Effects Of Symbiotics On The Bursa Of Fabricius And Internal Organs

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ABSTRACT

Probiotics play a central role in maintaining health and preventing many disorders. They are microbial food supplements that have an effect on the health of the host. They protect the body itself from infections, particularly of the gastrointestinal tract. The study of weight performance and the yield of slaughtered products (liver spleen) showed that the best growth performances were recorded with the probiotic-treated subjects, with significant differences ($p< 0.05$). Similarly, the microbiological study showed that *Lacidophilus* adapts well to *the* animal's digestive tract, interacting with the endogenous flora through a reduction in *E coli* in both phases. The experiment was carried out on two flocks of broilers of the ARBOR ACRES strain vaccinated against infectious bursal disease with a complex immune vaccine on the first day, with a view to assessing changes in the bursa of Fabricius during the various rearing phases (end of start-up and end of growth). At the same time, measurements of the bursal index were carried out on the bursa of chickens from the two buildings, treated and control. This study showed that the bursal index is heterogeneous within the same flock and between the two rearing buildings. 57.63% of the chickens were in the 'poor' class (bursal index < 0.15%). The state of bursal atrophy was moderate to very severe. Based on these field observations, we can conclude that broiler farms suffer from poor prophylactic management due in part to the various stresses caused during rearing, poor hygienic conditions and dependence on the choice of using the right vaccine strains against infectious bursal disease.

INTRODUCTION

The present study was carried out to determine the effects of L. acidophilus supplemented with drinking water on weight performance and the weight of internal organs such as the liver, larate and the bursa of Fabricius.

The bursa of Fabricius (BF) is an immune organ that plays a vital role in bird immunity (Toivanen et al 1987). The immune status of poultry depends on its physiological state, especially at the start of chick weight development. The various environmental stresses (stress, poor hygiene, vaccination, health problems, etc.) to which birds are subjected affect the anatomical and physiological development of the bursa of Fabricius (Siegvel, 1990), which can lead to immunodepression in some birds. To express their full genetic potential, birds need to be kept in perfect health, particularly in terms of their immune system. At the end of their growth, BF can reach a weight of 5 g and a diameter of 30 mm. The BF lymphocyte population is made up of 85-90% B cells, with less than 4% T cells and other lymphoid cells (Kim et al 2000**).** Immunosuppression is due to

selective damage to the B lymphocyte lineage. The clinical consequences are the appearance of opportunistic infections, reduced efficacy of vaccinations and growth retardation (van den Berg et al 2000).

The use of probiotics, prebiotics and symbiotics can have a positive influence on the state of health of the host in general and on the development and response of the bursa of Fabricius in birds. It is in this context that the present study was carried out, with the aim of determining the effect of the use of symbiotics on the development of the bursa of Fabricius in broilers.

Materials and methods

The experiment was carried out on ARBOR ACRES broilers.

Raised intensively in two buildings with the same technical parameters (construction materials, insulation, surface area, etc.), the walls are made of metal framework and sandwich panels containing glass wool, 40-50 cm thick, and the roof is double-layered. The buildings used for the experiments are of the closed type with dynamic ventilation (tunnel ventilation), measuring around 80 m long by 14 m wide and 2.5 m high for the side walls, and 4.4 m high in the middle, with a density of 35 kg per m² at 40 days old (weight≃ 2.5kg), i.e. 14 to 15 chicks per m2. 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. Feed for the first 4 to 5 days is poured onto cellulose paper throughout the building, covering more than 80% of the surface area used at the time of installation. The animals are reared on the floor on wood shavings, which limit heat loss by the animals and the absorption of moisture from excrement.

The chicks were vaccinated against Gumboro disease on day 1 with a complex immune vaccine by subcutaneous injection of 0.2 ml IM. The morphometric study of the bursae of Fabricius was carried out using the method of Kuney (1982). This consisted of taking at random: 10 chickens for each repetition of the same flock at the following ages: d 20, d 37 (end of start-up and end of growth) diagonally using the official technique of sampling from inside* the flock.

After autopsy, the BFs were removed, examined, weighed and measured using a "bursameter", an instrument that measures the diameter of the bursa (Figure 01) (Solvay Animal Health, 1992).

