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# Effects of *Silybum marianum* Seeds as a Feed Additive on Broiler Production Performance and Quality

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## ABSTARCT

**Background:** The use of growth-promoting antibiotics in poultry feed at sub-therapeutic doses can lead to the development of antibiotic resistance, which can reduce their effectiveness in treating both animal and human health issues. The study aimed to evaluate the effects of adding Milk thistle seeds to broiler feed and drinking water on zootechnical and production performance, as well as on various biochemical parameters.

**Methods:** One hundred Cobb 500 broiler chicks were weighed and divided into four equal groups of 25 chicks each. The control group was fed a conventional diet. The experimental groups were as follows: group 2 received a diet containing 3% ground Milk thistle seeds, group 3 was treated with water infused with 3% Milk thistle seeds and group 4 received a diet with 1.5% ground Milk thistle seeds and drinking water infused with 1.5% Milk thistle seeds. All chicks had unlimited access to food and water during the 43-day rearing period.

**Result:** The study revealed that the group labeled as control had the highest feed intake at 4300.32 g, while the chickens that were fed a ration containing 3% Milk thistle seeds consumed a lower quantity, approximately 3674.12 g. The inclusion of Milk thistle seeds at a rate of 1.5% in the feed and 1.5% in the drinking water significantly increased the slaughter weight of the chickens (2803.50 g) compared to the control group (2578.90 g) ($P<0.05$). The control group chickens, which were fed a conventional diet, had a lower carcass weight compared to those in group 4 ($P<0.05$). However, the inclusion of Milk thistle seeds in the different groups did not affect carcass yield, abdominal mass,biochemical profile ($P>0.05$).


**Key words:** Biochemical parameters, Broilers, Milk thistle seeds, Production performance, Zootechnical.

## INTRODUCTION

Chicken meat is preferred over other meats due to its higher protein content and lower cholesterol levels (Aman *et al*., 2023). Antibiotics have been added to poultry diets as growth-promoting feed additives. This is done to improve zootechnical performance, including increasing growth rate, decreasing mortality rate and reducing disease incidence. The goal is to increase the productivity of poultry farms and meet the growing demand for poultry meat (Askri *et al*., 2018). However, the use of growth-promoting antibiotics in poultry feed at sub-therapeutic doses can lead to the development of antibiotic resistance, which can reduce their effectiveness in treating both animal and human health issues (Dupraz, 2004; Mehboob *et al*., 2025). Indeed, in 2006, the European Union banned the use of antibiotics as growth promoters in animal feed (Alloui, 2011; Li, 2017). Faced with this ban, researchers are turning their attention to other nutritional solutions, with alternatives such as phytobiotics attracting increasing interest (Skomorucha *et al*., 2013). The use of phytobiotics has grown due to their pharmacological benefits, which include promoting feed intake, improving antioxidant status, increasing endogenous enzyme secretion, altering cell membrane fluidity and permeability and enhancing nutrient absorption (Vicente-Martínez *et al*., 2022). Milk thistle (*Silybum marianum*), which belongs to the Asteraceae family, is one of the medicinal plants that can be used for animal feed (Erisir *et al*., 2016). For over 2,000 years, its seeds have been used as a natural remedy to treat liver and bile disorders (Ding *et al*., 2001; Sayin *et al*., 2016; Wang *et al*., 2020). Silymarin is the biologically active substance in Milk thistle, a group of flavonolignan isomers

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(Kazazis *et al*., 2014). Bahmani *et al.* (2015), Fehér and Lengyel (2012), have shown in their studies that silymarin has hepatoprotective effects due to its antioxidant action; anti-inflammatory effect; membrane stabilization and inhibition of fibrogenesis, promoting Hepatocyte regeneration. In addition, silymarin has chemopreventive activity against bacterial, viral, fungal and chemical toxins (Opletal, 2010). Due to its chemical composition and biological properties, veterinarians prefer to use silymarin as a natural medicine for intensive livestock farming (Radko *et al*., 2007; Saadh, 2020), although Milk thistle is widely used in human and animal nutrition (Grela *et al*., 2020). Studies have shown that the addition of silymarin to livestock diets increases production performance and improves animal health (Tedesco, 2001). Ahmad *et al.* (2019), reported that the body weight and feed conversion ratio of broilers fed diets containing 10 and 15 g/kg Milk thistle, respectively, were increased. A positive effect of adding silymarin-phospholipid complex or silymarin to broiler diets on performance results was reported by Mojahedtalab *et al*., (2013).

