ANIMAL HEALTH AND WELL BEING

Supplementation of a water-soluble zinc–AA complex to reduce stress in broilers, pullets, and layers

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¹Funding for this project was provided by Zinpro Corporation.

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Abstract

This study consisted of three 5-wk experiments to test the effects of administering Zinpro-LQ (Zinpro-LQ, Zinpro Corporation, Eden Prairie, MN) for different time intervals in stressed broilers, pullets, and layers. Treatments included: nonstressed control (NC), stressed control (SC), stressed and supplemented Zinpro-LQ for 14 d prior to stressor (S1), 10 d prior (S2), 7 d prior to and 7 d during stressor (S3), and 5 d prior (S4). Birds included 1-d-old Cobb 500 male broilers (Experiment 1), 7-wk-old white Leghorn pullets (Experiment 2), and 50-wk-old white Leghorn layers (Experiment 3). All stressed birds were vaccinated against Newcastle Disease Virus (NDV) on day 28 and exposed to random feed and water withdrawal days 28 to 35 for 6 h/d. Pullets were beak trimmed on day 21, and layers were heat stressed days 28 to 35. Blood was sampled on day 35 to determine plasma chemistry, corticosterone (CORT), anti-NDV antibody titer, and heterophil/lymphocyte (H/L) ratio. Mortality, feed and water consumption, and BW were also recorded. In Experiment 1, CORT and H/L ratio were highest in SC ($P < 0.05$). In Experiment 2, CORT was higher in SC than S4 ($P < 0.05$) while all other treatments were statistically intermediate. NDV titer was lower in SC than S4 while all other treatments were statistically intermediate. Treatment differences were also observed for H/L ratio, and plasma uric acid and cholesterol in Experiment 2. In Experiment 3, treatment differences were observed for H/L ratio, plasma glucose, sodium, and chloride. H/L ratio was lower in NC, S1, and S4 compared with SC ($P < 0.05$); and NC and S1 were lower than S2 ($P < 0.05$). In conclusion, dietary inclusion of zinc AA complex for any length of time prior to a stressor helped reduce measures of stress in broilers. The S1 and S4 treatments helped reduce stress and improve humoral immune response in pullets and layers.

Key words: broiler, layer, pullet, stress, zinc–AA complex

Introduction

Zinc is an essential mineral involved in a wide array of physiological functions, including DNA and RNA synthesis and structure, humoral and cellular immune response, antioxidative function, cell membrane and connective tissue integrity, and enzyme and hormone function (reviewed in [Chien et al., 2006](#)).

In relation to stress mitigation, studies in mammals have shown that zinc modulates excitability of the brain through calcium channels, glutamate, GABA, dopamine, and serotonin receptors ([Piao et al., 2017](#)). Zinc is also a component of thymulin, a hormone which promotes T-cell function and differentiation ([Prasad et al., 1988](#)). Zinc deficiency can result in dysfunctional growth hormone production and IGF-1 signaling ([MacDonald,](#)

2000) as well as reduced IL-2 production, resulting in impaired natural killer cell and helper T-cell activity (Prasad, 2000).

Traditionally, inorganic sources of zinc (i.e., zinc oxide and zinc sulfate) have been added to the diet to meet nutritional recommendations (Naz et al., 2016). However, it has been hypothesized that zinc-AA complex is more nutritionally available and thus more efficient than zinc sulfate in maintaining physiological processes (Wedekind et al., 1992). Zinc distribution between the liver, lymphoid organs, and circulation is altered by exposure to *Eimeria* spp. (Richards and Augustine, 1988), *Escherichia coli* and stress (Tufft et al., 1988; Tufft and Nockels, 1991). However, zinc-AA complex has been shown to improve serum zinc concentrations despite exposure to *E. coli* (Kidd et al., 1994a) and to increase macrophage function in turkeys during a *Salmonella* challenge (Kidd et al., 1994b) and antibody titer in growing broilers after vaccination against NDV and Infectious Bronchitis (IB) (Moghaddam and Jahanian, 2009). Hamidi and Pourreza (2009) showed that excess zinc methionine supplementation improved humoral response in growing broilers challenged with coccidiosis. In layers, Kienholz et al. (1992) found that zinc methionine improved egg production and helped maintain egg weight when administered to hens during a low-calcium stress. Moreng et al. (1992) showed that the negative effects of drinking saline water on eggshell breaking strength, shell weight, and percent of shell defects were ameliorated with zinc methionine supplementation. Finally, zinc bacitracin (100 mg/kg) has shown potential for increasing heat tolerance in layers (Männer and Wang, 1991).

