



BAKIN

Agriculture Products Industry and Trade Ltd. Company



A good blend of poultry probiotics...



www.bakintarim.com.tr



A good blend of poultry probiotics...



About Us,

Our company was founded in 2002 with the aim of providing innovative and natural solutions in the field of animal nutrition. We focus on feed additives and biotechnological products, developing solutions that support both animal and, consequently, human health.

Since 2008, we have been conducting R&D studies on biotechnological feed additives in collaboration with universities. By expanding our facilities, we have increased our R&D area to 1,200 m² and our production area to 4,200 m². Specializing in poultry nutrition, we successfully completed two TÜBİTAK projects and were included in major project programs. After conducting preliminary research in our R&D poultry houses, we carried out field trials in commercial farms to test our products under real conditions.

With a research-driven and innovative approach, our company stands out in the industry by adhering to international standards and maintaining high-quality production. Our expert team, consisting of veterinarians and agricultural engineers, continues to develop sustainable solutions to meet the needs of the livestock sector. Our products are available not only in the domestic market but also in international markets, and we are continuously working to increase our export share. Through our R&D activities, we aim to offer innovative solutions to industry challenges and add value to the livestock sector.

FEED FORM

SMART PROLIVE

It is specially formulated for poultry.

With the supplementation of probiotics, prebiotics, and enzymes, the digestive system is maximally protected.

It strengthens the immune system and increases resistance to diseases.

Ammonia levels in the poultry house decrease, thus preventing upper respiratory infections.

Egg production increases, while feed consumption decreases.

The rate of dirty and hidden cracks in eggs is reduced.

It reduces abdominal fat accumulation, and fat is evenly distributed in the meat, resulting in more flavorful meat.

It enhances the effectiveness of vaccines.

Prevents issues such as undigested feed, intestinal damage, and intestinal sluggishness.

It prevents raw dropping and Salmonella carriage.

Supports economic farming, food safety, as well as preventive and therapeutic practices.

INGREDIENTS (g/kg):

Probiotics:

L. Acidophilus	1x 10 ¹⁰ CFU/g
P. Acidilactici	1x 10 ¹⁰ CFU/g
B. Subtilis	1x 10 ¹⁰ CFU/g

Prebiotics:

Inuline	100g/kg
MOS	200g/kg

Enzymes:

Xylanase	2,200,000 BXU/kg
Cellulase	270,000 FPUI/kg
Glucanase	100,000 FBG/kg
Phytase	1,000,000 PPU/kg

Carrier:

CaCO ₃	590g/kg
-------------------	---------

USAGE: It is used by adding 500 gr -1000 gr (500 gr-1000 gr/ton feed) to a ton feed.

PACKAGING : 25 kg a craft bag.

POWDER (Potable Water)

SMART PROLIVE

Intended Use :

It is specially formulated for poultry.

With the supplementation of probiotics, prebiotics, and enzymes, the digestive system is maximally protected.

It strengthens the immune system and increases resistance to diseases.

Ammonia levels in the poultry house decrease, thus preventing upper respiratory.

Egg production increases, while feed consumption decreases.

The rate of dirty and hidden cracks in eggs is reduced.

It reduces abdominal fat accumulation, and fat is evenly distributed in the meat, resulting in more flavorful meat.

It enhances the effectiveness of vaccines.

Prevents issues such as undigested feed, intestinal damage, and intestinal sluggishness.

It prevents raw dropping and Salmonella carriage.

Supports economic farming, food safety, as well as preventive and therapeutic practices.



INGREDIENTS (g/kg):

1. **PROBIOTICS (10 g)** : L. acidophilus, P. acidilactici, B. subtilis (each at least 1×10^{10} CFU/Kg). Includes adjunct and CE culture.

2. **PREBIOTICS (300 g)** : Inulin (100 g), MOS (200 g).

3. **ENZYMES (In 100 g conveyer)** : Xylanase (2,200.000 BXU/Kg), Cellulase (270.000 FPUi/Kg), Glucanase (100,000 FBG/Kg), Phytase (1.000.000 PPU/Kg).

4. **CARRIER**: Oligosaccharides (590 g).



Usage: It is enough to give 50 g daily for 10.000 chicks and chickens.

During the day, it is given for 6-8 hours by adding in the potable water.

Packaging: 50 gr in 1 box * There are 20 pieces.

LIQUID (Potable Water)

SMART PROLIVE LAYER

Usage:

Jerrycans should be shaken before use.

When added to drinking water, the water should not contain chlorine and antibiotics.

From the first day of the chick, 1 liter per ton of water is given every day for the first 4 weeks.

Between 4-18 weeks Half a liter per ton of water, given 1 day, 2 days break.

Between 18-120 weeks Half a liter per ton of water, 1 day, 3 days break.

When added to feed, it is used by adding 0.5 lt to 1 ton of feed by spraying the mixer.

Packaging: Jerrycans in 1 lt , 5 lt and 20 lt.



SMART PROLIVE BROILER

Usage:

Jerrycans should be shaken before use.

When added to drinking water, the water should not contain chlorine and antibiotics.

Broiler drinking water is mixed with 1 liter per ton of water for the first 15 days.

Between 16-35 days, half a liter per ton of water is added to the drinking water.

Between 16-35 days, the application is continued with a one day break.

Packaging: Jerrycans in 1 lt , 5 lt and 20 lt.



SMART PROLIVE BREEDER

Usage:

Use in feed: Used by spraying 0.5 lt per ton of feed.

Use in drinking water: 0,5 lt is added to 1 Ton of drinking water.

For maximum benefit, it should be used every day throughout the animal's life.

It can be given to the irrigation system line with a dosatron.

Jerrycans should be shaken before use.

When added to drinking water, the water should not contain chlorine and antibiotics

Packaging: Jerrycans in 1 lt , 5 lt and 20 lt..



Addition of Probiotics to Drinking Water in Laying Hens

EFFECT ON PERFORMANCE AND EGG QUALITY



SAKİNE YALÇIN¹, E. EBRU ONBAŞILAR², SUZAN YALÇIN³

¹ Ankara University Faculty of Veterinary Medicine, Animal Nutrition and Nutritional Diseases, Ankara

² Ankara University Faculty of Veterinary Medicine, Department of Animal Science, Ankara

³ Department of Food Hygiene and Technology, Selçuk University Faculty of Veterinary Medicine, Konya

ABSTRACT

In this study, it was aimed to determine the effects of probiotic supplementation to drinking water on performance and egg quality in laying hens. A total of 128 32-week-old Lohman Brown laying hens were used for this purpose.

The hens were divided into 1 control group and 1 experimental group, each containing 64 hens. The experiment lasted for 10 weeks. Probiotic (Smart Prolive Layer, 1×10^9 cfu/ml *Lactobacillus acidophilus* KUEN 1607; 1×10^9 cfu/ml *Pediococcus acidilactici* KUEN 1608) was added to the drinking water of the experimental group at 0.5% level. The effect of probiotic supplementation on feed intake, egg weight, number of dirty eggs and cracked and unshelled eggs was found to be statistically insignificant. Probiotic supplementation to drinking water during the ten-week experiment resulted in a 2.30% increase in egg production ($P=0.013$) and a 3.19% improvement in feed conversion ratio ($P=0.017$). Addition of probiotic to drinking water did not statistically affect shell quality characteristics and yolk color of eggs. White height in eggs ($P<0.001$), White height in eggs ($P<0.001$),

white index ($P=0.005$) and Haugh unit ($P<0.001$) were significantly increased with the addition of probiotics to the drinking water. *Salmonella* was not detected in the feces of chickens in both groups. In conclusion, probiotic supplementation of drinking water with *Lactobacillus acidophilus* KUEN 1607 and *Pediococcus acidilactici* KUEN 1608 improved egg production, feed conversion ratio and egg Haugh unit. It was concluded that the use of this probiotic supplement in commercial laying hen breeding would be important for both the breeder and the consumer as it would improve egg performance and egg internal quality.