The objective of this study was to determine the effect of incorporating Milk thistle seeds into the feed and drinking water of broiler chickens on zootechnical and production performance, as well as on certain biochemical parameters.

## MATERIALS AND METHODS

### Experimental animals and feeds

The experimental animals used in this experiment were: unsexed, vaccinated first-day-old chicks of the Cobb 500 strain, with an average weight of 45.2 to 47 g. The experiment was conducted at the Institute of Veterinary Sciences in Constantine. The ingredients and feed rations were prepared in the PADESCA research laboratory. Milk thistle seeds were ground and the feed ration for each group was calculated according to the nutritional value of the ingredients using Microsoft Excel to obtain an iso-energy iso-protein ration. To incorporate Milk thistle seeds into the drinking water, they were added to boiling water and left to infuse for a few minutes while maintaining the boiling point.

### Experimental design

The study was conducted at the rearing building of the experimental farm of the Institute of Veterinary Sciences-El Khroub, University of Constantine 1 in Algeria, from November 16 to December 28, 2022. One hundred chicks (n=100) were raised on a bed of wood shavings (3 kg/m$^2$). The rearing environment was maintained at a temperature of 33°C for the first three days, which was then gradually decreased by 2°C each week. At 43 days of age, the temperature was 21°C. The lighting system consisted of 24 hours of light for the first three days, followed by 6 hours of darkness and 18 hours of light for the remaining days of the rearing period (43 days). Static aeration ensured ventilation throughout. The environmental conditions and animal health were monitored daily during the rearing period. The chicks were weighed and randomly divided into four equal groups of 25 chicks each.

Treatments were applied throughout the rearing period as follows: Control group ″C″. received a basic diet + drinking water containing synthetic food additives; Experimental group ″2″. received a basic diet supplemented with 3% ground Milk thistle seeds + drinking water without additives; Experimental group ″3″. received a basic diet + drinking water containing 3% infused Milk thistle seeds; Experimental group ″4″. received a basal diet supplemented with 1.5% ground Milk thistle seeds + drinking water containing 1.5% infused Milk thistle seeds. All chicks had *ad libitum* access to food and water. The compositions of the starter, growth and finishing feeds distributed to birds in 4 experimental groups are described in Table 1.

### Data collection

Monitoring was conducted throughout the rearing period. Feed and water were weighed and distributed daily to the respective clean feeders and troughs for each group.

**Table 1:** Composition of starter, grower and finisher diet.

| | Starter diet | | | Grower diet | | | Finisher diet | | |
|---|---|---|---|---|---|---|---|---|---|
| Ingredients (%) | Ration (control, ration 3) | Ration 2 (3% Milk thistle) | Ration 4 (1.5% Milk thistle) | Ration (control, ration 3) | Ration 2 (3% Milk thistle) | Ration 4 (1.5% Milk thistle) | Ration (Control, ration 3) | Ration 2 (3% Milk thistle) | Ration 4 (1.5% Milk thistle) |
| Corn | 57 | 57.1 | 57.4 | 60.4 | 60.1 | 60.0 | 66 | 65 | 65.3 |
| Soya meals | 31.5 | 30.4 | 30.5 | 29 | 29 | 29.5 | 26.3 | 25.8 | 26.5 |
| Bran | 7 | 5 | 6.1 | 6 | 3.3 | 4.4 | 3 | 2 | 2 |
| Milk thistle seeds | 0 | 3 | 1.5 | 0 | 3 | 1.5 | 0 | 3 | 1.5 |
| Monocalcium phosphate | 1.7 | 1.7 | 1.7 | 1.3 | 1.3 | 1.3 | 0.8 | 0.8 | 0.8 |
| Calcium | 1.3 | 1.3 | 1.3 | 1.3 | 1.3 | 1.3 | 1.3 | 1.3 | 1.3 |
| Mineral complex vitamins | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Soybean oil | 0.5 | 0.5 | 0.5 | 1 | 1 | 1 | 1.6 | 1.1 | 1.6 |
| Metabolizable energy (Kcal/kg) | 2917.95 | 2914.12 | 2917.76 | 2950.42 | 2950.06 | 2950.66 | 3018.21 | 3010.07 | 3010.59 |
| Crude protein (%) | 19.86 | 19.50 | 19.50 | 18.77 | 18.80 | 18.99 | 18.77 | 18.80 | 18.99 |

