Research Notes P-99

Arm & Hammer Animal and Food Production

CELMANAX augmented *Salmonella* vaccine response in broilers challenged with *S. Heidelberg* or *S. Infantis*.

One of the more common methods to control *Salmonella* in commercial poultry is to vaccinate the flock using live or attenuated strains of *Salmonella*. However, a vaccine's efficacy against different serogroups is variable. CELMANAX™ has been shown to be efficacious at reducing *Salmonella* colonization in many published studies[1-7]. Thus, the objective of these two studies was to evaluate the effectiveness of CELMANAX with a live *Salmonella* vaccine to control *S. Heidelberg* (serogroup B) or *S. Infantis* (serogroup C1) challenge in broilers.

STUDY OVERVIEW

- Two broiler studies[8,9] evaluated the effect of live *Salmonella* vaccine, AviPro® Megan® Vac 1, alone or combined with CELMANAX SCP.
- Study 1 was a preliminary study conducted in two isolation rooms, each divided into three pens, with 35 birds/pen. Each of the three treatments (Table 1) were represented in both rooms.
- Study 2 was a pen trial with ten replicates per treatment and 50 birds/pen.
- Upon arrival, day-of-hatch Ross male broiler chicks received routine vaccinations, waited 30 minutes, and then were given Megan Vac 1 as a coarse spray, 0.25 ml/bird for treatments 2 and 3. Two hours after *Salmonella* vaccination, birds were placed on feed.

TABLE 1 Study design.

Treatment		Study 1: *S. Heidelberg* challenge	Study 2: *S. Infantis* challenge
1	Challenge control (unvaccinated)	Yes	Yes
2	Megan Vac 1 alone	Yes	Yes
3	Megan Vac 1 and CELMANAX SCP at 100 g/MT	Yes	Yes

- Broilers were fed standard non-medicated commercial starter and grower diets (control diet) for treatments 1 and 2, and control diet supplemented with CELMANAX SCP at a rate of 100 g/MT for treatment 3.
- All data were analyzed statistically.

Study 1[8]

- This was a preliminary study designed to evaluate the effect of vaccine alone or vaccine plus CELMANAX on protecting the broilers from a *Salmonella Heidelberg* challenge.
- At 2 days of age all birds were orally dosed (gavaged) with a $5x10^7$ colony forming units (CFU) nalidixic acid-resistant *Salmonella Heidelberg*.
- At 42 days of age, fifteen (15) birds per replicate (30/treatment) were taken from each individual pen, euthanized, and the ceca aseptically removed and cultured for prevalence and most probable number (MPN) for the challenge strain.

Study 2[9]

- This study was designed to evaluate the effect of vaccine alone or vaccine plus CELMANAX™ on protecting the broilers from a *Salmonella Infantis* challenge.
- At 5 days post vaccination, *Salmonella* was cultured from liver/spleen and ceca from one (1) bird/pen to measure vaccine uptake.
- At 5 days of age, twenty-five (25) seeder chicks per pen were tagged, color-coded and were orally dosed (gavaged) with a $2.3x10^7$ CFU *Salmonella Infantis* (direct challenge).
- At the end of the trial, *S. Infantis* was isolated and enumerated from ceca of five direct and five horizontally (indirect challenge) challenged birds/pen.

RESULTS

Study 1

Ceca *S. Heidelberg* Prevalence (42 days of age):

- The prevalence in the Megan Vac + CELMANAX SCP group was significantly lower than that of both the Megan Vac only group and the unvaccinated group, while the Megan Vac only group and the unvaccinated group were not significantly different from one another (Table 2).

TABLE 2 ***S. Heidelberg* prevalence (%) in ceca samples by treatment group. Ceca were collected from 15 birds in each of 2 pens per group on day 42.**

Treatment	No. samples	No. positive (%)	*P*
Challenge control (unvaccinated)	30	24 (80.0)[b]	<0.001
Megan Vac1 alone	30	23 (76.7)[b]	
Megan Vac1 and CELMANAX SCP at 100 g/MT	30	19 (63.3)[a]	

Percentages with a superscript in common do not differ with a level of significance of 5% over all comparisons.

Ceca *S. Heidelberg* MPNs– Taking the Culture-Negative Samples into Account (42 days of age):

- The estimated mean $\log_{10}$ MPN/g of the Megan Vac + CELMANAX SCP group was significantly lower than that of the unvaccinated group, while the mean of the Megan Vac alone group was intermediate and did not significantly differ from the other groups (Table 3).

