



## Impacts Of Probiotics On The Production Performance And Immune Status Of Broiler Chickens From Arbor Acre Strains

H. Arioua<sup>1</sup>, A. Berghiche<sup>2\*</sup>, N. Ait Isaad<sup>3</sup>, N. Mimoune<sup>4</sup>, F. Arioua<sup>5</sup>, R. Bouzid<sup>6</sup>, D. Khelef<sup>7</sup>

<sup>1,6</sup> Veterinary Institute, Chadli Ben Djedid University, El Taref, Algeria. amine-berghiche@yahoo.com

<sup>2\*</sup> Life sciences and techniques laboratory, Taoura Agro Veterinary Institute, Mohamed Cherrif Messaadia Souk Ahras University, Algeria

<sup>2</sup> Institut Agro Veterinary Taoura, Mohamed Cherrif Messaadia University Souk Ahras, Algeria.

<sup>3</sup> Veterinary Institute, Blida 1 University, Algeria.

<sup>3,4,5,7</sup> Animal Health and Production Laboratory (SPA), National Veterinary Higher School, Algiers, Algeria.

<sup>7</sup> National Veterinary Higher School Algiers, Algeria.

**\*Corresponding Author:** A. Berghiche

\* Life sciences and techniques laboratory, Taoura Agro Veterinary Institute, Mohamed Cherrif Messaadia Souk Ahras University, Algeria a.berghiche@univ-soukahras.dz,

	<b>Abstract</b>
<b>Article History</b> Received: 13/11/2023 Accepted: 16/03/2024 Published: 1/04/2024	<p>The aim of the present research was to evaluate the probiotic activity of <i>Lactobacillus acidophilus</i> in broilers of the ARBOR ACRE strain. The probiotic was administered in drinking water at a rate of 50 ml per 1000 chicks every 7 days during the breeding period. Growth performance, the effect on endogenous flora and on the structure of the gut and the bursa of Fabricius, as well as the immune response, were recorded at 20 and 37 days. We evaluated the effectiveness of <i>Lactobacillus acidophilus</i> added to broiler diets, a zootechnical study (mortality, growth rate, index of growth) and a clinical survey, homogeneity and antibody production. Zootechnical studies revealed that the best growth performances were achieved by the probiotic-treated chickens, with significant differences (<math>p &lt; 0.05</math>). which concluded with a positive effect on zootechnical performance compared with the controls. Similarly, the study of the organism's reaction showed a good response from the birds supplemented with <i>Lactobacillus acidophilus</i> in the animal's digestive tract, with interactions with the endogenous flora, compared with the control subjects (<math>P &lt; 0.05</math>). Antibody titration showed that the <i>Lactobacillus acidophilus</i>-based diet had a positive effect on antibody levels, resulting in a coverage rate higher than the average indicated by the baseline (1000-2500) and also higher than the control batch antibodies.</p>
CC License CC-BY-NC-SA 4.0	<b>Keywords:</b> Probiotics, lactobacillus acidophilus, zootechnical performance, antibody, Broiler chicken

## INTRODUCTION

Microbial microflora are generated during the first few days of life. From the age of four days, the number of bacteria increases considerably, and growth stabilizes from the second week of life. (Kuney, 1982).

For over twenty years, livestock farming has become increasingly industrialised, and antibiotics in particular have long been used successfully to improve the zootechnical and health performance of farm animals. (Gunal *et al.*, 2006 ; Berghiche *et al.*, 2019). The consequence of using these as growth promoters in chickens is the appearance of multi-resistant pathogenic bacteria, most of which are generally found in poultry droppings. (Moharrery et Mahzonieh, 2005 ; Berghiche *et al.*, 2020).

Bacteria spend an enormous amount of energy maintaining their resistance to antibiotics. The common practice in poultry farming of using antibiotics or replacing them with other drugs only exacerbates the problem, with the appearance of dysbiosis, where the undesirable microbial flore predominates the gastrointestinal compartment, reducing nutrient absorption and increasing the thickness of the mucosa as well as slowing down the passage of food, This disrupts the host's nutritional requirements and accelerates the turnover of enterocytes, while reducing the height of the villi and the depth of the crypts and ultimately reducing performance. (Kuney, 1982 ; Martinez, 2009)

To resolve such problems of bacterial multi-resistance and destabilisation of the microbiota, it is therefore necessary to enrich the diet with probiotics, which are essentially used to provide beneficial micro-organisms so that the chickens can benefit from the favourable effects of these micro-organisms. (Fuller, 1977).

Lactic acid bacteria such as *Lactobacillus acidophilus* have long been used, consciously or unconsciously, to maintain and improve health. (Coelho *et al.*, 2022).