INTRODUCTION

Probiotics are live microbial feed additives that positively affect animal performance by improving microbial balance in the digestive tract. It can reduce the population of *Escherichia coli* by lowering the pH through the production of lactic acid in the digestive tract. Produces enzymes and antibacterial compounds, reducing toxin release.

(Montes and Pugh, 1993; Shehata et al., 2022). In some studies, some probiotics (*Lactobacillus reuteri*, *Pediococcus acidilactici*) have been reported to improve egg production and nutrient absorption and feed conversion rate by improving intestinal health in laying hens (Quarantelli et al., 2008; Mikulski et al., 2012; Shanmugam et al., 2024). FSA (2023) reported that *P. acidilactici* CNCM I-4622 probiotic should be used in feed at a minimum level of 1×10^9 CFU in all animal species. *Pediococci* suppress the growth of enteric pathogens by producing bacteriocins such as lactic acid and pediocins (Daeschel and Klaenhammer, 1985).

Lactobacillus acidophilus is a non-spore-bearing lactic acid bacteria species that has been used to improve performance in laying hens (Galazzi et al., 2008; Kurtoğlu et al., 2004). *Lactobacillus* culture has also been reported to limit *Salmonella* colonization (Arreguin-Nava et al., 2019; El-Sharkawy et al., 2020). Some studies have shown that pro-biotic administration with water is more effective than feed administration (Karimi Torshizi et al., 2010; Ritzi et al., 2014). Therefore, the aim of this study was to investigate the effects of probiotic (Smart Prolive Layer, 1×10^9 cfu/ml *Lactobacillus acidophilus* KUEN 1607; 1×10^9 cfu/ml *Pediococcus acidilactici* KUEN 1608) added to drinking water on egg performance and egg quality in laying hens.

MATERIALS AND METHODS

A total of 128 32-week-old Loh-man Brown laying hens were used in the study. The hens were divided into 1 control group and 1 experimental group, each containing 64 hens. Each group consisted of 8 sub-groups, each containing 8 hens. Chickens were housed in 3 batteries with 3 floors. Each battery 4 cages (40 cmx49 cmx49) on each floor. The lighting program was set to 16 hours light and 8 hours dark. The experiment lasted 10 weeks. Compound feed with 15.94% crude protein and 2720 kcal/kg metabolizable energy was purchased from a commercial company in granule form.

Probiotic (Smart Prolive Layer, 1×10^9 cfu/ml *Lactobacillus acidophilus* KUEN 1607; 1×10^9 cfu/ml *Pediococcus acidilactici* KUEN 1608, Bakın Tarım Ürünleri San ve Tic Ltd Şti -Ankara) added to drinking water at 0.5% level in the experimental group. No additive was added to the drinking water of the control group. Drinking water for the hens controlled with 200 liter plastic tanks. Egg yield

were determined daily. The number of dirty eggs and the number of broken, cracked and shell-less eggs were also recorded daily. Feed consumption was determined in 2-week periods. Eggs collected on the last three days of each week were weighed and egg weight was recorded. Feed conversion ratio was calculated as the amount of feed consumed per kg of eggs for two-week periods. Chickens were monitored daily and the number of deaths was recorded. Autopsies were performed to determine the cause of death. Eggs collected for three days in the last week of the experiment were weighed individually and examined for internal quality (white height, white length, white width, yellow diameter, yellow height, Haugh unit, yellow color) and external quality characteristics (shape index, breaking strength, shell weight, shell thickness).

At the end of the experiment, fresh fecal samples were taken from the subgroups in each group to determine the presence of *Salmonella* (ISO, 2017) Statistical Analyses: T-test was used to compare egg quality traits and two-way analysis of variance was to compare egg production, feed consumption, egg weight and feed conversion ratio. Duncan's test was applied to determine the difference group. Statistical analyses were performed with SPSS (Inc.Chicagoll, USA $P < 0.05$ was accepted as significant.

FINDINGS

The effect of probiotic supplementation to drinking water on the performance of laying hens is given in Table 1. Probiotic supplementation significantly increased egg production by 2.30% ($P=0.013$) the 10-week study. Probiotic supplementation to drinking water caused a 0.92% decrease in feed consumption and a 0.08% increase in egg weight. However, there was no statistical difference between the groups in terms of feed consumption and egg weight. The amount of feed consumed per kg of egg decreased significantly ($P=0.017$) by 3.19% with pro-biotic supplementation. Feed consumption ($P=0.021$) and egg weight ($P=0.037$) with time. There was no group*period interaction in egg production, feed consumption, egg weight and feed conversion ratio when probiotic was added to drinking water. During the 10-week experiment, one death was observed in the control group and one in the probiotic group. As a result of the autopsy, it was seen that the deaths were caused by egg compression in the cloaca.



Total number of dirty eggs and total number of cracked-shell-free eggs were not statistically affected by the probiotic supplementation to the drinking water for ten weeks (Table 2). The effects of probiotic supplementation to drinking water of hens on external and internal quality characteristics of eggs are shown in Table 3 and Table 4, respectively. Egg weight, shape index, egg breaking strength, shell weight (membrane), shell weight, shell weight percentage, shell thickness and yellow color were not different between the groups. Addition of probiotic to drinking water increased white height ($P<0.001$) white index ($P=0.005$ and Haugh unit ($P<0.001$) in eggs. At the end of the experiment, Salmonella was not detected in fresh fecal samples taken from each subgroup in the groups.

DISCUSSION AND CONCLUSION

In this study, the addition of probiotics (Lacto-bacillus acidophilus KUEN 1607; Pediococcus acidilactici KUEN 1608) to drinking water caused a 0.92% decrease in feed consumption compared to the control group, but the difference was not statistically significant. Saleh et al. (2017) reported that when Aspergillus awamori and lactic acid bacteria were given separately to turkeys, feed consumption decreased compared to the control group, but the difference was not significant, but when the combination was given, feed consumption decreased significantly.

In the 10-week study, the addition of a probiotic combination containing Lacto-bacillus acidophilus KUEN 1607 and Pediococcus acidilactici KUEN 1608 to drinking water increased egg production compared to the control group.

2.30% increase ($P=0.013$). Similarly, Mikulski et al. (2012) showed that egg laying hens. They reported that the addition of P. acidilactici probiotic improved egg production by 2.8%. On the other hand, some researchers reported that the addition of P. acidilactici probiotic (Shanmugam et al., 2024), Lactobacillus acidophilus probiotic combination (Davis and Anderson, 2002) did not affect egg production.

