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Evaluation of the Reproduction Parameters of "Lohmann Brown" Strain Chickens Fed with *Cajanus cajan* Leaf Meal in the Republic of Congo

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Abstract

The objective of this study was to evaluate the reproductive parameters of "Lohmann Brown" strain chickens fed on *Cajanus cajan* leaves and to assess the viability of the chicks after hatching. 40 hens of the "Lohmann Brown" strain fed on *Cajanus cajan* leaf flour laid 219 eggs divided into four batches depending on the rate of incorporation of *Cajanus cajan* leaf flour in the rations (0%, 5%, 10% and 15%) were trained and introduced into the incubator. Results: the incubator indicates a hatching temperature of 38˚C to 37.2˚C and relative humidity of 60% to 70%. Hatching performance shows that: The 15% batch recorded the highest fertility rate 86.95% compared to 26.88% of the control batch. The CC 15% batch recorded the highest hatching rate 36.87. The lowest rate (17.18%) was recorded in the control batch (CC 0%). The highest embryonic mortality rate was recorded in the CC 15% batch. The lowest rate in the CC 10% batch. Concerning unfertilized eggs, the highest rate is 72.20% (CC 0%), and the lowest are (12.03%; 57.42% and 66.66%) recorded in CC batches. 15%, CC 5% and CC 10% respectively. For the shell mortality rate, the highest is 18.58% recorded in the CC 15% batch. The lowest shell mortality rate is 2.07%, obtained in the control batch (CC 0%); the dust mortality rate was 0% for all chicks. The study seems to indicate that the incorporation of *Cajanus cajan* leaf meal up to 15% into the reproduction type ration in hens does not cause any harmful effects on the reproduction performance of laying hens.

Keywords

Egg, Incubation, Hatching, Fertility, Mortality, Viability

1. Introduction

Protein-energy malnutrition is prevalent in most African countries. Given the vulnerability of large livestock to climatic and health hazards, livestock production development strategies are paying more and more attention to short-cycle animals, including poultry, which occupy a special place [1] [2]. Indeed, poultry farming is seen as a means of investment or capitalization making it possible to mobilize funds when necessary, and in addition to satisfying the animal protein needs of populations [2] [3]. It represents an interesting source of proteins of high biological value and makes it possible to quantitatively and qualitatively improve the diet of populations [4]. Poultry farming is an important activity for the development of a country's economy. In the Republic of Congo, poultry products (meat, eggs, milk and others) occupy an important place in household consumption. In fact, Congo imports approximately 50% of the total volume of animal products estimated at 45789.6 tonnes in 2007 [5].

In addition, the productivity of local breed chicken remains low despite significant actions to support the sector in the areas of health (vaccination and deworming campaign), food and housing and even strategies marketing [6]. A growth rate of 3% is recorded, a carcass weight of 0.8 to 1 kg [7] and an annual laying of 27 eggs per hen per year [8]. Multifactorial causes generally contribute to this low productivity, among which reproduction figures prominently. Indeed, natural brooding remains the main means of obtaining chicks in traditional poultry farming [8]. The use of artificial brooding remains marginal, especially in rural areas due to the unavailability of incubators adapted to this environment, mainly due to the absence of electricity and the rising cost of oil.

The shortage of livestock feed in terms of quality and cost constitutes an obstacle to the development of monogastric breeding in Congo [9]. In fact, feed represents 70% to 80% of the cost of poultry production. However, the availability of conventional raw materials (soybeans, peanuts and their derivatives, fish meal, corn, etc.) for poultry production clashes not only with the diet of humans but also with that of monogastric species. Under these conditions, the search for and development of alternative food resources available locally in chicken feed should make it possible to improve their productivity [10]. Among these alternative food resources, *Cajanus cajan* leaves feature prominently. Indeed, flour from *Cajanus cajan* leaves is a local and easily accessible non-conventional food resource (RANC) in Congo. They are relatively energetic, rich in proteins (22% to 30% DM) and amino acids, minerals, vitamins. Pigeon peas are also low in fat, making them a food of choice in both human and animal nutrition. Its nutritional value is very close to that of beans or peas so appreciated by many peoples,

and can be used as a supplement in the diet of chickens [11].