Our study looks at the effect of using the probiotic *Lactobacillus acidophilus* in drinking water on the zootechnical performance and stability of the endogenous flora of the intestinal microbiota of broilers of the Arbor acre strain, and on immune status.

The aim of this study is to determine, under our local rearing conditions, the value of adding probiotics during the two broiler rearing periods (start-up, growth), by studying the impact of this supplementation on growth and mortality, as well as on the conversion rate, immune status and intestinal integrity.

## MATERIALS AND METHODS

### • Bacterial strain

A symbiotic containing a prebiotic and a probiotic lactic acid bacterium (*Lactobacillus acidophilus*).

### • Animals and feed

Chicks of the ARBOR ACRE strain, purchased from a private hatchery and commercial feed manufacturer (Sarl Cheikh Fab Pro Azazga Wilaya Tizi. Ouzou) were weighed and randomly distributed over 2 buildings of average body weight ( $38 \pm 1$  g). The experimental group contained 14248 chicks, divided into 4 replicates of 3712 chicks each, and a control group of 15248 chicks, divided into 3812 birds for each group.

The pellet-type feed (start-up from D 1 to D 20 "Metabolizable energy: 2800 kcal/kg; Crude protein: 21%"), and growth from D 21 to D 37 "Metabolizable energy: 2900 kcal/kg; Crude protein: 19%") and water, these feeds were supplied ad-libitum in both buildings to ensure similar rearing conditions, only an addition of a symbiotic in the drinking water for the experimental building every 7 days from d1 until 35 days at a rate of 50 ml of product/week / 1000 subjects.

Both flocks were fed an identical diet adapted and formulated for each rearing period to meet the nutritional requirements of broilers (Dale, 1994), as shown in table 1. These diets are usually used by local producers.

**Table 1.** Diet composition of the two groups

Diet composition(%)	Start-up (1-20 days)	Grow-up (21-37 days)
Maize	60.7%	61,0%
Soya	32%	27,0%
Wheat bran	4.0%	9,0%
VMS	1.0%	1.0%
Phosphate	1.7%	1,5%
Calcareous sand	0.6%	0,5%
Chemical composition		
Metabolizable energy(Kcal)	2891%	29,08%
Crude protein	21.1%	19,5%

VMS: vitamin mineral supplement.

The chicks were vaccinated on day 1 with a complex live IBD vaccine containing the Winter field 2512G-61 strain of Gumboro disease complexed with anti-IBDV antibodies in lyophilised form.

#### • **Breeding methods**

The experiment was performed on a flock of broilers of the ARBOR ACRE strain, reared intensively in two buildings with the same technical parameters (construction materials, insulation, surface area, etc.) The walls were made of metal framework and sandwich panels containing glass wool, 40-50 cm thick, and the roof was double-layered. The buildings used for the experiments were of the closed type with dynamic ventilation (tunnel ventilation), measuring around 80 m long by 14 m wide, with side walls 2.5 m high and 4.4 m in the middle. The density was 35 kg per m<sup>2</sup> at 40 days of age (weight  $\approx$ 2.5kg), corresponding to 14 to 15 chicks per m<sup>2</sup>.

These buildings are equipped with a propane gas heating system and pad-cooling on the side walls at the entrance. They are also fitted with ultra-sophisticated environment control probes (humidity, temperature, carbon dioxide and ammonia) and a Scove brand "food chain" drinking and feeding system. During the first 4 to 5 days, feed is poured onto cellulose paper throughout the building, covering more than 80% of the surface area used at the time of installation. Rearing is then carried out on the floor on wood shavings, to limit heat loss by the animals and the absorption of moisture from excrement.

#### • **Measurement parameters**

Economic parameters : The average weight gain (ADG) equal to the average of the final weight minus the average of the initial weight over the number of days, the conversion index which is the ratio between the quantity of feed consumed and the final weight, the mortality and morbidity rate which is the ratio between the rate of dead or sick animals during the rearing period and the initial number of animals multiplied by 100, were evaluated according to the method described by Yusrizal and Chen, (2003), and were determined at the end of each rearing phase (20 days and 37 days).

The homogeneity (homogeneity formula) and immune status of the lots of broilers were assessed by evaluating the serum antibody titres of subjects from the two buildings (control and experimental) before and after vaccination.

The method consisted in randomly taking 22 subjects from the same hatchery for the two buildings, weighing them and then sacrificing them by bleeding, followed by the recovery of 2 ml of blood in dry tubes. For the second and third samples, taken at 20 and 37 days of age, twenty birds per replicate (4 replicates) for the two batches were taken at random in the form of a cross (type of sampling) inside each building. The birds were weighed and then punctured at the wing vein at a rate of 2ml/subject, then collected in dry tubes and kept in a refrigerator at 2° C to 8°C for a maximum of 24 hours before being sent to the laboratory for ELISA testing. This test is based on the use of a solid support (microplate wells, beads, microparticles). This is coated with antigen for antibody detection. The patient's serum is brought into contact with the coated support. An antigen-antibody complex is formed. This complex is revealed by the binding of an enzyme that transforms a specific substrate into a coloured compound or emits a signal. The signal is then detected using a spectrophotometer (Elfried *et al.*, 2017).