Feed and water remaining in the corresponding equipment were also weighed to determine feed intake (FI). Chicks were weighed weekly from arrival in the rearing building until the day of slaughter (live weight at slaughter) to determine average weight gain (AWG). A representative sample of animals in each group was weighed to calculate average group weight. Daily records of dead animals were kept to determine the mortality ratio (MR), for each phase and the entire trial period. Consumption Index (CI) was also calculated.

At the end of the rearing period (day 43), 10 birds were randomly selected from each group, identified and weighed to assess their live weight at slaughter. They were then slaughtered at the abattoir in Constantine, with feathers, paste and heads removed. The full carcasses were weighed and evisceration was carried out to weigh the empty carcasses and calculate the carcass yield (CY). The weight of liverand abdominal fat, was also calculated.

At the time of slaughter, blood was collected from 10 subjects per group individually in pre-identified heparinized tubes. The tubes were then centrifuged at 3,000 rpm for 10 minutes and the resulting plasma was collected in identified dry tubes and stored at a freezing temperature of -18°C. Biochemical analyses were conducted at the private laboratory 'EL AZIZA' in Constantine to evaluate the impact of Milk thistle seeds on the biochemical profile, specifically liver function, by measuring : Aspartate AminoTransferase (AST), Alanine AminoTransferase (ALT) and glucose levels.

### Statistical analysis

The collected data were statistically processed using IBM SPSS Statistics 22. The statistical test used was the Analysis of One Factor Variance (ANOVA) test for most of the parameters studied. After validating the conditions of application, such as normality and homogeneity, the Tukey test (post-hoc test) was applied for comparison of multiple treatments. The 'Welch' test was used to analyze the 'carcass yield' parameter, correcting for homogeneity where h0 is rejected, indicating a lack of equality of variances. The non-parametric 'Kruskal-Wallis' test was used to analyze the 'ALT' parameter, as its normality was invalid.

The data are presented as mean values±standard deviation, with a statistically significant difference considered at $p<0.05$.

## RESULTS AND DISCUSSION

### Zootechnical performance and mortality ratio

The results regarding the effects of incorporating Milk thistle seeds into broiler feed and drinking water on zootechnical Performance are summarized, in Table 2.

The highest "FI" was noted for the "control" group with a value of 4300.32 g and the lowest was noted for experimental group "2" (3674.12 g), while values of 4241.21 g and 3818.68 g were noted for experimental groups "3" and"1", respectively. Although Milk thistle seeds incorporated into the feed and drinking water increased feed intake compared with other "experimental groups" it remained lower than that of the "control" group.

In contrast, Zaker-Esteghamati *et al*. (2020) reported that consumption of 0.8% heat-treated Milk thistle seeds increased "FI" in broilers of the Cobb 500 strain. Similarly, Fani *et al.* (2013) showed that Milk thistle's active ingredient, silymarin, had a positive effect on feed intake.

The group with the highest live weight at slaughter was recorded as group "4", with an average of 2803.50 g. The lowest live weight, at 2493.20 g, was recorded for group "3", while group "C" and "2" recorded values of 2578.90 g

**Table 2:** Effects of incorporating Milk thistle seeds into broiler feed and drinking water on zootechnical performance.