TABLE 3 **Estimated mean (SE) *Salmonella* $\log_{10}$ MPN/g in ceca samples by treatment based on a Tobit censored regression model.**

Treatment	Samples, n	Mean (SE) $\log_{10}$ MPN/g	Mean MPN/g
Challenge control (unvaccinated)	30	0.18[b] (0.17)	1.51
Megan Vac 1 alone	30	-0.02[ab] (0.17)	0.95
Megan Vac 1 and CELMANAX SCP at 100 g/MT	30	-0.52[a] (0.18)	0.30

[a,b] Means with a superscript in common do not differ with a level of significance of 5% over all comparisons.

A Tobit regression model was used to estimate the true mean MPN/g based on the distribution of MPNs in the culture-positive samples as well as the proportions of culture-negative samples in the different treatment groups.

SE = Standard Error

Study 2

Vaccine recovery (5 days of age)

- *Salmonella* vaccine was not recovered from the ceca of any birds, or from the liver/spleen of control (unvaccinated) treatment group. *Salmonella* vaccine was recovered from the liver/spleen of Megan Vac 1 alone and CELMANAX + Megan Vac 1 group with no statistical difference between the two treatments.

Ceca *S. Infantis* Prevalence (44 days of age):

- The prevalence of *S. Infantis* in the Megan Vac + CELMANAX™ SCP group was significantly lower than the challenge control, with Megan Vac alone group being intermediate (Table 4).

TABLE 4 **Prevalence of *S. Infantis* in ceca collected from 5 indirect challenged birds and 5 direct challenged birds in each of 10 pens per treatment group.**

Treatment	Challenge status		
	Indirect	Direct	Total
Challenge control (unvaccinated)	38/50 (76)	37/50 (74)	75/100 (75)[b]
Megan Vac 1 alone	27/50 (54)	23/50 (46)	50/100 (50)[ab]
Megan Vac 1 and CELMANAX SCP at 100 g/MT	18/50 (36)	24/50 (48)	42/100 (42)[a]
Total	**83/150 (55.3)[a]**	**84/150 (56)[a]**	**167/300 (56)**

Marginal percentages with a superscript in common do not differ with a level of significance of 5% over all comparisons.

Ceca *S. Infantis* MPNs– Taking the Culture-Negative Samples into Account (44 days of age):

- The estimated mean $\log_{10}$ MPN/g of the Megan Vac + CELMANAX SCP group was significantly lower than that of the challenge control, while the mean of the Megan Vac alone group was intermediate and did not significantly differ from the other groups (Table 5).

TABLE 5 **Estimated mean (SE) $\log_{10}$ *Salmonella Infantis* (MPN/g) based on a Tobit regression model with culture-negative ceca samples censored at a lower limit of -0.5 $\log_{10}$ MPN/g.**

Treatment	Challenge status			
	Indirect	Direct	Total	MPN/g
Challenge control (unvaccinated)	0.27 (0.31)	0.10 (0.31)	0.18[b] (0.28)	1.51
Megan Vac 1 alone	-0.51 (0.32)	-0.90 (0.33)	-0.71[ab] (0.29)	0.20
Megan Vac 1 and CELMANAX SCP at 100 g/MT	-0.94 (0.32)	-0.85 (0.32)	-0.89[a] (0.30)	0.13
Total	**-0.39[a] (0.19)**	**-0.55[a] (0.19)**	**-0.47 (0.17)**	**0.34**

Samples were collected from 5 indirect challenged birds and 5 direct challenged birds in each of 10 pens per treatment group.
A Tobit regression model was used to estimate the true mean MPN/g based on the distribution of MPNs in the culture-positive samples as well as the proportions of culture-negative samples in the different treatment groups.
Marginal means with a superscript in common do not differ with a level of significance of 5% over all comparisons.

CONCLUSION

- CELMANAX did not negatively impact live *Salmonella* vaccine Megan Vac 1 uptake in broilers.
- The combination of Megan Vac 1 + CELMANAX tended to be more effective in reducing the prevalence and load of *S. Heidelberg* or *S. Infantis* in broiler ceca.
- There appears to be a synergistic or additive effect with these two combined interventions to reduce cecal *Salmonella* belonging to different serogroups.

To learn more about CELMANAX contact your nutritionist, veterinarian or ARM & HAMMER™ representative or visit AHfoodchain.com.

1 Poultry Science 2017; 96:2684-2690
2 Poultry Science, 2018; 97:1412–1419
3 Presented as an abstract at PSA, 2019 #204
4 Poultry Science, 2013; 92 :655–662,
5 International Poultry Scientific Forum 2020, P289,
6 Journal of Applied Poultry Research 2018; 27:453–460
7 International Poultry Scientific Forum, 2020; P304
8 *Adapted from a study done at a private Poultry Research Center.*
9 *Adapted from a study done at a private Poultry Research Center.*