The enzyme conjugated to the secondary antibody can be a peroxidase. This is an oxidase-type enzyme, allowing the degradation of peroxides. Oxidation of the various substrates yields a chromogenic compound as the final product (Zuchuat, 2014).

#### • **Statistical treatment**

The results are described by the mean and standard error (SE). The homogeneity of the variance between treatments was verified by Bartlett's test. The results were subjected to a one-factor analysis of variance to determine the effect of probiotic supplementation on the parameters described. Differences between treatments were compared using a Student-Newman-Keuls test. Any p-value less than 0.05 is considered significant. The data collected for this study were analysed using *Statview* software (Abacus Concepts, 1996, Inc., Berkeley, CA94704-1014, USA), in order to assess the efficacy of the probiotic *lactobacillus acidophilus* on Growth Performance and the immune response of broiler chickens.

## **RESULTS**

#### • **Net body weight and average daily gain (ADG)**

Starting with a chick weight of 38±1g at start-up, compared with a weight of 44g for the 2022 standard strain, the weights recorded by the replicates that received a symbiotic showed a dominant positive trend (**P=0.05**) compared with the other replicates of the control batch in both rearing phases, as shown in the **table 2** below.

**Table 2.** Weight evolution of broilers during the start-up and growth phases of rearing as a function of whether or not symbiotics are added to the feed ration.

Experimental lot				Control lot				
Repetitions								
1	2	3	4	1	2	3	4	
Initial weight (g)	38	38	38	38		38	38	
	38	38						
Start-up phase (1 to 20 days)								
Weight (g) at 20 days	686	728	728	724.4	674	692.5	625.5	703
ADG 1-20 days (g/d/subject)	32.4	34.5	34.5	34.32	31.8	32.725	29.375	33.25
Growth phase (21 to 37 days)								
Weight (g) at 37 days	2370	2359	2395	2514	2248	2364	2189	2364
ADG 21-37 days (g/d/subject)	99.05	95.94	98.05	105.27	92.58	98.32	91.97	97.70
Phases 1+2 (1 à 37 days)								
Final body weight (g)	2370	2359	2395	2514	2248	2364	2189	2364
ADG(g/d/subject)	64.05	63.75	64.72	67.94	60.75	63.89	59.16	63.89

**ADG : average daily gain**

At the end of the rearing period, group 4 of the experimental batch performed best, with a final weight of 2514g and an ADG of 67.94g/d, significantly better ( $P=0.05$ ) than the control and even the experimental batch replicates. However, lower weights and growth rates were recorded for the control replicates.

During the start-up phase, the results showed a significant superiority ( $P=0.05$ ) in weight and growth rate, reflected by a higher average ADG in the experimental lot with its four replicates, equal to 33.93g/day. On the other hand, a delay in growth was recorded for the control batch in all these repetitions, with an average ADG equal to 31.78g/d. The standard 2022 ADG for the strain at this age is 42.09g/d.

At the end of the first phase, the experimental batch remained significantly superior and dominated the control batch with an average weight of 716.6 g compared with 673.75 g and 928 g for the standard. The ADG for the start-up phase showed superior weight growth in the chicks receiving the symbiotic, in contrast to the batch without the symbiotic, which showed significant growth retardation.

During the growth phase, the growth rate reported for all the replicates of the treated batch, represented by the ADG, was higher than for the replicates of the control batch ( $P=0.05$ ) with an average ADG of 99.57 g, in contrast to the groups of the control batches which continued their growth with an ADG of 95. At 14 g/d, both ADGs were higher than the 2022 standard for the strain, which is 91.64 g/d. Weight development during the growth phase was higher for the treated batch ( $P=0.05$ ), with a significantly higher value for group 4 (ADG=105.27 g/d) compared with the other groups in the experimental and control batches.

#### • Indices de conversion

Feed intake during the first rearing phase (Table 3) was almost similar for the two batches, with an average of 946.97g for the experimental batch and 937.64g for the control batch compared with 1057g of feed intake for the standard strain, with a small difference in the growth phase, with 2616g of feed intake for the experimental batch compared with 2510g for the control batch and 2554g for the standard strain.

With respectively for the average conversion indices, start-up 1.312 treated batch against 1.394 control batch and 1.139 standard of the strain, in the growth period 1.496 experimental batch against 1.517 untreated batch against 1.639 for the standard.