Work carried out by [12] [13] showed that it is possible to improve the reproductive performance of hens by giving them conventional feed. However, the mobilization of conventional resources requires a fairly heavy investment which breeders in the sector almost do not have. However, there are unconventional food resources (*Cajanus cajan*; *Cassia tora*, *Leucaena leucocephala*, *Moringa oleifera*, etc.) that can replace conventional protein sources [14] [15].

Studies carried out by various authors [12] [13] [16]-[19] showed that *Cajanus cajan* leaves are rich in nutrients, particularly proteins, minerals and vitamins. They have been incorporated up to 5% to 20% respectively in the feed of poultry, pigeons and guinea fowl without any harmful effect on the productivity and health status of the animals. It is in this context that this study was undertaken. The general objective of this work is to contribute to the search for ways and alternatives to improve the diet and productivity of hens.

2. Material and Methods

2.1. Site and Period of the Experiment

The experiment took place at the experimental farm of the Veterinary Clinic of the Congolese Association for the Development of Livestock (A. CO. D. EL) during the period from September 2020 to January 2021. It is a experimental farm which is located within the Clinic (Nkombo district), in the Brazzaville department.

2.2. Incubation and Rearing Equipment

2.2.1. Incubator

For our study, we used a VLAIS brand incubator with a capacity of 1100 eggs per incubation cycle (**Figure 1**). This machine operates automatically with predefined programs (temperature, relative humidity, air speed, turning frequencies). The operating system of the incubator is conditioned on an energy source (current or generator). The device is equipped with a water tray to ensure good relative humidity.

Figure 1. Incubator used for experimentation.

2.2.2. Livestock Building, Breeding Equipment and Performance Control

The experimental building was emptied, cleaned with soapy water and disinfected with bleach and sleet two weeks before the placement of the subjects, *i.e.* just after the chicks hatched. The breeding equipment (feeders, drinkers, etc.) was also washed and disinfected. A second disinfection of the building was carried out with a virocide (VIRUNET) one week after the first. The experimental setup was set up with mesh cages which made it possible to continue the experiment (Figure 2). On the eve of the placement of the subjects, the surface of the henhouse was covered with a thick layer of litter (wood shavings). A thermometer was installed for temperature control and a footbath was placed at the entrance to the door of the experimental building. Feeders, drinkers and other performance monitoring equipment (scale, identification ring and data collection sheets) have been placed.

Figure 2. Device used in the survival study.

2.2.3. Biological Material

The experiment involved 219 eggs from 40 hens of the "Lohmann Brown" strain fed with meal from the leaves of *Cajanus cajan* on the farm of the National Higher School of Agronomy and Forestry. These eggs were subsequently transported to the ACODEL Veterinary Clinic where the incubation of the eggs took place (Figure 3).

Figure 3. Sample of fertilized eggs for incubation.

3. Methods

3.1. Artificial Incubation Method

Before incubation, several operations were carried out. The first operation consisted of selecting the eggs on the basis of their physical characteristics: cleanliness, shell quality, size and shape. Thus, only eggs that were clean and had a smooth shell, a regular shape and an average size were retained for incubation.

The sorted eggs were then candled using a pocket flashlight to ensure the presence of an embryonic disc and the absence of micro cracks. This first mirage was necessary given the mode and means of transport used. A second candling was carried out on the 7th day of incubation to check the fertility of the eggs. This second candling allowed the clear (unfertilized) eggs to be removed from the incubator. A final candling was carried out on the 18th day of incubation during the transfer and made it possible to determine embryonic mortalities and rotten eggs.

3.2. Incubation of Eggs

This phase first consisted of commissioning the incubator. Thus, 3 hours before the start of the experiment, the incubator was put into service empty in order to ensure its proper functioning. The incubated eggs come from the four batches (0%, 5%, 10% and 15%) previously identified. The quality of the eggs was first checked before their introduction into the incubator (**Figure 4**).

Figure 4. Arrangement of eggs in batches.

3.3. Measurement of Temperature, Relative Humidity and Egg Transfer

Recordings of the temperature and relative humidity of the incubator were made weekly. In fact, every week the temperature and humidity were recorded using the cards. The temperature and humidity readings provided by the incubator were taken throughout the incubation period (21 days).