| | Group "C" | Group "2" | Group "3" | Group "4" | ANOVA test |
|---|---|---|---|---|---|
| Feed intake (g) (FI) | 4300.32 | 3818.688 | 3674.12 | 4241.211 | / |
| Live weight at slaughter (g) | 2578.90±252. | 2708.60±297.46 | 2493.20±212.34 [a] | 2803.50 ±201.47[a] | P<0.05 |
| | Max | Max | Max | Max | |
| | 3083 | 3294 | 2878 | 3120 | |
| | Min | Min | Min | Min | |
| | 2315 | 2366 | 2223 | 2494 | |
| Consumption Index (CI) (%) | 1.66 ±0.15[b] | 1.40±0.13 [b] | 1.47±0.12 | 1.51±0.15 | P<0.05 |
| | Max | Max | Max | Max | |
| | 1.85 | 1.61 | 1.65 | 1.70 | |
| | Min | Min | Min | Min | |
| | 1.39 | 1.15 | 1.27 | 1.35 | |
| Average weight gain (g) (AWG) | 58.87±5.86 | 61.91±6.92 | 56.91±4.93[c] | 64.11±4.70[c] | P<0.05 |
| | Max | Max | Max | Max | |
| | 3037 | 3244 | 2834 | 3075 | |
| | Min | Min | Min | Min | |
| | 22690 | 2316 | 2173 | 2448 | |
| Cumulative mortality ratio (%) (MR) | 0 | 4 | 0 | 4 | / |

Values on the same line with the same superscript letters are significantly different (P<0.05). Max= Maximum values, Min= Minimum values.

and 2708.60 g, respectively. The ANOVA test (P<0.05) showed that the incorporation of Milk thistle seeds in the feed (1.5%) and drinking water (1.5%) had an effect on live weight at slaughter (2803.50 g) compared to groups ″C, 2 and 3″. A significant difference was observed between group “3” and group ″4″, according to the Tukey post-hoc test.

Our results are in agreement with Janocha *et al*. (2021), who demonstrated that incorporating ground Milk thistle seeds (2/3%) into the diets of Ross 308 broilers throughout the rearing period (42 d) significantly increased live weight at slaughter. Similarly, Gawel *et al*. (2003) reported an increase in live weight at slaughter of broilers when they received silymarin. However, according to Šastník *et al*. (2016), the addition of Milk thistle seed cake to the feed of Ross 308 broilers at 5% and 15% did not affect the average body weight. In their study, the control group had a weight of 2320.31 g, while the experimental groups at 5% and 15% had weights of 2166.69 g and 1988.78 g, respectively.

Regarding the ″CI″, group 3 had the lowest ″CI″ (1.40), while group C had the highest ″CI″ (1.66). Groups ″3″ and ″4″ had CIs of 1.47 and 1.51, respectively. The inclusion of ground Milk thistle seeds in the broiler feed (3%) significantly reduced the Consumption Index (CI) (P<0.05) compared to the other groups. A significant difference was observed between group “C” and group ″2″, as determined by the Tukey test.

Kralik *et al*. (2015), demonstrated that adding 3% Milk thistle oil to the diet of Cobb 500 broilers increased the”CI”. Similarly, the introduction of 0.5% Milk thistle in the diet of broilers decreased feed conversion compared to other chickens fed a feed without additives; Milk thistle, state Kalantar *et al*. (2014). Our results are not in agreement with those of Zaker-Esteghamati *et al*. (2020), who obtained a slightly higher feed conversion ratio in the 0.4% heat-treated raw Milk thistle seed diet (1.37) than in the 0.8% heat-treated Milk thistle seed diet (1.36).

The recorded ″AWG” for group ″C″ was 58.87 g, while group ″2″ had a value of 61.91 g. Group ″4″ had the highest ″AWG″ (64.11 g), while group ″3″ showed a significant drop in ″AWG″ (56.91 g).

The inclusion of 1,5% Milk thistle seeds in both feed and drinking water significantly increased average gain (P<0.05) compared to the other groups. The Tukey test revealed a significant difference between groups ″3″ and ″4″.

Our findings differ from those of Suchı *et al.* (2008), who reported that incorporating 0.2% and 1% Milk thistle Seed Cake in Ross 308 broiler feed resulted in a decrease in average gain. In contrast, Ahmad *et al.* (2019) obtained similar results to ours, finding significantly higher mean weight gain (P<0.05) for the groups fed a ration containing 10 g/kg and 15 g/kg of Milk thistle seed powder. During the 2$^{nd}$, 3$^{rd}$, 4$^{th}$ and 5$^{th}$ weeks, the addition of 15 g/kg of Milk thistle seed powder to the broiler feed significantly increased the average weight gain, followed by the group fed 10 g/kg of Milk thistle seed powder.

During the entire test period, groups ″C″ and ″3″ did not experience any mortalities. However, groups ″2″ and ″4″ had a ″MR″of 4%. The observed health issues included aerosacculitis, fibrinous pneumonia, perihepatitis and pericarditis. Additionally, a sporadic case of colibacillosis was suspected. No treatment was administered to living subjects, as they did not exhibit any symptoms.