**Tableau 3.** Evolution of the conversion index (CI) during the rearing period in broilers as a function of symbiotic supplementation or not.

(CI) conversion index								
Experimental lot				Control lot				
Repetitions	1	2	3	4	1	2	3	4
1		2	3	4				
Food ingredient start-up (QI)	946.97				937.64			

CI start-up (1-20 days)	1.37 0	1.291	1.291	1.297	1.3 91	1.353	1.49 9	1.333
<b>Food ingredient growth (QI)</b>		<b>2616</b>				<b>2510</b>		
CI growth (21-37 days)	1.51 9	1.567	1.534	1.431	1.5 57	1.468	1.56 7	1.477
<b>Food ingredient total (QI)</b>		<b>3562.97</b>				<b>3447.64</b>		
Total conversion index	1.52 1	1.528	1.505	1.434	1.6 08	1.530	1.65 2	1.530
<b>QI : Food ingredient quantity, IC : conversion index</b>								

Concerning the total conversion index (Table 3) and based on the ADGs performed and the quantities of feed ingested, we found that the replicates of the experimental batch had the best conversion index throughout the rearing period with an average of (1.497) compared with 1.58 for the control batch and 1.452 for the strain standard. These replicates were significantly better ( $P=0.05$ ) than the replicates of the control batch, which were unable to better translate their feed intake during the two rearing phases into weight gain. Furthermore, the replicates of the control batch showed almost similar conversion indices, while the best performing index throughout the rearing period was recorded at the fourth replicate of the experimental batch for all batches at 1.434.

#### • Mortality rates

With regard to the mortality rate during the experiment (Table 4), the results showed that the replicates of the batch that received the symbiotic presented the lowest mortality rates evaluated at 4.04% on average throughout the rearing period, compared with  $\approx 5\%$  that were observed for the untreated batch, with an average of 1.36% start-up phase experimental batch compared with 1.275% of the control batch and growth phase 2.75% treated batch compared with 3.79% control batch.

**Table 4.** Mortality rates recorded during the two breeding phases.

Repetitions	Experimental lot				Control lot			
	1	2	3	4	1	2	3	4
	64	50	54	36	57	55	46	37
Start-up phase (number and Mortality rate %)	1.72 4	1.3	1.45	0.96	1.49	1.44	1.2	0.97
Growth phase (number and Mortality rate %)	92 2.55 9	108	113	84	147	123	134	161
Total mortality	156	158	167	120	204	178	180	198
Overall mortality rate %.	4.2	4.2	4.49	3.23	5.35	4.66	4.72	5.19
		5						

The results quoted above show that the mortality rate evolved inversely with the replicates that received the symbiotic compared with the others that did not, especially in the growth phase.

#### • Immunological status

The serological results for the two farms are shown in the Table 5 below.

**Table 5:** Serological statistics for anti-Gumboro antibodies in each phase

Repetitions	Experimental lot				Control lot				
	1	2	3	4	1	2	3	4	
<b>Parameters</b>									
<b>Just after hatching (a single sample for the 2 buildings)</b>	MT			4831					
	GMT			3869					
	CV%			61					
<b>Start-up phase</b>	MT	450	308	368	324	410	193	317	205
	GMT	313	146	183	184	256	109	156	175
	CV%	81	113	102	109	101	73	115	59

<b>Growth phase</b>	<b>MT</b>	701 7	600 7	7960	5293	4423	564 2	743 8	6102
	<b>GMT</b>	677 5	583 6	7836	5091	4251	552 5	461 1	5762
	<b>CV%</b>	30	27	19	30	33	2	51	36

**MT:** arithmetic mean of antibody titre, **GMT:** geometric mean of titre, **CV:** coefficient of variability.

**A. For AOM** (antibodies of maternal origin) all the sera were positive and the average antibody titres of the chicks that had recently come from the hatchery was very high compared with the protective threshold (1000-2500), which explains the success of the vaccination of the parents.

However, the coefficient of variability is slightly high (61%), which means that homogeneity needs to be improved in terms of immune response, which means that the broiler flock is old.

#### **B. For post-vaccination antibodies**

- Vaccination: The flock was vaccinated with a complex immune type vaccine on the first day by a subcutaneous injection at a rate of 0.1ml/chick for the two buildings (treated and control):

#### **C. At the end of the start-up phase**

the mean antibody titres of the replicates in the two buildings were below the positivity threshold, with a slight superiority of the mean of the replicates in the experimental batch (TM=370) against (TM=281.25).

With a poor coefficient of variation for the two buildings, expressed by the mean of the experimental batch replicates (CV=101.25%) compared with (CV=87%) for the control batch replicates.