The transfer took place on the 18th day after incubation. It is an operation which consists of removing the eggs from the incubation trays to place them in the hatching trays. To do this, newspaper was placed in the rack so that the eggs were placed on top. Finally, the traps were stacked and placed in the hatcher.

3.4. Hatching

Upon hatching, the chicks were identified by batch (Figure 5) and kept for 48 hours in the incubator while they dried. Then they were transferred to a building for the survival study. Unhatched eggs were also identified by batch and their contents checked for scrap.

Figure 5. Identification of eggs and hatched chicks.

3.5. Analysis of Unhatched Egg Breakage

It consisted of checking the contents of the unhatched eggs during the experiment. It was done as follows: a bucket containing the mixture of tap water and bleach to avoid contamination and odors produced by the latter. Each egg was broken using forceps and checked simultaneously for its contents (Figure 6).

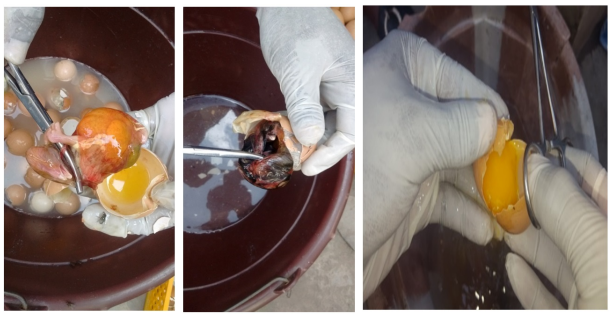


Figure 6. Study of breakages (early death on the left; late death in the middle; light egg on the right).

3.6. Formulas Used for Calculating Different Hatching Parameters

The different hatching parameters were calculated according to the formulas below:

$$\textbf{TOC} = \frac{\text{Number of clear eggs}}{\text{Total number of eggs incubated}} \times 100$$

$$\textbf{TF} = \frac{\text{Number of fertile eggs}}{\text{Total number of eggs incubated}} \times 100$$

$$\textbf{TME} = \frac{\text{Number of eggs with dead embryos}}{\text{Total number of eggs incubated}} \times 100$$

$$\textbf{TM}\ \text{intra-shell chick} = \frac{\text{Number of dead chicks in shell}}{\text{Total number of eggs incubated}} \times 100$$

$$\textbf{TEA} = \frac{\text{Number of chicks hatched}}{\text{Total number of eggs incubated}} \times 100$$

$$\textbf{TER} = \frac{\text{Number of chicks hatched}}{\text{Total number of eggs incubated}} \times 100$$

3.7. Method for Studying the Survival of Hatched Chicks

After hatching, the chicks were monitored for thirty (30) days during which they were fed the standard food. They were vaccinated against Newcastle disease, Gumboro disease, infectious bronchitis and treated against coccidiosis, according to the prophylaxis program shown in Table 1.

Table 1. Medical prophylaxis program applied to chicks.

Age (day)	Actions	Products used
D4	Vaccination against Newcastle disease	HB1 (drinking water)
D5-6	Administration of vitamins	Amin's total (in drinking water)
D7	Vaccination against Gumboro	AVIBD inter 500 (in drinking water)
D8-9	Administration of vitamins	Amin' total
D15-16-17	Administration of collibacillosis	Trisulmycine (in drinking water)
D18	vaccination reminder Newcastle	HB1 (drinking water)

3.8. Arrival and Settling in the Chicks

Before the subjects were installed, a physical examination was carried out to ensure the physical fitness of the animals and then identification was made using identification marks.

3.9. Feeding and Watering Program

Food was served in the xiphoid-type feeders at a rate of twice a day (morning and evening). Drinking water was distributed ad libitum in the plastic siphoid type drinkers with a capacity of ten liters. These were cleaned at a rate of once a day.