In this study conducted for ten weeks, egg weight was 62.93 g in the control group, while it was 62.93 g in the probiotic group. 62.98 g and the difference between the groups was not statistically significant. On the other hand, some studies found that the addition of P. acidilactici probiotic (Shanmugam et al., 2024) and L. acidophilus probiotic combination (Davis and Anderson, 2002) increased egg weight. The amount of feed consumed per kg of eggs increased with probiotic supplementation. decreased significantly ($P=0.017$) at the level of 3.19%. Similarly, some researchers (Mikulski et al., 2012; Shanmugam et al., 2024) reported that the addition of P. acidilactici probiotic improved the feed conversion ratio. Since the utilization of energy in the feed increased with the addition of P. acidilactici probiotic, it was emphasized that this energy was used to improve egg production and feed utilization (Mikulski et al., 2020). Similar to the findings of this study, Quarantelli et al (2008) reported that feeding P. acidilactici did not affect feed intake and egg weight but improved egg production and feed conversion ratio. Alaqil

Table 1. Effect of probiotic supplementation to drinking water on performance of laying hens

Period	Group	Egg yield, %	Feed consumption,g/d ay	Egg weight, g	Feed conversion ratio, kg feed/kg eggs
1	Control	93.02	107.82	61.99	1.88
	Probiotic	93.04	106.56	62.08	1.85
2	Control	93.08	110.49	62.39	1.91
	Probiotic	95.90	107.82	62.95	1.79
3	Control	93.33	109.74	62.85	1.88
	Probiotic	95.99	109.23	62.85	1.81
4	Control	93.64	110.10	63.47	1.86
	Probiotic	96.17	111.22	63.06	1.84
5	Control	93.66	112.85	63.97	1.89
	Probiotic	96.40	111.13	63.99	1.80
1		93.03	107.19 ^b	62.04 ^b	1.86
2		94.49	109.16 ^{ab}	62.67 ^{ab}	1.85
3		94.66	109.49 ^{ab}	62.85 ^{ab}	1.84
4		94.91	110.66 ^a	63.26 ^{ab}	1.85
5		95.03	111.99 ^a	63.98 ^a	1.85
	Control	93.35	110.20	62.93	1.88
	Probiotic	95.50	109.19	62.98	1.82
Standard error		0.422	0.455	0.196	0.013
P (Significance)					
Period		0.575	0.021	0.037	0.991
Group		0.013	0.272	0.898	0.017
Period*Group		0.806	0.745	0.960	0.695

a, b: the difference between groups with different letters in the same column is statistically significant ($P<0.05$)



Table 2. Effect of probiotic addition to drinking water on the total number of dirty eggs and total number of cracked-shell-free eggs during the ten-week experiment

Group	Total number of dirty eggs, %	Total number of cracked-shell-free eggs, %
Probiotic	1.10±0.07	0.76±0.08
Control	1.10±0.18	0.77±0.07
P	0.991	0.952

Table 3. Effect of probiotic addition to drinking water on quality characteristics of eggs

Group	Shape index, %	Crushing strength, kg/cm ²	Shell weight, g	Shell thickness, µm	Shell weight, %
Probiotic	78.98±0.39	3.71±0.13	7.22±0.09	378±4	11.36±0.12
Control	78.78±0.50	3.63±0.11	7.21±0.06	369±4	11.41±0.12
P	0.758	0.617	0.942	0.104	0.787

Table 4. Effect of probiotic addition to drinking water on quality characteristics of eggs

Group	Ak height, mm	Yellow color	Yellow index, %	Ak index, %	Haugh unit, %
Probiotic	7.57±0.08	10.44±0.09	40.04±0.42	9.07±0.13	85.93±0.49
Control	7.15±0.07	10.44±0.09	39.47±0.38	8.51±0.14	83.41±0.42
P	<0.001	1.000	0.317	0.005	<0.001

(2020) the use of *Lactobacillus acidophilus* probiotic at a level of 3×10^9 cfu/kg reduced feed intake and improved egg production, egg weight and feed conversion ratio in hens. This improvement can be attributed to the ability of *Lactobacillus* culture to improve the intestinal microbial balance, adhere to the intestinal mucosa, secrete active metabolites and antagonize and competitively eliminate some pathogenic bacteria (Boostani et al., 2013). In addition, the enzymes produced by *Lactobacillus* culture increase the digestion and absorption of nutrients, resulting in a greater improvement in performance (Jin et al., 2000). Differences in research results may be due to the type and dose of probiotic used and the method of obtaining probiotic (Saleh et al., 2017).

In the ten-week experiment, no difference was observed between the groups in terms of mortality rate. Quarantelli et al. (2008) reported that mortality rate was 1.39% in the control group, while it was 0% with the addition of *P. acidilactici* to the feed.

In the ten-week trial, the number of dirty eggs and total number of cracked and unshelled eggs in the probiotic group were similar to the control group. Similar to the findings of the study, Quarantelli et al. (2008) reported that the addition of *P. acidilactici* to the feed did not make a difference in the rate of dirty eggs.

At the end of the ten-week experiment, no difference was observed in egg shape index, breaking strength, shell weight, shell weight percentage, thickness and yellow color when probiotic was added to drinking water compared to the control group. Egg white height ($P < 0.001$), white index ($P = 0.005$) and Haugh unit ($P < 0.001$) increased in probiotic water consumption. This situation will be important for consumers. Similarly, in the study conducted by Shanmugam et al. (2024), the addition of *P. acidilactici* probiotic

They noted that there an improvement in Haugh unit, but there was no difference in egg breaking strength, egg shell thickness and relative egg weight.

Probiotic consumption has been reported to reduce the colonization of pathogenic bacteria in the digestive tract (Kabir et al., 2004). In the present study, *Salmonella* was not found in the feces of either the control group or the probiotic supplemented groups. Jazi et al (2018) reported that the addition of additives containing *P. acidilactici*, mannan oligosaccharide, butyric acid or its combination reduced *Salmonella Typhimurium* colonization, especially the combination was much more effective.

As a result, 0.5% probiotic supplementation of drinking water with *Lactobacillus acidophilus* KUEN 1607 and *Pediococcus aci- dilactici* KUEN 1608 improved egg production, feed conversion ratio and egg Haugh unit in laying hens. It was concluded that probiotic supplementation containing *Lactobacillus aci- dophilus* KUEN 1607 and *Pediococcus acidilactici* KUEN 1608 can be used to increase egg yield and quality in laying hen breeding.



ACKNOWLEDGEMENTS

We would like to thank the staff of Bakır Taş - rım Ürünleri San ve Tic Ltd Şti -Ankara for their help in conducting the trial.