#### **D. At the end of the growth phase**

- The value (CV=26.5%) is correct, the uniformity of the replicates in the experimental batch is excellent compared with the control batch (CV=35.5%).

- The arithmetic mean titre of the 4 replicates was fairly high (MT=6569.25) compared with the control batch (MT=5901.25) and with the baseline (1000-2500) of the MClabovet Algeria laboratory.

## **DISCUSSION**

### **1. Impact of probiotics on growth performance**

In broiler chickens, probiotics can improve growth performance and control diseases such as salmonellosis, necrotic enteritis and coccidiosis. (M'Sadeq, S.A et al., 2015).

During the start-up phase, with a start-up weight of 38g, the results show that the weights and growth rates achieved by the replicates of the experimental batch (average weight = 686g) were almost similar to each other ( $p = 0.05$ ), with lower values observed in group 1, but still higher than the control, which suffered a significant growth retardation ( $p = 0.05$ ) observed in chicks not supplemented with the lactbacillusacidophilus strain. Similarly, the highest ADG was achieved by groups 2 and 3 of the treated batch (34.5g/d) as opposed to group 4 (33.25g/d) of the control batch. Nevertheless, we can deduce that in the start-up phase the young chicks reacted relatively positively to the addition of probiotics in the drinking water compared with the standard weight of the strain at this age (=928g started with 44g).

Contrary to the many experiments that have been conducted involving the incorporation of probiotics, once only, especially at start-up, into the drinking water, because of the cost of the product and the stress involved in handling the livestock, especially on traditional farms, our results show that the introduction of probiotics throughout the rearing period (every 7 days) is strongly recommended, especially when using antibiotics that have an impact on the digestive microbiota. (Maldonado et al., 2017).

During the growth phase, the groups receiving the probiotic continued to grow at an accelerated rate, with a marked superiority for repetition 4 (2514g) compared with (2364g) for repetitions 2 and 4 of the control batch. This is probably due to the good assimilation and use of nutrients. Better performance was obtained with the probiotic-supplemented subjects, with an average weight of 2409.5 g close to the 2022 standard for the strain with (2486g) and an average ADG of 99.69g/d, which slightly exceeds the standard (99g/d), followed by 2291.25g average weight and 95.14g/d ADG for the control.

### **2. Impact on food intake and conversion index**

Intestinal health goes hand in hand with the health of the host and therefore its performance, so it makes sense to try to achieve a balance in the flora that would be able to ensure a symbiotic relationship between the flora and intestinal function, this harmonious relationship between these two entities will help maintain wellbeing

and performance by ensuring optimal digestion and absorption, integrity of the intestinal mucosa and good neuroendocrine and motor function (Celi *et al.*, 2017), and therefore optimal nutrient conversion.

Studies have shown that the addition of *L. reuteri*, *B. licheniformis* and *B. subtilis* improved weight gain and conversion index in broilers. (Bhagoju *et al.*, 2021).

The use of multiple strains of probiotics (*E. faecium*, *Bifidobacterium animals*, *P. acidilactici*, *L. reuteri*, and *L. salivarius*) significantly improved weight gain and conversion index in broilers. (Palamidi *et al.*, 2016).

With the prohibition on the use of antibiotics as growth promoters, in addition to the drop in performance, the poultry industry has also seen the emergence of certain pathologies such as necrotic enteritis. The use of *B. subtilis* has made it possible to improve the digestive flora of poultry with necrotic enteritis problems (Whelan *et al.*, 2019), which obviously had a positive effect on the host's digestive health and consequently on its health in general and therefore on its performance. This was the case in our present trial, where we observed an improvement in the homogeneity between the experimental batches, in the conversion index, in the mortality rate and in weight gain compared with the control batch.

It is interesting to note that during the start-up phase, feed intake was almost identical for the two batches, with a value of (QI=946.97g) for the experimental and (937.64 g) for the control, both values being below the standard value for the strain (1057 g). It appears that the batch that received a dose of symbiotic proved to be the most sensitive to variations in feed intake. However, this slight increase in feed intake correlated with the body weights achieved shows an improvement in the conversion index of the treated batch compared with the control.

With regard to the growth phase, there was a moderate increase in the feed intake of the experimental batch (2616 g) compared with the control (2510 g), both of which were lower than the feed intake of the standard strain (3611 g). As a result, the feed conversion of group 4 in the experimental batch was considerably lower, at 1.434 compared with the other replicates and including the groups in the control batch. We can conclude that probiotic supplementation was very beneficial for broilers in the growth phase.

We can conclude that the total ingested quantities evolve proportionally with the addition of symbiotic and consequently the feed conversion indices were positively influenced by the probiotics following the moderate increase in feed intake and the good final weights obtained.