3.10. Data Processing and Analysis

The analysis of the results obtained and the comparison of the means between the different treatments were carried out by the analysis of variance test (ANOVA)

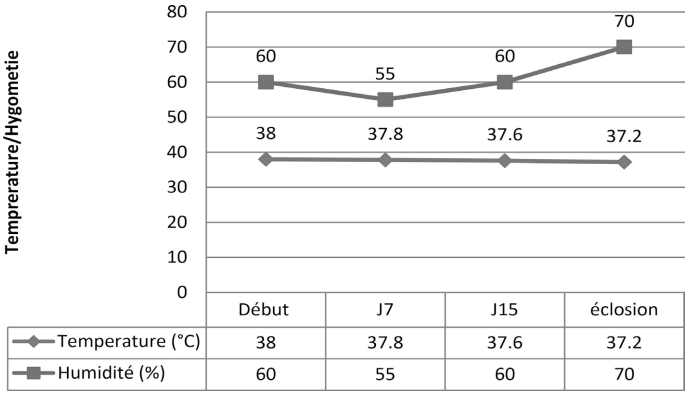
using the Statistical Package for the Social Science (SPSS) software and completed by the test of Duncan when the ANOVA test showed a significant difference.

4. Results

4.1. Temperature and Humidity of the Incubator

Temperature and humidity are important factors that allow us to monitor and understand variations during our experiment.

Figure 7 shows the curves of variations in temperature and relative humidity as a function of time. As the number of days advances, the temperature decreases slightly from 38˚C to 37.2˚C and the relative humidity increases over time. The temperatures recorded at our incubator are contrary to those (37.7˚C and 37.8˚C) reported by Sauveur (1988) and L'Amoulen (1988). These results indicate that the temperature was homogeneous and constant at all points in the incubator during incubation. The results showed that the relative humidity was more or less respected in the incubator.



	Début	J7	J15	éclosion
Temperature (°C)	38	37.8	37.6	37.2
Humidité (%)	60	55	60	70

Figure 7. Variation in temperature and humidity of the incubator.

4.2. Fertility Rate (FR)

The fertility rates according to food rations are recorded in Table 2. It appears from this table that the animals of the 15% batch recorded the highest fertility rate (86.95%) followed by the 5% batch. The lowest fertility rate was recorded in the control batch (26.88%). There is a clearly significant difference between the rates of different batches $P < 0.05$).

Table 2. Fertility rates recorded in different batches.

Treatment	FR
CC 0%	26.88 ± 0.32a
CC 5%	43.95 ± 1.30c
CC 10%	33.20 ± 0.87b
CC 15%	86.95 ± 0.77d

a, b, c followed by different letters within the same line are significantly different at the threshold of 5%. The same as all tables.

4.3. Hatching Rate (HR)

The hatching rates of the different batches are recorded in Table 3. It appears that the CC 15% batch recorded the highest rate 36.87% followed by the CC 10% and 5% batch. The lowest hatching rate (17.18%) was recorded in the control batch (CC 0%).

Table 3. Hatching rates recorded in different batches.

Treatment	HR
CC 0%	17.18 ± 0.34a
CC 5%	22.05 ± 1.03b
CC 10%	25.96 ± 0.72c
CC 15%	36.87± 0.88d

4.4. Embryonic Mortality Rate (EMR)

The embryonic mortalities recorded throughout the experiment are presented in Table 4. The highest embryonic mortality rates were recorded in the CC 15% batch (30.58%) followed by the CC 5% batch (19.28%) and the control batch CC 0% (10.74%). The lowest rate of embryonic mortality was recorded in the CC 10% batch (2.10%). These rates are statistically significantly different ($P < 0.05$).

Table 4. Embryonic mortality rates recorded in different batches.

Treatment	EMR
CC 0%	10.74 ± 0.82b
CC 5%	19.28 ± 0.42c
CC 10%	2.10 ± 0.89a
CC 15%	30.68 ± 0.79d

4.5. Clear Egg Rate (CER)

Table 5 presents the rates of unfertilized eggs in the different batches of the experiment. The rate of 72.20% represents the highest rate (CC 0%). The lowest rates of unfertilized eggs were recorded in the experimental batches (12.03%; 57.42% and 66.66%) respectively in the CC 15%, CC 5% and CC 10% batches. These rates are significantly different ($P < 0.05$).

Table 5. Rate of unfertilized eggs recorded in different batches.