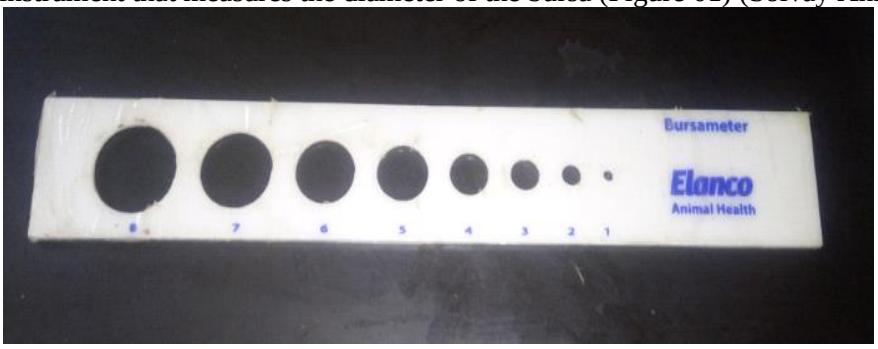

Figure 01 - Bursameter for measuring the Fabricius bursa (Solvay animal health, 1992)
Plastic tool with 8 holes (T) calibrated from the smallest (1) to the largest (8) and representing the diameter T1 = 3 mm T2 = 6 mm T3 =10 mm T4 =14 mm T5 = 17 mm T6 = 20 mm T7 = 23 mm T8 = 26 mm

MEASURED PARAMETERS

Analysis of the carcasses was carried out on subjects taken at random and weighed on the spot. 40 subjects were taken for each batch, including 10 subjects for each compartment, with 4 replicates in each batch. After 6-7 hours of fasting, the animals were weighed and then slaughtered, de-feathered and eviscerated. The production parameters studied during start-up and growth were:

- **Zootechnical parameters** :

Body weight was calculated at the end of each phase (end of start-up and end of growth) and the overall mean weight was assessed using the method described by Yusrizal and Chen (2003).
-Average live weight (g) = Total weight of subjects (g)/ Number of subjects

2. Slaughter products: following the standard procedure (slaughter, de-feathering, evisceration) the internal organs (liver, spleen) were immediately removed and weighed separately, with their yield calculated.

3. Morphometry: (Weight and size of the Fabricius bursa)

To determine the immune status and degree of homogeneity of broilers, the bursal index of each flock is calculated according to Bennett (2002)**:** BF weight / body weight x 100.

STATISTICAL PROCESSING

The results are described by the mean and the 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 symbiotic supplementation on the parameters described. Differences between treatments were compared at 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 Probiotiquelactobacillusacidophiluss on growth performance, intestinal flora balance and the immune response of broiler chickens (Berghiche et al., 2023).

Results and discussion

1. Harvester efficiency

Analysis of the slaughter product results shows a significant difference (P<0.05) between the results of the different diets tested (Table 01).

Table 01: Average weight of liver and spleen according to the addition or non-addition of lactobacillus acidophilus

	Chick weight 38g first day							
	End of start-up 20 days							
	Exper 1	**Exper2**	**Exper 3**	**Exper 4**	**Control1**	**Control 2**	**Control 3**	**Control 4**
Liver	**22(g) 3.42 %**	**22.02(g) 3.1 %**	**24.06(g) 3.33 %**	**22.04(g) 3.35 %**	**18.96(g) 2.95 %**	**18.86(g) 3.06 %**	**19.06(g) 3.20 %**	**21.4(g) 3.11 %**
Rate	**0.64(g) 0.099 %**	**0.7(g) 0.098 %**	**0.82(g) 0.11 %**	**0.62(g) 0.094 %**	**0.52(g) 0.08 %**	**0.5(g) 0.08 %**	**0.63(g) 0.10%**	**0.564(g) 0.082 %**
	End of growth 37 days							
Liver	**72(g) 3.19 %**	**61(g) 2.65%**	**59(g) 2.55 %**	**64(g) 2.64 %**	**52g 2.4 %**	**58g 2.55 %**	**57g 2.66 %**	**58g 2.56 %**
Rate	**2.196(g) 0.097 %**	**2.225(g) %0.096**	**1.92(g) 0.083 %**	**2.76(g) 0.11 %**	**1.742g 0.08 %**	**2.28g 0.10 %**	**1.79g 0.092 %**	**2.26g 0.10 %**