### 3. Effect on immunity and mortality

Concerning the mortality rate recorded during our experiment, the results show that probiotic supplementation leads to a reduction in mortality when they are introduced at the recommended dose. This reduction is probably due to the improvement in the birds' immune resistance and the absence of any alteration to the digestive tract. During the start-up period, we noted that the average mortality rate was around 1.36% for the treated batch compared with 1.275% for the control, whereas during the growth phase, mortality reached 2.75% for the experimental batch compared with 3.79% for the control batch.

Galdeano *et al.*, (2019) explain that probiotics fight pathogenic bacteria and prevent them from attaching to the mucus, since they can attach to the mucus membrane and help adjust the host's immune response. They can also hinder the progression of pathogenic bacteria, as they are able to produce antimicrobial substances such as volatile fatty acids, hydrogen peroxide and bacteriocins, which reinforce the host's resistance to the pathogen. (Plaza-Diaz *et al.*, 2020).

Probiotics also appear to improve the expression of tight junction proteins in the intestine (Jiang *et al.*, 2011) in addition to having anti-inflammatory properties (Lehtoranta *et al.*, 2020).

The administration of probiotics led to an increase in the level of immunoglobulins such as immunoglobulins M and A, at the same time as an increase in the antioxidant capacity of serum. (Wang *et al.*, 2018).

According to Walker (2008) bacteria that interact with the gastrointestinal mucosa can interact with the mucosa and lymphoid elements of the mucosa to stimulate intestinal defences, supplementation of the diet with *Bacillus subtilis* DSM32315 attenuated the negative effect on the performance and balance of the intestinal microbiota of broiler chickens challenged by *Clostridium perfringens*. (Bortoluzzi *et al.*, 2019).

### 4. Impact on the production of anti-Gumboro antibodies

In our study farms, we noted that the chickens had suffered a vaccine failure or a delay in the production of post-vaccination antibodies against Gumboro disease, which fills the immunological gap between the AOM and the vaccine antibodies. The type of vaccine and its method of releasing antigens from the immune complex may be responsible for this delay, This explains the titres below the positivity threshold at the end of the start-up period (17 days) when the result is completely negative for both batches, with a slight superiority of antibody titres in the treated batch (supplemented with probiotics) compared with the control, with less mortality as well. (Kurukulasuriya, 2017).

Messaï *et al* (2019) reported that vaccine failures can be due to an inadequate vaccination method; in other words, the vaccine is not used, does not contain the right strain, non-compliance with the vaccine storage cooling chain, and incorrect use of the vaccine at the time of vaccination.

On the other hand, Salhi *et al* (2021) reported that risk factors such as poor hygiene, a lack of biosecurity measures on farms and an inadequate vaccination programme aggravate viral diseases and can lead to huge economic losses in terms of production due to high morbidity and mortality rates.

In replicates of the experimental batch, the mortality rate was found to be lower than in the untreated batch, which explains the undeniable effect of probiotics in supporting the body against viral attacks. (Zuchuatet, 2014).

In addition to their direct antagonistic effect on pathogenic germs, probiotics have also been shown to have a positive regulatory effect on the immune response (Bilal *et al.*, 2021; Terada *et al.*, 2019), by stimulating the production of cytokines by several subclasses of immune cells. In fact, the addition of probiotics has been shown to prevent digestive diseases such as salmonellosis, coccidiosis and necrotic enteritis (El-Sharkawy, 2020 ; El-Sawah, *et al.*, 2020). An improvement in digestion and the caecal microbial population was observed in broilers supplemented with probiotics (Khalid *et al.*, 2021); other author observed an improvement in growth rate, the immune system and the quantity of antioxidants when *Lactobacillus casei* and *Bifidobacterium* were added to the ration. (Zhang *et al.*, 2021).

## CONCLUSION

The results obtained after the addition of probiotics during this trial are only a glimpse, due to the lack of data or benchmarks, of the performance of Arbor Acre broilers under typical Algerian rearing conditions under well-controlled conditions.

However, the addition of probiotics to the chicken's drinking water shows an improvement in growth performance and immune response.

ELISA is a relevant tool for completing a diagnosis of avian pathology in the field, providing information on the proper functioning of the immune system, especially in the event of aggression caused by a vaccinal or wild virus.

Before each vaccination, it is advisable to assess the pathogenicity of the vaccine viruses, so that an appropriate prophylaxis programme can be drawn up. The choice of vaccine strain and vaccination date cannot be standardised, as there are so many parameters that affect the vaccination strategy.

Our study has shown that the use of probiotic bacteria has made it possible to reduce the use of antibiotics as a conventional means of treating or preventing disease in broiler rearing, which is why it is strictly recommended to add them, especially after each antibiotic treatment, for the stability of the broiler's digestive microflora and intestinal integrity.