Treatment	CER
CC 0%	72.20 ± 0.96d
CC 5%	57.42 ± 0.65b
CC 10%	66.66 ± 0.69c
CC 15%	12.03 ± 0.67a

4.6. Shell Mortality Rate (SMR)

Shell mortalities are generally low in all batches (Table 6). However, the rate of 18.58% was recorded in the CC 15% batch. The lowest shell mortality rate was obtained in the control batch (CC 0%). These rates are significantly different ($P < 0.05$).

Table 6. Shell mortality rates recorded in different batches.

Treatment	SMR
CC 0%	2.07 ± 0.53a
CC 5%	5.90 ± 0.58b
CC 10%	6.17b± 0.41b
CC 15%	18.58 ± 0.24c

a, b, c followed by different letters within the same line are significantly different at the threshold of 5%.

5. Discussion

The temperature during our experiment varied between 38˚C to 37.2˚C. The temperatures recorded at our incubator are similar to those (37.7˚C and 37.8˚C) reported by [20] [21]. These results indicate that the temperature varied slightly in a decreasing manner as time progressed. Our results are similar to those of [22] who believe that the optimal temperature must be maintained at 38.9˚C, during the first two weeks of incubation, and reduced to 36.1˚C from the 19th day of incubation because the chicks also produce heat. Our results are different from those of [23], who thinks that this temperature should be between 39 and 39.5˚C (102˚f - 103˚f) and also be constant during incubation. According to [20] [21], for incubators with forced ventilation, the ideal temperature for better development of the embryo and optimal hatching is 37.7˚C to 37.8˚C. This temperature is also decisive for the correct growth of the chick after hatching. Low temperatures delay hatching but are more dangerous than high temperatures [22]. Humidity varied in an increasing direction (60% to 70%). This development ensures the proper development of the embryo but also facilitates digging by making the shell more fragile. Our results corroborate those [20] [21] [24], which stipulate that the best incubation results are obtained with a relative humidity of 50% to 60% during the first 18 days and at more than 75% during the last three days of incubation.

The fertility rates depending on the rations in our study varied significantly between 26.88% and 86.95%. This variation in fertility rates could be explained by the level of mobility and the breeding conditions which in a certain way caused stress to the animals. The most interesting rates (78% and 91%) have been reported in lighter breeding conditions [25] [26]. According to [22] [25] [27], the sex ratio is generally one rooster for 10 hens with variations depending on the breed. [28], rather recommends that to have good fertilization, it takes 8%

to 8.5% of roosters of the number of hens to obtain 90% to 92% of fertilized eggs from the 26th week of age and 94% to 97% from the 28th week. In chickens, the higher the laying rate, the higher the percentage of fertile eggs. The hens that lay the most eggs would also be the ones that get the most attention [23]. The hatching rates obtained in this study are generally low and vary between 36.87% and 17.18%. This low hatching rate could be explained on the one hand by the untimely power outages causing variations in temperatures and relative humidity at the level of the incubator, and on the other hand by the rearing conditions of the breeders in experimental cages undoubtedly facilitating the breeding of females by males. Higher rates have been reported by various authors in Africa. Indeed, it would be 80% [29] [30] [31] respectively in Nigeria, Senegal and Cameroon. However, it shows a strong variation depending on the country. In Mali, it varies between 60% - 70% [32] and 42% - 80% in Guinea according to [33]. This variation would be due, in addition to possible errors linked to the data collection methodology, but also to the season. For this purpose, the hottest seasons would be the most unfavorable, undoubtedly because of the poor conservation of eggs due to high ambient temperatures [32] [34].

The embryonic mortalities recorded in our study varied between 2.10% and 30.68%. These rates were significantly different depending on the treatments. This difference could be due on the one hand to power outages during the incubation period, consequently causing temperature variations, and on the other hand, to the frequency of relative humidity control during the incubation phase incubation. Note also that these high embryonic mortality rates could be the result of poor egg porosity in these batches. Indeed, too great a porosity would lead to excessive evaporation with the consequence of dehydration of the embryo and death, and too low porosity would cause insufficient evaporation and consequently the embryo dies by drowning.