**Production performance**

The results regarding the effects of incorporating Milk thistle seeds into feed and drinking water on broiler production performance are summarized, in Table 3.

The impact of Milk thistle seeds on carcass weight and liveris significant (P<0.05). Group ″4″ recorded the highest average weights for full carcass (2327 g), empty carcass (1968.30 g) and liver (54.70 g), compared with those of group ″3″, which represented the lowest values (2050 g, 1728.90 g, 43 g), respectively.

Group ″2″ had weights of 2239 g for full carcass, 1889 g for empty carcass and 51.90 g for liver, followed lastly by group ″C″ at 2221.10 g, 1881.60 g and 49.70 g, respectively. The incorporation of Milk thistle seeds in feed and water (1.5%) “group 3″ significantly increased liver weight (p<0.05). The Tukey test revealed a significant difference between group ″3″ and ″2″.

Our results are similar to those of Tavakolinasab *et al.* (2020), who concluded that Milk thistle extract added at 250 mg/kg to broiler feed resulted in an increase in liver weight. However, introducing 15% Milk thistle seed cake into the broiler diet increased liver weight compared to control groups and chickens fed a diet containing 5% Milk thistle, but the effect was not statistically significant (P>0.05).

The addition of Milk thistle seeds to the feed and drinking water did not significantly affect ″CY″, abdominal fat weight (P>0.05). In our experiment, the highest “CY” was found in group ″C″ (73.11%) compered to group ″3″ (69.39%). No significant difference was found between the different groups (P>0.05) according to Welch’s test.

Our results are similar to those of Schiavone *et al.* (2007), who found that the addition of 40 and 80 ppm silymarin to the feed of Ross 308 strain broilers decreased ″CY″, however the control group recorded the highest yield (75.04%). Similarly, Šastník *et al.* (2016), found in their experiment that the use of 5% and 15% Milk thistle seeds did not have a positive impact on ″CY″, with the control group still recording the highest yield.

Group ″2″ recorded the highest average abdominal fat weight of 37.40 g, compared to 34.40 g for the control group, whose subjects had the least amount of fat. The inclusion of Milk thistle seeds in the ration and/or drinking water did not have a positive impact on abdominal fat. A research study carried out by Rashidi *et al.* (2015), shows that the incorporation rates 0.5%, 1%, 1.5%, 2% of Milk thistle seeds in broiler feed do not have a significant effect on abdominal fat weight. Similarly, a significant reduction in abdominal fat was recorded in broilers fed a diet

containing 250 mg/kg Milk thistle extract, Tavakolinasab *et al.* (2020).

**Biochemical parameters**

The results regarding the effects of incorporating Milk thistle seeds into broiler feed and drinking water on certain biochemical parameters are summarized in Table 4.

Incorporation of Milk thistle seeds into the feed and/or drinking water had no significant effect (P>0.05) on AST, ALT and glucose. Regarding AST, group ″2″ had the highest average (390 IU/L) compared with (366.80 IU/L) for the Control group but this increase was not significant.