For organ yield, there were significant variations between the groups of the two diets during both periods. Organ yield was higher in chickens fed the probiotic supplemented diet compared to the unsupplemented diets, with a liver weight peak in the start-up phase of 24.06g in experimental group 03 compared to 21.4g in control group 4, and a spleen weight peak of 0.82g peak for group 4 of the treated batch compared with 0.63g for group 3 of the control batch. In the growth phase, the peak liver weight was 72g for group 01 of the treated batch compared with 58g for groups 2 and 4 of the control batch, and the peak spleen weight was 2.76g for group 4 of the treated batch compared with 2.The organ weight results are reported in Table 01. This shows that, throughout the rearing period, there is a positive effect (P<0.05) of the addition of the probiotic on organ weights, especially for liver and spleen weights, during both rearing periods. This is due to the remarkable effect of Lactobacillus acidophilus on the balance of intestinal flora and consequently intestinal integrity and consequently liver and spleen weights.

This probably means that the livers of chickens fed the diet containing Lactobacillus acidophilusa increased metabolic functions for better performance.

Although the spleen weight results were better in the probiotic-supplemented chickens than in the control batches. This probably means that the spleens of the chickens fed the probiotic increased their reaction and defence capacities following consumption of the probiotic.

- **Weight performance and morphometry of the Fabricius bursa**

2.1. Weight Trends:
The weight evolution of the chicks of the batches receiving the probiotic measured regularly every 7 days, showed the best results ($p<0.05$), and however the first and the second phase of rearing compared to the control batches according to figure 02, with a peak of 720.75g for repetition 3 at the end of starting and 2418g of group 4 at the end of growth of the experimental batch against a peak of 687.75(g) at the end of starting and 2263(g) at the end of growth repetition 04 of the control batch tables 02

2.2 Morphometry of the Fabricius bursa
In each replicate, changes in the weight of the LFB, its diameter and the body weight of the chickens were measured and transferred to Table 02.

	End of start-up 20 days							
	Exper 1	**Exper2**	**Exper 3**	**Exper 4**	**Control1**	**Control 2**	**Witness3**	**Control 4**
Body weight	**642.75 (g)**	**708.75 (g)**	**720.75 (g)**	**656.75 (g)**	**640.75(g)**	**614.75 (g)**	**594.75 (g)**	**687.75(g)**
diameter of B F (the average)	**16.25mm**	**16.4mm**	**16.4mm**	**14.6mm**	**16.4 mm**	**15.2mm**	**15.2mm**	**14.6mm**
weight of B F (the average)	**2.125(g)**	**1.76(g)**	**1.88(g)**	**1.28(g)**	**1.666(g)**	**1.55(g)**	**1.454(g)**	**1.38(g)**
Bursal index according to Bennett (2002): BF weight / body weight x 100	**0.33%**	**0.24%**	**0.25%**	**0.19%**	**0.26%**	**0.25%**	**0.24%**	**0.2%**
	End of growth 37 days							
Body weight	**2255 (g)**	**2296 (g)**	**2311 (g)**	**2418(g)**	**2158(g)**	**2267(g)**	**2136(g)**	**2263(g)**
diameter of B F (the average)	**14 mm**	**14.4 mm**	**15.8 mm**	**14.75 mm**	**12.4mm**	**13.2mm**	**13.2mm**	**13.8mm**
weight of B F (the average)	**1.272(g)**	**1.475(g)**	**1.62(g)**	**1.3(g)**	**1.146g**	**1.084g**	**1.002g**	**1.308g**
Bursal index according to Bennett (2002): BF weight / body weight x 100	**0.05%**	**0.06%**	**0.07%**	**0.05%**	**0.05%**	**0.04%**	**0.04%**	**0.05%**

Table 02: Average body weight, bursal weight and diameter of the bursa of Fabricius of broilers in the two rearing phases observed (n = 10)

In our experiment, we observed that the average weight and average diameter of the bursa decreased with age and average body weight respectively. The work carried out by Kuney (1982) and Huapaya (1995) shows that BF weight and height are proportional to the age and weight of the subject. The development of BF weight in relation to body weight gives a correlation coefficient r2 = 0.54(Figure xx). This is a negative and

significant correlation ($p \leq 0.001$), which explains why the weight of the LFB decreased as a function of age and live weight.