REFERENCES

- Alaqil AA et al. (2020). Dietary supplementation of probiotic *Lactobacillus acidophilus* modulates cholesterol levels, immune response, and productive performance of laying hens. *Animals*, 10, 1588.
- Arreguin-Nava MA et al. (2019). Isolation and identification of lactic acid bacteria probiotic culture candidates for the treatment of *Salmonella enterica* serovar enteridis in neonatal Turkey poults. *Animals*, 9, 696.
- Boostani A et al. (2013). Growth performance, carcass yield and intestinal microflora populations of broilers fed diets containing thepax and yogurt. *Rev Bras Cienc Avic* 15, 1-6.
- Daeschel MA, Klaenhammer TR (1985). Association of a 13.6-megadalton plasmid in *Pediococcus pentosaceus* with bacteriocin activity. *Applied Environmental Microbiology*, 50, 1538-1541.
- Davis GS, Anderson KE (2002). The effects of feeding the direct-fed microbial, primalac, on growth parameters and egg production in Single Comb White Leghorn hens. *Poultry Science*, 81, 755-759.
- El-Sharkawy H et al. (2020). Evaluation of *Bifidobacteria* and *Lactobacillus* probiotics as alternative therapy for *Salmonella typhimurium* infection in broiler chickens. *Animals* 10, 2023.
- Galazzi D et al. (2008). Effects of *Lactobacillus acidophilus* D2/CSL on laying hen performance. *Italian Journal of Animal Science*, 7, 27-37.
- ISO (2017). Microbiology of the Food Chain - Horizontal Method for the detection, Enumeration and serotyping of *Salmonella* - Part 1: Detection of *Salmonella* spp. (ISO 6579-1). International Organization for Standardization, Geneva, Switzerland.
- Jazi V et al. (2018). Effects of *Pediococcus acidilactici*, mannan-oligosaccharide, butyric acid and their combination on growth performance and intestinal health in young broiler chickens challenged with *Salmonella typhimurium*. *Poultry Science*, 97, 2034-2043.
- Jin LZ et al. (2000). Digestive and bacterial enzyme activities in broilers fed diets supplemented with *Lactobacillus* cultures. *Poultry Science*, 79, 886-891.
- Kabir SML et al. (2004). The Dynamics of probiotics on growth performance and immune response in broilers. *International Journal of Poultry Science*, 3, 361-364.
- Karimi Torshizi MA et al. (2010). Assessing the effect of administering probiotics in water or as a feed supplement on broiler performance and immune response. *British Poultry Science*, 51, 2, 178-184.
- Kurtoğlu V et al. (2004). Effect of probiotic supplementation on laying hen diets on yield performance and serum and egg yolk cholesterol. *Food Additive Contam*, 21, 817-823.
- Mikulski D et al. (2020). Effects of dietary probiotic (*Pediococcus acidilactici*) supplementation on productive performance, egg quality, and body composition in laying hens fed diets varying in energy density. *Poultry Science*, 99, 2275-2285.
- Mikulski D et al. (2012). Effects of dietary probiotic (*Pediococcus acidilactici*) supplementation on performance, nutrient digestibility, egg traits, egg yolk cholesterol, and fatty acid profile in laying hens. *Poultry Science*, 91, 2691-2700.
- Montes AJ, Pugh DG (1993). The use of probiotics in food-animal practice. *Veterinary Medicine*, 88, 282-288.
- Quarantelli A et al. (2008). Effects of the administration of *Pediococcus acidilactici* to laying hens on productive performance. *Veterinary Research Communications*, 32(suppl 1), S359-S361.
- Ritzi MM et al. (2014). Effects of probiotics and application methods on performance and response of broiler chickens to an *Eimeria* challenge. *Poultry Science*, 93, 2772-2778.
- Saleh et al. (2017). Synergistic effect of feeding *Aspergillus awamori* and lactic acid bacteria on performance, egg traits, egg yolk cholesterol and fatty acid profile in laying hens. *Italian Journal of Animal Science*, 16, 1, 132-139.
- Shanmugam S et al. (2024). Supplemental effect of *Pediococcus acidilactici* CNCM I-4622 probiotic on the laying characteristics and calcium and phosphorus metabolism in laying hens. *Scientific Reports*, 14,1, 12489.
- Shehata AA et al. (2022). Probiotics, prebiotics, and phytochemical substances for optimizing gut health in poultry. *Microorganisms*, 10, 395.



P¹² The Effect of Probiotic Use on Gut Microbiota and Body Weight of Broilers

Akın Ünal¹, Serdar Özlü², Okan Elibol², Mehmet Akan¹

¹Ankara University, Faculty of Veterinary Medicine, Department of Microbiology, Ankara, Türkiye

²Ankara University, Faculty of Agriculture, Department of Animal Science, Ankara, Türkiye

Abstract

Probiotics are frequently used to regulate gut microbiota and improve performance. In this study, it was aimed to investigate the effects of drinking water and probiotic use on gut microbiota and live weight in broilers. The study was carried out in a total of 128 Ross 308 broilers. The probiotic was used throughout the entire production period. On the 7th, 14th, 21st, 28th, 35th and 42 nd days of the production period, broilers were individually weighed and body weight calculations were made.

For microbiome analysis, a total of 16 greyhound feces samples were collected, 8 representing each group in the experimental and control groups. The digestive tract microbiota was determined by next-generation sequencing and metagenomic analysis. When the body weight findings were evaluated, the body weights of the

experimental group given probiotics were found to be higher than the control group in all weeks of weighing. It was observed that the live weight was 52 g higher in the group given probiotics at the slaughter stage. As a result of microbiome analysis, Firmicutes, Actinobacteriota and Gastranaerophilales bacterial phyla were found to be higher in the experimental group compared to the control group, while

Bacteroidales bacterial phyla were found to be lower. As a result, it was seen that the use of probiotics increased the beneficial bacterial phyla that make up the intestinal microbiota and showed a uniform distribution, and accordingly, the body weight in the experimental group was higher than in the control group.

Keywords: Body weight, broiler, gut microbiota, probiotic

Introduction

The digestive tract of broilers harbors a diverse and complex microbiota that plays a vital role in nutrient digestion and absorption, immune system development and pathogen exclusion [1,2]. Different broiler gut microbiota are known to play an important role in broiler metabolism, nutrient digestion, growth performance and host health. Probiotics are frequently used to maintain a balanced and healthy gut microbiota [3]. Probiotics, defined as live microorganisms, are one of the main feed additives used in broiler production for a long time due to the benefits they provide to the host when administered in appropriate amounts [4]. Probiotics for broilers can increase feed consumption and digestive efficiency by increasing the activity of digestive enzymes, maintain the balance of bacteria in the gastrointestinal (GI) tract and support intestinal integrity. Thus, probiotics can improve growth performance and health of broilers [5,6]. In addition, probiotics have also been shown to maintain microbiota balance in the gut by reducing the proliferation of pathogenic species and reduce the risk of disease by increasing resistance to infection [7,8].

The aim of this study was to investigate the effect of probiotic supplementation with drinking water on intestinal microbiota and body weight in broilers

Materials and Methods

Broiler chicks: A total of 128 one-day-old broiler chicks (Ross 308, 50% male-female separation) were used in the experiment. The study was conducted in two experimental and two control groups with 32 chicks (16 broilers/m²) in each pen (1x2 m; 2 m²).

Feed: All feed used during the production period (coded as 4 different periods, 0-12, 12-22, 23-37, 38-42 days) was commercially available as reported in the genotype booklet.

Probiotic: Smart Prolive Broiler Liquid (Bakın Tarım, Ankara) was used as probiotic in chickens with drinking water as recommended (1/1000).



Experiment: The study was conducted as an experimental (probiotic treated) group and a control group. After individual numbering and weighing, the chicks were placed in each pen in the poultry house with 32 chicks (16 females, 16 males; 16 chicks/m²) and reared for 42 days. In the study, care and management practices (temperature, ventilation, feed/water, lighting, etc.) were carried out in a way to include standard practices related to broiler care and management. Body weights were calculated by individual weighing on days 7, 14, 21, 28, 35 and 42. The performance data obtained in the experimental and control groups were analyzed using SPSS statistical package program.