**Table 3:** Results of incorporating Milk thistle seeds into feed and drinking water on broiler production performance.

| | Group ″C″ | Group ″2″ | Group ″3″ | Group ″4″ | ANOVA test | Welch test |
|---|---|---|---|---|---|---|
| Full carcass weight (g) | 2221.10±182.077 | 2239.00±265.39 | 2050.00 ±194.82[a] | 2327.00±165.60[a] | P<0.05 | |
| | Max | Max | Max | Max | | |
| | 2561 | 2760 | 2440 | 2620 | | |
| | Min | Min | Min | Min | | |
| | 2000 | 1980 | 1830 | 2050 | | |
| Empty carcass weight (g) | 1881.60±162.28 | 1889.00±236.27 | 1728.90±141.45[b] | 1968.30±167.80[b] | P<0.05 | |
| | Max | Max | Max | Max | | |
| | 2185 | 235 | 1969 | 2257 | | |
| | Min | Min | Min | Min | | |
| | 1712 | 1622 | 1569 | 1740 | | |
| Carcass yield (%) | 73.11±4.30 | 69.64±1.06 | 69.39±1.40 | 70.32±5.60 | | P>0.05 |
| | Max | Max | Max | Max | | |
| | 80.11 | 71.55 | 70.84 | 84.32 | | |
| | Min | Min | Min | Min | | |
| | 67.99 | 68.55 | 66.71 | 62.64 | | |
| Abdominal mass weight (g) | 34.40±14.5 | 37.40±10.8 | 35.30±10.49 | 36.40±12.5 | P>0.05 | |
| | Max | Max | Max | Max | | |
| | 68 | 55 | 52 | 63 | | |
| | Min | Min | Min | Min | | |
| | 17 | 21 | 20 | 22 | | |
| Liver weight (g) | 49.70±7.13 | 51.90±7.04 | 43.00± 8.75[c] | 54.70±7.42[c] | P<0.05 | |
| | Max | Max | Max | Max | | |
| | 60 | 67 | 59 | 66 | | |
| | Min | Min | Min | Min | | |
| | 40 | 44 | 30 | 45 | | |

Values on the same line with the same superscript letters are significantly different (P<0.05).

P>0.05= Difference not significant. Max= Maximum values, Min= Minimum values.

**Table 4:** Effects of incorporating Milk thistle seeds into broiler feed and drinking water on certain biochemical parameters.

| | Group ″C″ | Group ″2″ | Group ″3″ | Group ″4″ | Test ANOVA | Kruskal-wallis test |
|---|---|---|---|---|---|---|
| AST(IU/L) | 366.80±69.15 | 390.00±113.08 | 302.60±62.61 | 332.60±105.68 | P>0.05 | |
| | Max | Max | Max | Max | | |
| | 455 | 637 | 441 | 560 | | |
| | Min | Min | Min | Min | | |
| | 255 | 280 | 210 | 201 | | |
| ALT(IU/L) | 3±0 | 3.50±1.58 | 3±0 | 4.10±2.3 | | P>0.05 |
| | Max | Max | Max | Max | | |
| | 3 | 8 | 3 | 9 | | |
| | Min | Min | Min | Min | | |
| | 3 | 3 | 3 | 3 | | |
| Glucose (g/l) | 2.05± 0.20 | 2.00±0.21 | 1.93±0.23 | 1.99±0.24 | P>0.05 | |
| | Max | Max | Max | Max | | |
| | 2.37 | 2.35 | 2.36 | 2.37 | | |
| | Min | Min | Min | Min | | |
| | 1.77 | 1.57 | 1.61 | 1.56 | | |

P>0.05= Difference not significant. Max= Maximum values, Min= Minimum values.

For ALT, the highest value was recorded in group ″4″, where animals received Milk thistle in their feed ration and drinking water, *i.e*. 4.10 IU/L, while group ″C″ recorded the lowest value, 3 IU/L.

The results show that the highest blood glucose level was obtained in the control group (2.05 g/l), while the lowest glucose level was reported in group ″3″ (1.93 g/l).

The results obtained by Tavakolinasab *et al*. (2020), show that adding Milk thistle extract at 250 mg/kg to broiler feed significantly reduced (P<0.05) glucose (164.40 mg/dl), AST (4.53 IU/L) and ALT (212.67 IU/L) levels, respectively, compared to the control group, which recorded 191.23 mg/ml glucose, 300.33 IU/L ALT and 8 IU/L AST.

## CONCLUSION

The results indicate that the inclusion of Milk thistle (*Silybum marianum*) seeds in the diet and drinking water of Cobb 500 broilers had a significant effect (P<0.05) on certain zootechnical and production parameters. The incorporation of whole Milk thistle seeds in the feed (1.5%) and drinking water (1.5%) of chickens had a positive impact on live weight at slaughter, ″AWG″, full and empty carcass weight and liver weight. The results also show that incorporating ground Milk thistle seeds at a rate of 3% into the chickens' feed significantly (P<0.05) reduced ″CI″. The results of this experiment show that the Milk thistle seeds used did not have a significant effect on the biochemical profile. However, nutritional status and liver function were maintained.

Milk thistle seeds may be recommended as a natural feed additive for poultry and livestock farmers, replacing synthetic feed additives and minimizing their harmful effects on animal and human health.

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### Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

### Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

### Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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