The average bursa weight was 0.21 g at d 7 and 4.90 g at d 42 according to Morales (2002). However, it was 1.76 g in the treated batch compared with 1.51 g in the control batch at 20 days and 1.41 g in the experimental batch compared with 1.135 g in the control batch at 37 days in the chickens in our study. This difference in weight demonstrates the atrophy of the BF and the heterogeneous state of growth of the chickens in the different replicates studied. The bursal diameter was also small compared with the results observed by Kuney (1982) (26 mm vs 14.75 mm average of the treated batch replicates and 13.15mm control at d 35).

According to the evaluation grid established by Bennett (2002) and representing the IB: the BF weight/body weight ratio x100 (0.20 % represents the ratio of a 4 g BF/weight of a 2000 g chicken x 100).

Class :	> of 0.20%.	0.18%< IB< 0.20%	0.15% < IB< 0.18%	< of 0.15%
	Excellent	Medium	Mediocre	Bad

The bursal index calculated during the two rearing phases for the two batches of chickens showed that all the birds belonged to the excellent class at the end of the start-up phase, with an average bursal index of 0.25% for the treated batch compared with 0.23% for the control batch, whereas it was poor at the end of the growth phase, with an average of 0.05% for the experimental batch and 0.045% for the control batch, confirming the heterogeneity and immunosuppression in the flocks at the end of the growth phase (Table 2).

Discussion

In our experience, the addition of probiotics to the broiler diet has modified their growth performance, as reported by several studies contributing to improving the nutritional value of symbiotic-based feeds, which have shown the efficacy of adding alactobacillus acidophilus

Furthermore, in our trial, the increase in body weight of chickens at 20 days of age observed on the overfed diet compared to the control is comparable to that reported by (Bai et al., 2018 ; Ariouaet al., 2024)

The major positive effect of feeding a diet supplemented by the symbiotic occurred mainly during the last three weeks of the production cycle. This increase in body weight of the chickens is thought to be linked to a good assimilation of the feed thanks to the intestinal microflora.(Abd El Hack et al., 2020; Borda-Molina et al., 2018)

M'Sadeq et al., 2015. find that in broilers, probiotics can improve growth performance and control diseases such as salmonellosis, necrotic enteritis and coccidiosis.

The increase in the size of the intestinal villi amplifies the absorption process, allowing nutrients to enter the bloodstream and be taken to the liver, where they are processed before being distributed to the rest of the body. The nutrients are then used up or, when the body's needs are met, the excess is accumulated to build up reserves. This is why our results show significant differences ($p<0.05$) in liver weight between the two diets. The results indicate that chickens fed the symbiotic supplemented diet had greater liver weights than those fed the non-symbiotic diet.

The atrophy of the BF at the end of growth in chickens (of small weight and size) leads us to look for the causes of this atrophy. According to Kuney (1982) and Benett (2002), it can be caused by several factors (infectious bursitis, caused by a wild or vaccinated virus, reovirosis, bacterial infections, stress, mycotoxicoses).

Farmers sometimes do not apply any biosecurity measures (disinfection, deratting, disinsectisation, sanitary barriers, etc.), or they may vaccinate their livestock incorrectly against infectious diseases (unqualified people, inappropriate or out-of-date vaccine, etc.), or they do not respect the cold chain, which can lead to vaccination failures.

The atrophy observed in the bursae may be caused either by a wild Gumboro disease virus that infected the chicks as soon as they arrived in the poorly decontaminated buildings, or by the vaccine strain used. Research has shown that, despite a high level of maternal antibodies in chicks, there can be early and lasting colonisation of the bursa of Fabricius by a wild strain (Allamigeon and Comte, 2003)**.** There is an optimum time for vaccination that is difficult to determine. Sufficient maternal antibodies are needed to control a possible wild strain, but not so much that the vaccine virus is neutralised (Goutebroze et al. 2003; Lemière 2003).