At the slaughter stage (day 42), a total of 16 fresh fecal samples, 8 in each group, were taken in separate containers and coded. The coded samples were delivered to the laboratory under cold chain (2-8 °C) conditions. The microbiome in the samples was determined by next generation sequence and metagenomic analysis.

Details of microbiome analysis are presented below.

The coded samples were filtered using Sterivex-GP (Millipore, MA) with a membrane pore size of 0.22 µm. After filtration, the membrane retaining environmental DNA was directly used for DNA extraction. The resulting filtrate was homogenized in bead-containing tubes using a dry ice homogenizer and DNA extraction was performed using the Qiagen Blood & Tissue DNA Purification Kit. All extractions were performed in 3 replicates and isolates were pooled together.

The 16SV3 gene region of the obtained DNA for the bacteria was amplified. Approximately 200 bp long V3 fragment of 16S rRNA was amplified with primers 16SV3F (5'- ACTCTACGGGAGGCAGCAGT-3') and 16SV3R (5'- ACCGCGCTGCTGGCAC-3') containing Illumina universal adapters. All amplifications were performed in triplicate and pooled and used in the library phase. PCR amplifications were performed with GoTaq® Flexi DNA Polymerase (Promega) in a total volume of 25 µl on a T100 Thermal Cycler (Bio-Rad) according to the breeder protocol. Amplified DNA fragments were evaluated with a 2% agarose gel in 1x TAE and quantified with a ThermoFisher Qubit 4 Fluorometer.

The obtained PCR products were combined and AMPure XP beads (Beckman Coulter) was purified using Illumina Using Nextera XT Index Kit v2 and "16S Metagenomic Library preparations were carried out following the "Sequencing Library Preparation " protocol. ThermoFisher After quantitative measurement was performed with Qubit 4 Fluorometer , library sizes were checked on the BioAnalyzer 2100 system and Illumina was analyzed using 2x150 PE chemistry. Sequenced on the iSeq 100 platform.

Bioinformatic analysis was completed via Linux/Unix terminal. Quality control of forward and reverse read sequences in ". fastq " format was performed using FASTQC program. The analyses were continued using " ObiTools " package. Sequences with Phred score above 20 were aligned and merged with " illumina pairedend ". After that, unmerged reads were cleaned (obigrep), both forward and reverse primers were removed (perl), duplicated data were cleaned (obiuniq) and unnecessary data were removed from each sample header, respectively (obiannotate). Then, sequences with more than 2 repeats and longer than 100 base pairs were recorded (obigrep) and the abbreviation 'c10.1100' was used for them. ". fasta " files obtained from ObiTools package were blasted via SILVA and Geneious Prime interface . For the evaluation of SILVA and Blast results, only results with match rate above 97% were taken into consideration.

Findings

The live weight findings recorded in the experimental and control groups are presented in Table 1. The average of the groups given probiotics on all days was found to be higher than the experimental group. It was observed that the live weight was 52 g higher in the group given probiotics at the slaughter stage. Although there was a numerical difference between the findings obtained in all periods, the difference between the findings was found to be statistically insignificant.

Table 1. Live weight findings

Group	Live Weight, g (Mean±St.Error)					
	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Attempt	180.0 ± 2.19	510.1 ±6.66	1084 ±14.40	1821 ±22.9	2611 ±31.5	3364 ±38.1
Control	176.0 ± 2.19	497.2 ±6.66	1060 ±14.28	1780 ±22.5	2565 ±30.9	3312 ±37.4

Microbiome analysis findings: Obtained from fresh feces provided by the experimental and control groups. The microbiome analysis findings are presented in Table 2. In the experimental group, Firmicutes, Bacteroidales, Actinobacteriota, Protobacteria, Gastranaerophilales and Desulfovibrionaceae bacterial phyla constituted the microbiome, while in the control group, Firmicutes, Bacteroidales, Actinobacteriota, Protobacteria, Gastranaerophilales bacterial phyla constituted the microbiome (Figure 1). In the experimental group, the average Firmicutes, Actinobacteriota and While the bacterial phyla Gastranaerophilales were higher than the control group averages, Bacteroidales and Protobacteria bacterial phyla were found to be lower (Figure 2). In addition, when the samples were evaluated individually, it was noted that the distribution in the control group was more widespread (Figure 3).

Table 2. Microbiome analysis findings (averages for groups)

Bacterial phyla	Group (%)	
	Attempt	Control
<i>Firmicutes</i>	89,54	85,87
<i>Bacteroidales</i>	5,83	10,62
<i>Actinobacteriota</i>	2	1,19
<i>Protobacteria</i>	1	1,7
<i>Gastranaerophilale</i>	1,23	0,62
<i>Desulfovibrionaceae</i>	0,4	0

Figure 1. Microbiome analysis findings (individual)

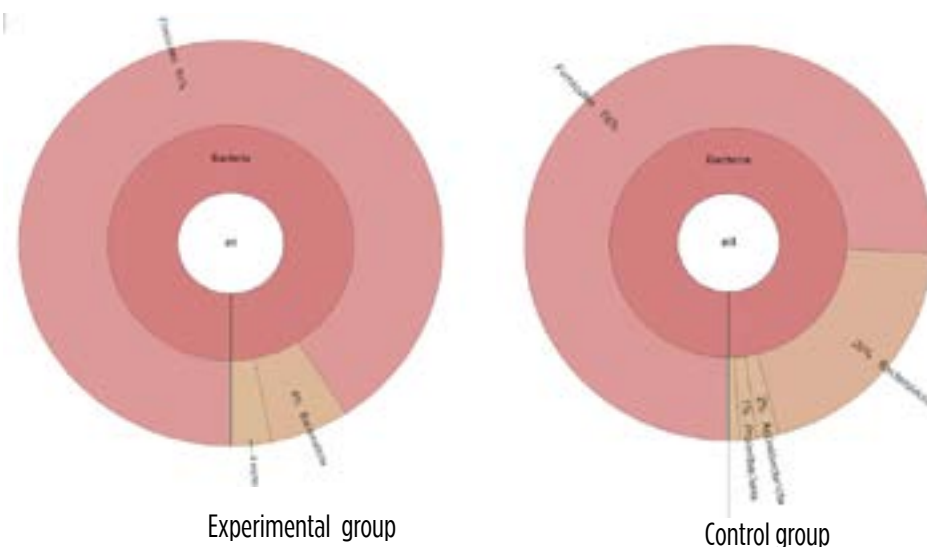


Figure 2. Microbiome diversity in the Trial Control group

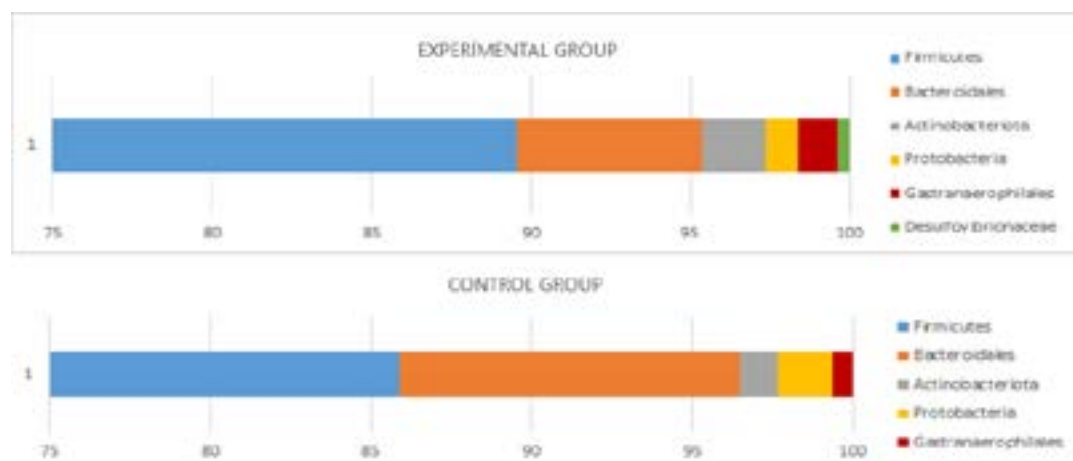


Figure 3. Distribution graph of the Firmicutes bacterial phylum



Discussion and Conclusion

According to the findings, the body weight was higher in the probiotic group than in the control group. This result was similar to the results of Hrnar et al. [9] and Fesseha et al. [10], who investigated the effect of probiotic use on body weight.