Several studies have already evaluated the effects of zinc supplementation in broilers (Downs et al., 2000; Jahanian et al., 2008; Moghaddam and Jahanian, 2009), but not in pullets or layers. Only one study has evaluated dietary inclusion of zinc AA complex during heat stress in broilers (De Grande et al., 2019). Overall, there are few studies which demonstrate the effects of zinc-AA complex on health and stress susceptibility in poultry, or the efficacy of administering zinc-AA complex in the drinking water. The purpose of this study was to evaluate supplementation of zinc AA complex (Zinpro-LQ, Zinpro Corporation, Eden Prairie, MN) in the drinking water for various lengths of time before and during a stress event on indicators of stress and immune function in broilers, pullets, and layers.

Materials and Methods

General Experimental Design

All animals were cared for in accordance with the requirements outlined by The Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010) and study protocols were approved by the Texas A&M University Animal Care and Use Committee (AUP2017-0174). All experiments followed a randomized complete block design (RCBD), with similar protocol with regard to stressors and data collection. Some exceptions to rearing procedures are explained in the following sections. Feed and water were refilled as needed and provided ad libitum to stressed treatments days 0 to -27 and were removed for 6 h/d with a random start time days 28 to 35. Nonstressed control birds retained ad libitum access to feed and water days 0 to 35. Zinpro-LQ (Zinpro Corporation, Eden Prairie, MN) was added to the water from a stock solution according to the manufacturer's recommendations (200 g/7.57 L stock solution), and was supplemented to S1 days 14 to 27, to S2 days 17 to 27, to S3 days 23 to 27, and to S4 days 21 to 35. In Experiment 1, only stressed treatments were vaccinated against Newcastle Disease Virus (NDV) on day 28 (COMBOVAC-30,

Merck), whereas in Experiments 2 and 3 all treatments were exposed to the vaccination stress. Waterers were removed overnight, and the vaccine was mixed into the drinking water and added back to stressed pens.

Blood samples were collected from 40 randomly selected birds per treatment on the morning of day 35. Treatments were sampled at random according to the order of the RCBD in each block of cages or pens in order to minimize sampling bias. Blood samples were used to assess plasma corticosterone (CORT) and heterophil/lymphocyte ratio (H/L ratio). Two to three milliliters of blood were collected from the brachial vein: one drop was used to make a blood smear, and the rest was placed in a lithium heparin vacutainer with plasma separation gel (BD368056, BD, Franklin Lakes, NJ). Vacutainers were stored in ice during collection and then centrifuged at 4000 RPM for 15 min (Beckman GS-6R, Beckman Coulter, Brea, CA). Plasma was then stored in microcentrifuge tubes at -19 °C until analysis. Approximately 0.5 mL of each plasma sample was sent to Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL) on day 35 for plasma chemistry analysis.

Commercially available ELISA kits were used to measure plasma CORT (ADI-901-097, Enzo Life Sciences, Farmingdale, NY) and plasma anti-NDV antibody titers (ProFlock 96-6547, Zoetis, San Diego, CA). Absorbance was read at 450 nm using a microplate absorbance reader (Tecan Sunrise, Tecan Trading AG, Männedorf, Switzerland) and analyzed using the Magellan Tracker software program. Dry blood smear slides were stained using a commercial hematology stain kit (25034, PolySciences, Inc., Warrington, PA) and analyzed under light microscopy at 40× magnification (Omax DCE-2, Kent, WA) using oil immersion. An area of the slide where cells appeared in a single layer was analyzed and individual heterophils and lymphocytes were counted using a key stroke counter until a total of 100 cells per slide was reached.