A good vaccine strain should be selected for its exceptional balance between efficacy and safety from 1 day of age, as well as for its high immunogenicity.

CONCLUSION

The protective effects of dietary supplementation with L. acidophilus on birds result from a strengthening of cellular and humoral immunity and an improvement in the function of the intestinal barrier. These effects led to improved growth performance and reduced mortality in the birds. L. acidophilus can be used as an intervention strategy to reduce viral or bacterial infection in broilers. However, the mechanism by which dietary supplementation with L. acidophilus exerts protective effects against intestinal microflora and disease requires further investigation.

The size and bursal index of the bursa of Fabricius are two effective means of completing a diagnosis of avian pathology in the field. They provide information on the physiological state of the BF, especially in the event of an attack caused by a vaccinal or wild virus. It should be pointed out that the success of any avian surveillance programme, such as Gumboro disease in a given farm, does not automatically mean success, because each farm has its own conditions and factors for success. The choice of vaccine strain and the date of vaccination cannot be standardised, as the parameters conditioning the vaccination strategy are multiple.

REFERENCES

1. Abd El-Hack, M. E., El-Saadony, M. T., Shafi, M. E., Qattan, S. Y., Batiha, G. E., Khafaga, A. F., ... & Alagawany, M. (2020). Probiotics in poultry feed: A comprehensive review. Journal of animal physiology and animal nutrition, 104(6), 1835-1850.
2. Allamigeon M.F., Comte S., 2003: Afrique Agriculture, 292, 82 - 83.
3. Arioua, H., Berghiche, A., Isaad, N. A., Mimoune, N., Arioua, F., Bouzid, R., & Khelef, D. (2024). Impacts Of Probiotics On The Production Performance And Immune Status Of Broiler Chickens From Arbor Acre Strains. Journal of Advanced Zoology, 45(4).
4. Bai, S. P., Wu, A. M., Ding, X. M., Lei, Y., Bai, J., Zhang, K. Y., & Chio, J. S. (2013). Effects of probiotic-supplemented diets on growth performance and intestinal immune characteristics of broiler chickens. Poultry science, 92(3), 663-670.
5. Bennet C., 2002. Univ. Crescent WinniPeg, Http://Www.Gov.Mb.Ca/Agriculture/Livestock/Poultry/
6. Berghiche, A., Zeghdoudi, M., Asnoune, Z., Djebrane, C., Labied, I., Attia, K., & Bouzid, R. (2023). Modeling a capital approach to biological risk assessment: An updated overview of the entropic scenario and the outcome of the upcoming microbial and ecological crises. Tobacco Regulatory Science (TRS), 4752-4771.
7. Borda-Molina, D., Seifert, J., & Camarinha-Silva, A. (2018). Current perspectives of the chicken gastrointestinal tract and its microbiome. Computational and structural biotechnology journal, 16, 131-139.
8. Huapaya.J.R., 1995. Thesis. University Of Lima. Peru.
9. Kim I. J., You S.K., Kim H., Yeh H.Y., Sharma J.M., 2000. Journal of Virology, 74: 884-892).
10. Kuney D., 1982. Western Poultry Disease Conf. Proceedings, Usda, Vol, 29, 45-47.
11. Morales C.M., 2002. Http:/Members.Tripod.Com.Pe/Morales
12. Rehman, A., Arif, M., Sajjad, N., Al-Ghadi, M. Q., Alagawany, M., Abd El-Hack, M. E., ... & Swelum, A. A. (2020). Dietary effect of probiotics and prebiotics on broiler performance, carcass, and immunity. Poultry science, 99(12), 6946-6953.
13. Siegvel B.P., 1990. Poultry Digest, Vol. 4: 38-42.
14. Solvay Animal Health. 1992. Poultry Manual Solvay, Mendotta, Set.
15. Toivanen P., Naukkarinene H., Vannino 0. 1987. AvianImmunology, Vol. 1: 79-92).
16. Van Den Berg T.P., Eterradossi N., Toquin D., Meulemans G., 2000. Rev. Sci. Tech. Off. Int. Epiz. 19 (2), 527-543.
17. Yusrizal, t., chen, t. c., 2003.Int. J.Poult.Sci, 2, 214- 219.