microbiome analysis, Firmicutes, Actinobacteriota were found in the probiotic group. And While the bacterial phyla Gastranaerophilales were higher than the control group averages, Bacteroidales and Protobacteria bacterial phyla were found to be lower. Wang et al. [11] found that the microbiota changed in favor of the Firmicutes bacterial phylum in their study using probiotics as a feed additive, while Memon et al. [12] found that Firmicutes, Bacteroidales and Protobacteria increased after the use of probiotics. It was stated that bacterial phyla were the most affected and that beneficial bacterial groups increased proportionally.

In this study, the live weight was found to be high in the probiotic group at all stages from the first week until the slaughter stage, and the digestive system microbiota. It was explained by the presence of uniform beneficial bacteria. This result showed that performance could be improved by regulating the digestive system microbiota.

References

1. Broom LJ, Kogut MH. The role of the gut microbiome in shaping the immune system of chickens. *Vet Immunol Immunopathol*. 2018; 204:44-51.
2. Shang Y, Kumar S, Oakley B, Kim WK. Chicken gut microbiota : importance and Detection technology *Front. Vet. Sci*. 2018; 5:254.
3. Hack MEA, Saadony MT, Shafi ME, Qattan SYA, Batiha GE, Khafaga AF, Moneim AME, Alagawany M. Probiotics in poultry feed : a comprehensive review. *J Anim Physiol Anim Nutr*. 2020;104:1835-1850.
4. Markowiak P, Liewska K. The role of probiotics, prebiotics and synbiotics in animals nutrition. *Gut Pathog*. 2018; 10 :1-20.
5. Grave SML. The Dynamics of probiotics in enhancing poultry meat production and quality *Int. J. Poult. Sci*. 2009;3: 361-364
6. Soomro RN, Abd El- Hack ME, Shah SS, Taha AE, Alagawany M, Swelum A, Tufarelli V. Impact of restricting feed and probiotic supplementation on growth performance, mortality and carcass traits of meat-type quails. *Anim Sci J*. 2019; 90:1388-1395.
7. Thacker PA. Alternatives to antibiotics as growth Promoters for use in swimming production : a review. *J Anim Sci Biotechnol*. 2013; 4: 35.
8. Yadav S, Jha R. Strategies to modulate the intestinal microbiota and their effects on nutrients utilization, performance, and health of poultry *J Anim Sci Biotechnol*. 2019; 10:2
9. Hrnar C, Weis J, Mindek S, Bujko J. Effect of probiotics addition to drinking water on body weight and body measurements of broiler chickens. *Anim Sci Biotechnol*. 2014; 47 : 249-253.
10. Fesseha H, Demlie T, Mathewos M, Eshetu E. Effect of Lactobacillus species probiotics on growth performance of dual purpose chicken. *Vet Med Res Rep*. 2021;12: 75-83.
11. Wang Y, Sun J, Zhong H, Li N, Xu H, Zhu Q, Liu Y. Effect of probiotics on the meat Flavour and gut microbiota of chicken. *science Rep*. 2017; 7 : 1-13.
12. Memon FU, Yang Y, Lv F, Soliman AM, Chen Y, Sun J, Si H. Effects of probiotics and Bidden's pilosa on the performance and gut health of chicken during induced Eimeria Tenella infection *J. Appl. Microbiol*. 2021 131 : 425-434.

Diagnostic and Analysis Report

Report No:	AVT-541464	Report Date:	15.12.2023
------------	------------	--------------	------------

Material Information

Sending Institution : BAKIN TARIM
Sample Registration : 541464 Sample Protocol:2023-4001
Number
Sample Acceptance
Date / Time : 11.12.2023 : 12:00:00
Sample Type : Chicken
Sample : Smart Prolive (Probiotic)
Analysis Start Date/Time : 12.12.2023 12:15:00
Reason For Revision :

Analysis Results:

The reconstitution results are given in the table below according to temperature and time.

Direct	9,1 x 10 ⁹
65°C-30 min.	8,2 x 10 ⁹
72°C-15 sec.	8,1 x 10 ⁹

Date	Report Prepared by	Experiment Supervisor	Responsible Manager
15.12.2023	Utkucan Sarıpinar	Betül Küçük	Merve Özdal Salar



This report may not be partially copied or reproduced without the written permission of the laboratory. Unsigned and unsealed reports are invalid. Test

results are valid only for the specified sample.

Our laboratory has no responsibility for the Sampling stage and the samples sent by the customer are analyzed.

* Revision Reason will be filled in for revised reports.

Diagnostic and Analysis Report

Report No:	AVT-542225	Report Date:	08.02.2024
------------	------------	--------------	------------

Material Information

Sending Institution : BAKIN TARIM
Sample Registration Number : 542225 Sample Protocol:2024-537
Sample Acceptance Date / Time : 08.02.2024 : 20:29
Sample Type : Chicken
Sample : Smart Prolive
Analysis Start Date/Time : 08.02.2024 20:50:00
Reason For Revision :

Analysis Results:

Sample No	Sample Information	Analysis	Conclusion
633796	<i>Pediococcus acidilactici</i>	Total Bacteria Count	$2,1 \times 10^9$ cfu
633797	<i>Lactobacillus acidophilus</i>	Total Bacteria Count	$1,9 \times 10^8$ cfu
633798	<i>Bacillus subtilis</i>	Total Bacteria Count	$1,2 \times 10^8$ cfu

Date	Report Prepared by	Experiment Supervisor	Responsible Manager
08.02.2024	Utkucan Sarıpınar	Betül Küçük	Merve Özdal Salar



This report may not be partially copied or reproduced without the written permission of the laboratory. Unsigned and unsealed reports are invalid. Test results are valid only for the specified sample.

Our laboratory has no responsibility for the Sampling stage and the samples sent by the customer are analyzed.

* Revision Reason will be filled in for revised reports.