Gumboro disease is generally endemic in regions of intensive poultry farming, probably because of its great resistance to physical conditions and chemical products. This resistance gives it great persistence in the environment. However, it is vital to put in place strict biosecurity and hygiene plans, with rigorous vaccination practices, including the right strain, and the use of probiotics to boost the birds' immunity by keeping the digestive tract intact and functional.

## REFERENCES

1. BERGHICHE, A. (2019). *Impact d'utilisation anarchique des médicaments vétérinaires sur les poulets chair dans l'Est Algérien* (Doctoral dissertation, Université de Souk Ahras-Mohamed Chérif Mesaadia).
2. Berghiche, A., Khenenou, T., Kouzi, A., & Labiad, I. (2018). An investigation on the predominant diseases, its diagnosis, and commonly used drugs in the poultry farms in the North-Eastern regions of Algeria. *Veterinary world*, 11(7), 986.
3. Bhogoju, S.; Khwatenge, C.; Taylor Bowden, T.; Akerere, G.; Kimathi, B.; Donkor, J.; Nahashon, S. Effects of *Lactobacillus reuteri* And *Streptomyces coelicolor* Growth Performance of Broiler Chickens. *Microorganisms* 2021, 9, 1341. [CrossRef][PubMed].
4. Bilal, M.; Si, W.; Barbe, F.; Chevaux, E.; Sienkiewicz, O.; Zhao, X. Effects of novel probiotic strains of *Bacillus pumilus* and *Bacillus subtilis* on production, gut health, and immunity of broiler chickens raised under suboptimal conditions. *Poult. Sci.* 2021, 100, 100871.
5. Bortoluzzi, C.; Vieira, B.S.; Dorigam, J.C.D.P.; Menconi, A.; Sokale, A.; Doranalli, K.; Applegate, T.J. *Bacillus subtilis* DSM32315 Supplementation Attenuates the Effects of *Clostridium perfringens*



