3.67±0.67	3.00±0.58	0.831
EOS(%)	3.67±0.88	3.67±0.88	4.67±0.88	2.67±0.88	4.00±0.58	0.575
BAS(%)	0.00	0.33±0.33	0.33±0.33	0.33±0.33	0.33±0.33	0.903

a,b,cTreatments with similar superscript in the row are not significantly different ($p>0.05$)
PCV (Packed cell volume), Hb (Haemoglobin), RBC (Red blood cell), WBC (White blood cell), PLT (Platelet), LYM (Lymphocyte), HET (Herophil), MON (Monocyte), EOS (Eosinophil), BAS (Basophil)

IV. DISCUSSION

In the present study, the weight of newly hatched chicks was higher (39.40g, 37.27g, 37.23g) following the *in-ovo* glucose injection compared to the control (36.80g) and sham groups (36.57g). It has been established in previous studies that the initial weight of chicks at hatching is a critical indicator of their eventual market weight, although this relationship may vary among different strains. The relevance of initial hatch weight to final market weight is becoming more pronounced with broiler breeding companies' ongoing selection for rapid growth traits [16]; [17]and [18]. This observation is consistent with findings from [19], which indicated that carbohydrate treatments significantly alter the body weight of one-day-old chicks or during the subsequent breeding period.

Blood parameters are said to be diagnostic tools that shows major indices of physiological, pathological and nutritional status of an animal and can be used to interpret the effect of nutritional managements in veterinary medicine. Several factors such as environment, physiological, age, dietary constituents and so on are said to have significant effect on the blood indices of farm animals [25]. In this study, it was observed that *in-ovo* injection of varying levels of glucose in experiment 1 did not significantly influenced the haematological indices at week 6 post hatch in Arbor acre broiler chickens but the PCV, RBC, WBC, platelets, lymphocytes and heterophil were significantly influenced at week 3. However, these values were within the normal ranges for broiler chickens [26][27] which suggests that the chicks were not physiological stressed based on the treatment groups. The non-significance of the PCV and Hb parameters implies the adequacy of dietary nutrients for normal haematopoiesis without any sign of anaemia. This agreed with the previous reports by [28] that the number of erythrocytes in poultry birds varies with age, sex, diets and clinical conditions of the animal. According to [29], fluctuations in avian blood haematological values are depending on the physiological status of the birds. This could also explain why the broiler chicks PCV values varied in the present study. In this study, the *in-ovo* injection of glucose did not exhibit any influence on blood serum protein levels. This finding is in agreement with the findings of [30]; [31] who reported non-significance in blood protein of chickens *in-ovo* injected. Non-significant difference in serum glucose among broilers injected glucose at week 3 and 6 indicates that energy metabolism was not impaired.

Conclusion and Application

1. In-ovo administration of glucose did not have any deleterious effect on the blood parameters of Arbor Acrebroiler chickens.

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