Diagnostic and Analysis Report

Report No:	AVT-542225	Report Date:	08.02.2024
------------	------------	--------------	------------

Material Information

Sending Institution : BAKIN TARIM
Sample Registration Number : 542225 Sample Protocol:2024-537
Sample Acceptance Date / Time : 08.02.2024 : 20:29
Sample Type : Chicken
Sample : K.B.T 01
Analysis Start Date/Time : 08.02.2024 20:50:00
Reason For Revision :

Analysis Results:

Sample No	Sample Information	Analysis	Conclusion
633796	<i>Pediococcus acidilactici</i>	Total Bacteria Count	3,1 x 10 ⁸ cfu
633797	<i>Lactobacillus acidophilus</i>	Total Bacteria Count	1,2 x 10 ⁷ cfu
633798	<i>Bacillus subtilis</i>	Total Bacteria Count	2,5 x 10 ⁸ cfu
633799	<i>Salmonella spp.</i>	ISO-6579	Negative

Date	Report Prepared by	Experiment Supervisor	Responsible Manager
08.02.2024	Utkucan Sarıpinar	Betül Küçük	Merve Özdal Salar





This report may not be partially copied or reproduced without the written permission of the laboratory. Unsigned and unsealed reports are invalid. Test results are valid only for the specified sample.

Our laboratory has no responsibility for the Sampling stage and the samples sent by the customer are analyzed.

* Revision Reason will be filled in for revised reports.

Ankara Üniversitesi Teknokent Ekalanı Kalaba Mah. Küttükçi Alibey Cad. No:2B/201 Keçiören/ANKARA
Phone/Fax:0312 359 10 55 Office Phone:+90 552 299 10 55
Web: www.avitekarge.com Mail: info@avitekarge.com

Diagnostic and Analysis Report

Report No:	AVT-542225	Report Date:	08.02.2024
------------	------------	--------------	------------

Material Information

Sending Institution : BAKIN TARIM
Sample Registration Number : 542225 Sample Protocol:2024-537
Sample Acceptance Date / Time : 08.02.2024 : 20:29
Sample Type : Chicken
Sample : K.B.T 01
Analysis Start Date/Time : 08.02.2024 20:50:00
Reason For Revision :

Analysis Results:

Sample No	Sample Information	Analysis	Conclusion
633796	<i>Pediococcus acidilactici</i>	Total Bacteria Count	3,1 x 10 ⁸ cfu
633797	<i>Lactobacillus acidophilus</i>	Total Bacteria Count	1,2 x 10 ⁷ cfu
633798	<i>Bacillus subtilis</i>	Total Bacteria Count	2,5 x 10 ⁸ cfu
633799	<i>Salmonella spp.</i>	ISO-6579	Negative

Date	Report Prepared by	Experiment Supervisor	Responsible Manager
08.02.2024	Utkucan Sarıpinar	Betül Küçük	Merve Özdal Salar



This report may not be partially copied or reproduced without the written permission of the laboratory. Unsigned and unsealed reports are invalid. Test results are valid only for the specified sample.

Our laboratory has no responsibility for the Sampling stage and the samples sent by the customer are analyzed.

* Revision Reason will be filled in for revised reports.

CERTIFICATE OF ANALYSIS		
SMART PROLIVE (FEED FORM)		
Probiotics (Total Bacteria Count: 1×10^{10} CFU/g)		
<i>L. Acidophilus</i>	1×10^{10}	CFU/g
<i>A. Acidilactici</i>	1×10^{10}	CFU/g
<i>B. Subtilis</i>	1×10^{10}	CFU/g
Prebiotics		
MOS	100	g/kg
Inulin	200	g/kg
Enzymes		
Xylanase	2200000	BXU/kg
Cellulase	270000	FPU/kg
Glucanase	100000	FBG/kg
Phytase	1000000	PPU/kg
Carriers		
Calcite (Razmol)	590	g/kg

DIRECTIONS FOR USE

It is used adding 500 gr - 1000 gr/ton (Ton feed 500 gr - 1000 gr) to a ton feed.

THIS IS NOT A DRUG, IT IS A FEED ADDITIVE. IT CAN NOT BE USED FOR TREATMENT.

It should be stored in a cool, non-humid and non-direct sunlight place.

PROD. DATE:

Batch Number:

EXP. DATE:

The product can be stored for a period of 12 months.

NET 25 KG

BAKIN AGRICULTURE PRODUCTS INDUSTRY AND TRADE LTD. COMPANY

Head Office & Factory: Fatih Mah. Turgut Özal Bulvarı No:24 Kahramankazan/ANKARA

Phone: (+90 312) 480 20 61 • Fax: (+90 312) 480 20 91

www.bakintarim.com.tr - info@bakintarim.com.tr

CERTIFICATE OF ANALYSIS		
SMART PROLIVE 50 GR		
	Total of amount	Unit
PROBIOTICS :		
<i>B. Subtilis</i> (each one at least, 1x10 ¹⁰ CFU/g)	10	gr
<i>L. Acidophilus</i> (each one at least, 1x10 ¹⁰ CFU/g)		gr
<i>P. Acidilactici</i> (each one at least, 1x10 ¹⁰ CFU/g)		gr
Includes adjunct culture and CE culture		gr
PREBIOTICS:		
Inulin (100 g)	300	gr
MOS (200 g)		
ENZYMES:		
Xylanese (2.200.000 BXU/kg)	100	gr
Cellulase (270.000 FPUI/kg), Glucanase (100.000 FBG/kg)		
Phytase (1.000.000 PPU/kg)		
CARRIERS:		
Oligosaccharides	590	gr

DIRECTIONS FOR USE: 50 g per day is sufficient for 10.000 chicks and chickens.

It is given by adding to water or feed for 6-8 hours during the day.

The product is stored in its original packaging, tightly closed, in a cool and dry place away from sunlight.
SHELF LIFE IS 12 MONTHS.

THIS IS NOT A DRUG, IT IS A FEED ADDITIVE. IT CAN NOT BE USED FOR TREATMENT.

BAKIN AGRICULTURE PRODUCTS INDUSTRY AND TRADE LTD. COMPANY

Head Office & Factory: Fatih Mah. Turgut Özal Bulvarı No:24 Kahramankazan/ANKARA

Phone: (+90 312) 480 20 61 • Fax:(+90 312) 480 20 91

www.bakintarim.com.tr - info@bakintarim.com.tr

CERTIFICATE OF ANALYSIS			
SMART PROLIVE LAYER (LIQUID)			
NAME	Activity (u/g.ml) Colony forming unit FTU/g	Amount (gr.ml)	U.CFU/kg Drinking water
PROBIOTICS			
<i>Lactobacillus Acidophilus</i>	1 x 10 ¹⁰ CFU/gr	1x10 ¹² CFU/lt (20.000 mg/lt)	1x10 ⁹ CFU
<i>Pediococcus Acidilactici</i>	1 x 10 ¹⁰ CFU/gr	1x10 ¹² CFU/lt (100.000 mg/lt)	1x10 ⁹ CFU
CARRIERS			
Inulin MOS Water		850.000 mg/lt	

DIRECTIONS FOR USE : From the first day of laying, 1 litre of water per to ton is given every day for the first 4 weeks to regulate the intestinal flora to ensure better feed utilization.

4-18 weeks, halfa liter per ton of water is given 1 day and 2 days break is given.

8-120 weeks, halfa liter is added to ton water and given 1 day and 3 days break.

When added to feed, it is used by adding 0,5 liters to 1 ton of feed by spraying the mixer.

THIS IS NOT A DRUG, IT IS A FEED ADDITIVE. IT CAN NOT BE USED FOR TREATMENT.

The product is stored in its original packaging, tightly closed, in a cool and dry place away from sunlight.

SHELF LIFE IS 6 MONTHS.