The rates of unfertilized eggs in the different experimental batches varied between 72.20% and 12.03%. The high rates of unfertilized eggs in our study would undoubtedly be linked to the quality of the males. Indeed, males whose sexual characteristics of interest (absence of dewclaw, weakly developed and leaning crest, asymmetry of the barbels) in reproduction are not well developed must be excluded. In our study, the limited number of males did not allow for adequate selection.

Generally speaking, the incorporation of *Cajanus cajan* leaf flour into the ration of breeders did not cause any adverse effects on the health status of the chicks produced from the latter's eggs. Indeed, no mortality was observed during the entire thirty (30) day study period. The 0% mortality rate suggests that the incorporation of sun-treated *cajanus cajan* leaf meal does not have negative impacts on the general functioning of the reproductive organs of chickens.

6. Conclusions

The leaves of *Cajanus cajan*, like those of other legumes, constitute an important source of nutrients. They are relatively rich in nutrients, particularly proteins,

minerals and vitamins. They have a better benefit of essential amino acids (lysine, phenylalanine, valine, leucine and isoleucine). The grains are edible (India, Kenya, etc.) and rich in fatty acids, the main ones being linoleic acid and palmitic acid. Our study involved 219 eggs from 40 subjects fed *Cajanus cajan* leaf flour as a substitute for soybean meal at the ENSAF School farm. It appears from this study that the reproductive parameters of these laying hens are good. There was no difference between the two foods distributed (control and experimental) to the laying hens regarding the start of laying, the fertility rate of 86.95 (CC 15%); 43.95% (CC 5%); 33.20% (CC 10%) and 26.88 (CC %). A hatching rate of 36.8% (CC15%); 25.96 (CC 10%); 22.05 (CC 5%) and 17.18 (CC 0%), the shell mortality rate per batch was 2.10 (CC 10%); 10.74 (CC 0%); 19.28 (CC 5%) and 30.68 (CC 15%).

At the end of our study, we can conclude that the incorporation of *Cajanus cajan* leaf meal up to 15% in the reproduction type ration in hens, had no harmful effect on the reproduction performance of laying hens. Congo. On the contrary, it improved growth parameters and increased reproductive parameters. It did not cause any harmful effects on the health of the chicks produced from the latter. Taking into account the performances obtained in this study, it can be concluded that the incorporation of *Cajanus cajan* leaf meal can be recommended up to 15% in the breeding type feed in laying hens.

7. Comments and Perspectives

1) Summarize and comment on the challenges related to poultry farming:

In the Republic of Congo, despite a strong demand for poultry, the level of production remains low and does not cover the needs of the populations. This situation increases Congo's dependence on the outside world for meat products, giving way to massive imports [35] (DJOMBO, 2016). Consequently, the quantity of imports increases every year, it was estimated at 200 billion in 2011 [36] (FAO, 2012). This deficit in meat products is linked not only to the low productivity of local breeds but also to the insufficient mastery of production techniques and to the demographic explosion that the Congo is experiencing [37] (AKOUANGO, 2010). To reverse this trend, the desire of the Congolese State is to ensure the sustainability of agricultural activities through the modernization of family farming towards commercial agriculture, that is to say, to make this sector more productive in order to diversify its economy [38] (PDAC, 2017). Since 2002, we have seen a revival of the poultry sector, particularly in peri-urban and rural areas. Despite these efforts, the poultry sector in Congo faces a food problem. The success of poultry farming depends on several factors including nutrition. These are, in a few lines, the challenges of poultry farming in the Republic of Congo.

2) Does the addition of *Cajanus cajan* leaf flour influence the nutritional components of eggs?

In order to answer this relevant question, our study did not evaluate the quality of eggs from chickens fed *Cajanus cajan* leaves. However, we raise this ques-

tion as a perspective to determine the nutritional quality of eggs from chickens fed on *Cajanus cajan* leaves.

3) Does the addition of *Cajanus cajan* leaf meal influence the health of chickens?

In our study, it appears that the study of the survival of chicks from chickens fed on *Cajanus cajan* leaves showed a 0% chick mortality rate. From these results, we can conclude that the incorporation of *Cajanus cajan* leaves up to 15% does not influence the health status of poultry.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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