- Challenge on the Growth Performance and Intestinal Microbiota of Broiler Chickens. *Microorganisms* 2019, 7, 71.
6. Celi, P.; Cowieson, A.; Fru-Nji, F.; Steinert, R.; Klueber, A.-M.; Verhac, V. Gastrointestinal functionality in animal nutrition and health: New opportunities for sustainable animal production. *Anim. Feed Sci. Technol.* 2017, 234, 88–100. [CrossRef].
  7. Coelho, M. C., Malcata, F. X., & Silva, C. C. (2022). Lactic acid bacteria in raw-milk cheeses: From starter cultures to probiotic functions. *Foods*, 11(15), 2276.
  8. Dale N 1994 .National research council, nutrient requirements of poultry. Journal of Applied Poultry Research. Ninth revised edition. 3, 101-101.
  9. Elfried Angeingrid late lawson, (2017). Comparaison des performances des Tests de Diagnostic Rapide de l'hépatite B au CHUD/OP et du test ELISA de référence. Mémoire de fin d'études spécialité de génie de biologie humaine. Université d'abomey-calavi, 38p
  10. El-Sawah, A.A.; Aboelhadid, S.M.; El-Nahass, E.N.; Helal, H.E.S.; Korany, A.M.; El-Ashram, S. Efficacy of probiotic *Enterococcus faecium* in combination with diclazuril against coccidiosis in experimentally infected broilers. *J. Appl. Microbiol.* 2020, 129, 1020–1028.
  11. El-Sharkawy, H.; Tahoun, A.; Rizk, A.M.; Suzuki, T.; Elmonir, W.; Nassef, E.; Shukry, M.; Germoush, M.O.; Farrag, F.; Bin-Jumah, M.; et al. Evaluation of Bifidobacteria and Lactobacillus Probiotics as Alternative Therapy for Salmonella typhimurium Infection in Broiler Chickens. *Animals* 2020, 10, 1023.
  12. Fuller R. 1977. the importance of Lactobacilli in maintaining normal microbial balance in the crop. *British Poultry Science*, 18:85-94.
  13. Gunal, M., Yayli, G., Kaya, O., Karahan, N., Sulak, O., 2006. *Int. J. Poult. Sci.*, 5, 149- 155.
  14. Jiang, M.; Dai, C.; Zhao, D.-H. VSL#3 probiotics regulate the intestinal epithelial barrier in vivo and in vitro via the p38 and ERK signaling pathways. *Int. J. Mol. Med.* 2011, 29, 202–208.
  15. Khalid, A.H.; Ullah, K.S.; Naveed, S.; Latif, F.; Pasha, T.N.; Hussain, I.; Qaisrani, S.N. Effects of spray dried yeast (*Saccharomyces cerevisiae*) on growth performance and carcass characteristics, gut health, cecal microbiota profile and apparent ileal digestibility of protein, amino acids and energy in broilers. *Trop. Anim. Health Prod.* 2021, 53, 252.
  16. Kuney D., 1982. Western Poultry Disease Conf. Proceed., Usda, Vol., 29, 45-47
  17. Kurukulasuriya, S. N. (2017). *Efficacy of vaccines against variant infectious bursal disease viruses to control immunosuppression in the broiler chicken industry in Canada* (Doctoral dissertation, University of Saskatchewan).
  18. Lehtoranta, L.; Latvala, S.; Lehtinen, M.J. Role of Probiotics in Stimulating the Immune System in Viral Respiratory Tract Infections: A Narrative Review. *Nutrients* 2020, 12, 3163.
  19. M'Sadeq, S.A.; Wu, S.; Swick, R.; Choct, M. Towards the control of necrotic enteritis in broiler chickens within-feed antibiotics phasing-out worldwide. *Anim. Nutr.* 2015, 1, 1 [CrossRef].
  20. Maldonado Galdeano, C.; Cazorla, S.I.; Lemme Dumit, J.M.; Vélez, E.; Perdígón, G. Beneficial Effects of Probiotic Consumption on the Immune System. *Ann. Nutr. Metab.* 2019, 74, 115–124.
  21. Martinez, J. L. (2009). The role of natural environments in the evolution of resistance traits in pathogenic bacteria. *Proceedings of the Royal Society B: Biological Sciences*, 276(1667), 2521-2530.
  22. Messai CR, Salhi O, Khelef D, Lounes A, Mohamed-Cherif A, Kaidi R, Ait\_Oudhia k (2019): Serological, clinical, and risk factors of the Newcastle disease on broilers flocks in Algeria, *Veterinary World*, 12(7): 938-944.
  23. Palamidi I, Fegeros K, Mohnl M, et al. Probiotic form effects on growth performance, digestive function, and immune related biomarkers in broilers. *Poult Sci* 2016; 95:1598-608. <https://doi.org/10.3382/ps/pew052>.
  24. Plaza-Diaz, J.; Ruiz-Ojeda, F.J.; Gil-Campos, M.; Gil, A. Mechanisms of Action of Probiotics. *Adv. Nutr.* 2019, 10, S49–S66, Correction in *Adv. Nutr.* 2020, 11, 1054.
  25. Salhi O, Messai CR, Ouchene N, Bousaadi I, Kentouche H, Kaidi R, Khelef D (2021) Indicators and risk factors of infectious laryngotracheitis in layer hen flocks in Algeria, *Veterinary World*, 14(1): 182-189
  26. Solvay Animal Health. 1992. Poultry Manual Solvay, Mendotta, Set.
  27. Terada, T.; Nii, T.; Isobe, N.; Yoshimura, Y. Effects of Probiotics *Lactobacillus reuteri* and *Clostridium butyricum* on the Expression of Toll-like Receptors, Pro- and Anti-inflammatory Cytokines, and Antimicrobial Peptides in Broiler Chick Intestine. *J. Poult. Sci.* 2019, 57, 310–318.
  28. Walker, W.A. Mechanisms of Action of Probiotics. *Clin. Infect. Dis.* 2008, 46, S87–S91. [CrossRef].

29. Wang, Y.; Dong, Z.; Song, D.; Zhou, H.; Wang, W.; Miao, H.; Wang, L.; Li, A. Effects of microencapsulated probiotics and prebiotics on growth performance, antioxidative abilities, immune functions, and caecal microflora in broiler chickens. *Food Agric. Immunol.* 2018, 29, 859–869.
30. Whelan, R.A.; Doranalli, K.; Rinttilä, T.; Vienola, K.; Jurgens, G.; Apajalahti, J. The impact of *Bacillus subtilis* DSM 32315 on the pathology, performance, and intestinal microbiome of broiler chickens in a necrotic enteritis challenge. *Poult. Sci.* 2019, 98, 3450–3463.
31. Yusrizal, Y., & Chen, T. C. (2003). Effect of adding chicory fructans in feed on broiler growth performance, serum cholesterol and intestinal length.
32. Zhang, L.; Zhang, R.; Jia, H.; Zhu, Z.; Li, H.; Ma, Y. Supplementation of probiotics in water beneficial growth performance, carcass traits, immune function, and antioxidant capacity in broiler chickens. *Open Life Sci.* 2021, 16, 311–322.
33. Zuchuatet Sandrine. (2014). 14 : Le test ELISA - BiOutils. [www.bioutils.ch](http://www.bioutils.ch). [En ligne] Université de Genève, 2014. [Citation: 17 06 2020.] <https://www.bioutils.ch/protocoles/14-le-test-elisa>.