BAKIN AGRICULTURE PRODUCTS INDUSTRY AND TRADE LTD. COMPANY

Head Office & Factory: Fatih Mah. Turgut Özal Bulvarı No:24 Kahramankazan/ANKARA

Phone: (+90 312) 480 20 61 • Fax:(+90 312) 480 20 91

www.bakintarim.com.tr - info@bakintarim.com.tr

CERTIFICATE OF ANALYSIS			
SMART PROLIVE BROILER (LIQUID)			
NAME	Activity (u/g.ml) Colony forming unit FTU/g	Amount (gr.ml)	U.CFU/kg Drinking Water
PROBIOTICS			
<i>Bacillus Subtilis</i>	1,6 x 10 ¹⁰ CFU/gr	8x10 ¹¹ CFU/lt (50.000 mg/lt)	8x10 ⁸ CFU
<i>Pediococcus Acidilactici</i>	1 x 10 ¹⁰ CFU/gr	1x10 ¹² CFU/lt (100.000 mg/lt)	1x10 ⁹ CFU
CARRIERS			
Inulin MOS Water		850.000 mg/lt	

DIRECTIONS FOR USE : In order to regulate the intestinal flora to ensure better utilization of the feed, 1 lt per ton of drinking water of broilers 0-15 days per ton of water.
It is recommended to use 0,5 lt per ton of water after 16-35 days.
After 16-35 days, the application is continued with a one day break.

THIS IS NOT A DRUG, IT IS A FEED ADDITIVE. IT CAN NOT BE USED FOR TREATMENT.
The product is stored in its original packaging, tightly closed, in a cool and dry place away from sunlight.
SHELF LIFE IS 6 MONTHS.

BAKIN AGRICULTURE PRODUCTS INDUSTRY AND TRADE LTD. COMPANY

Head Office & Factory: Fatih Mah. Turgut Özal Bulvarı No:24 Kahramankazan/ANKARA

Phone: (+90 312) 480 20 61 • Fax:(+90 312) 480 20 91

www.bakintarim.com.tr - info@bakintarim.com.tr

CERTIFICATE OF ANALYSIS			
SMART PROLIVE BREEDER (LIQUID)			
NAME	Activity (u/g.ml) Colony forming unit FTU/g	Amount (gr.ml)	U.CFU/kg Drinking water
PROBIOTICS			
<i>Lactobacillus acidophilus</i>	1 x 10 ¹⁰ CFU/gr	1x10 ¹² CFU/lt (20.000 mg/lt)	1x10 ⁹ CFU
<i>Pediococcus acidilactici</i>	1 x 10 ¹⁰ CFU/gr	1x10 ¹² CFU/lt (100.000 mg/lt)	1x10 ⁹ CFU
CARRIERS			
Inulin MOS Water		850.000 mg/lt	

Used in feed : 0,5 lt per ton of feed.

Use in drinking water : 0,5 lt is added to 1 ton of drinking water.

For maximum benefit, it should be used every day throughout the animal's life.

It can be given to the irrigation system line with a dosatron.

Drums should be shaken before use.

When added to drinking water, the water should not contain chlorine and antibiotics.

The product is stored in its original packaging, tightly closed, in a cool and dry place away from sunlight.

THIS IS NOT A DRUG, IT IS A FEED ADDITIVE. IT CAN NOT BE USED FOR TREATMENT.

The product is stored in its original packaging, tightly closed, in a cool and dry place away from sunlight.

SHELF LIFE IS 6 MONTHS.

BAKIN AGRICULTURE PRODUCTS INDUSTRY AND TRADE LTD. COMPANY

Head Office & Factory: Fatih Mah. Turgut Özal Bulvarı No:24 Kahramankazan/ANKARA

Phone: (+90 312) 480 20 61 • Fax:(+90 312) 480 20 91

www.bakintarim.com.tr - info@bakintarim.com.tr



CORRECT CHOICE FOR HEALTHY FLOCKS



ProLive

PROBIOTIC
PREBIOTIC
ENZYME

NATURAL
GUT
FLORA
REGULATOR



ProLive Liquid



BREEDER



BROİLER



LAYER



Head Office & Factory
Fatih Mah. Turgut Özal Bulvarı No:24/A Kahramankazan/ANKARA
Phone: (+90) 312 480 20 61 • Fax: (+90) 312 480 20 91

www.bakintarim.com.tr • info@bakintarim.com.tr

List of Feed Additives Approved by the Ministry of Agriculture and Forestry

Category	Functional Group	Substance	Name of the Authorization Holder	Additive	Composition, Chemical Formula, Description, Analytical Method	Animal Species or Category
4	b	Zooteknik Additives / Gut Flora Regulator	Bakim Tarım Ürünleri San. ve Tic. Ltd. Şti.	Polibacillus acidophilus 1x10 ⁹ CFU/ml KUEN 1608	Additive Composition: Polibacillus acidophilus 1x10 ⁹ CFU/ml KUEN 1608 strain (2.1x10 ⁹ CFU/ml) in liquid form as a feed additive. - Characterization of the Active Substance: Live cells of Polibacillus acidophilus 1x10 ⁹ CFU/ml KUEN 1608 strain. Analytical Method: The determination of the active substance in feed additives, premixes, feed materials, and water is performed using the Polibacillus acidophilus Chrom Select Agar (Sigma 92325) spread plate method.	Broiler chickens Laying hens
4	b	Zooteknik Additives / Gut Flora Regulator	Bakim Tarım Ürünleri San. ve Tic. Ltd. Şti.	Polibacillus acidophilus 1x10 ⁹ CFU/ml KUEN 1608	Additive Composition: Polibacillus acidophilus 1x10 ⁹ CFU/ml KUEN 1608 strain (2.1x10 ⁹ CFU/ml) in liquid form as a feed additive. - Characterization of the Active Substance: Live cells of Polibacillus acidophilus 1x10 ⁹ CFU/ml KUEN 1608 strain. Analytical Method: The determination of the active substance in feed additives, premixes, feed materials, and water is performed using the Polibacillus acidophilus Chrom Select Agar (Sigma 92325) spread plate method.	Broiler chickens
4	b	Zooteknik Additives / Gut Flora Regulator	Bakim Tarım Ürünleri San. ve Tic. Ltd. Şti.	Lactobacillus acidophilus 1x10 ⁹ CFU/ml KUEN 1607	Additive Composition: Lactobacillus acidophilus 1x10 ⁹ CFU/ml KUEN 1607 strain (2.1x10 ⁹ CFU/ml) in liquid form as a feed additive. - Characterization of the Active Substance: Live cells of Lactobacillus acidophilus 1x10 ⁹ CFU/ml KUEN 1607 strain. Analytical Method: The determination of the active substance in feed additives, premixes, feed materials, and water is performed using the Lactobacillus acidophilus Chrom Select Agar (Sigma 92325) spread plate method.	Laying hens

**A FIRST IN
TURKEY!**

**PROBIOTICS APPROVED
BY THE MINISTRY!**

www.tarimorman.gov.tr >Ministry-Approved Feed Additives List



BAKIN

Agriculture Products Industry and Trade Ltd. Company



Head Office & Factory

Fatih Mah. Turgut Özal Bulvarı No:24/A Kahramankazan/ANKARA

Phone: (+90) 312 480 20 61 • Fax: (+90) 312 480 20 91

info@bakintarim.com.tr

www.bakintarim.com.tr