Experiment 1: Broilers

Day-of-hatch male Cobb 500 broilers ($n = 360$) were randomly assigned to each treatment and grown to 35 d of age according to Cobb management guidelines. There were 12 pens with 30 birds per pen; thus, there were 60 birds in each treatment. Pens measured 1.83×1.83 m and were lined with 3 to 5 cm of pine shavings. Each pen was supplied with one tube feeder and one 18.93 L drinker bucket with nipples on the bottom. Birds received standard broiler diets in the form of a crumbled starter (days 0 to 13), pelleted grower (days 14 to 27), and pelleted finisher (days 28 to 35). Photoperiod consisted of 24 h of light for the first 3 d, and 20 h of light followed by 4 h of darkness for the remainder of the experiment. Room temperature was maintained at 31 °C for the first week, 29 °C for the second week, and reduced 2.5 °C each week until ambient temperature (22 to 24 °C) was reached. Birds were weighed on a per-pen basis (UFM-F120, UWE Scales, Cape Town, South Africa) on day 0 and on the morning of day 35 to measure BW gain. On day 35, birds were weighed in the morning, ~12 h after the end of the previous random feed withdrawal period. Feed was weighed as it was added to each pen and residual feed was weighed on diet transition days in order to measure feed intake. Feed conversion ratio (FCR) was calculated by dividing total feed intake by total BW gain on a per-pen basis and correcting for mortality. Water consumption for each pen was also measured.

Experiment 2: Pullets

Seven-week-old white Leghorn W-36 pullets ($n = 120$) were randomly assigned to each treatment and housed in wire cages providing 516.1 cm² per bird (40 cages with three birds per

cage) until 11 wk of age. Birds received a standard mash pullet diet and each cage had access to an 18.93 L water bucket with nipples on the bottom. The photoperiod consisted of 12 h of light followed by 12 h of darkness, and room temperature was maintained between 22 and 24 °C. Birds were vaccinated and stressed as previously described. Additionally, pullets were beak trimmed on day 21.

Experiment 3: Layers

Healthy-looking 50-wk-old white Leghorn W-36 layers ($n = 120$) were randomly assigned to each treatment and housed in wire cages providing 516.1 cm² per bird (40 cages with three birds per cage) until 55 wk of age. Birds received a standard mash layer diet and each cage had access to an 18.93 L water bucket with nipples on the bottom. Photoperiod consisted of 16 h of light followed by 8 h of darkness. Room temperature was maintained between 22 and 24 °C on days 0 to 27 of the experiment. Hens were then exposed to heat stress at 32 to 33 °C for 12 h/d on days 28 to 35. Birds were vaccinated and otherwise stressed as previously described.

Statistical Analysis

All data were analyzed using one-way ANOVA in the General Linear Model procedure in Minitab 17.1.0, with treatment as the main effect. Mean separation was performed using the LSD post hoc procedure. A significant difference was defined as $P \leq 0.05$.

Results

Experiment 1: Broilers

Data for BW, FCR, and water consumption are presented in Table 1. No treatment differences were observed for day 42 BW ($P > 0.05$) or water consumption ($P > 0.05$). Differences were observed in FCR, which was lowest in NC ($P < 0.05$) compared with all other treatments. Data for stress measures and plasma chemistry are shown in Table 2. Plasma CORT was highest in SC ($P < 0.05$) compared with all other treatments, whereas all supplemented treatments did not differ from NC ($P > 0.05$). H/L ratio was highest in SC ($P < 0.05$). Plasma glucose in S3 was not significantly different ($P > 0.05$) from S1 or from S3 and S4, but S1 was higher than S2 and S4 ($P < 0.05$). Meanwhile glucose was lower in NC and SC than all supplemented treatments ($P < 0.05$). Cholesterol was higher in S1 than SC, S2, and S3 ($P < 0.05$), whereas NC and S4 were statistically intermediate. Sodium in S4 was lower than NC, S1, S2, and S3 ($P < 0.05$), and NC was higher than SC ($P < 0.05$). Plasma sodium did not differ between SC and S4, nor were there differences between NC, S1, S2, and S3. Plasma chloride was lower in SC than NC, S1, S2, and S3 ($P < 0.05$), but SC did not differ from S4. Plasma chloride in NC was higher than S4 ($P < 0.05$), but NC did not differ from S1, S2, or S3 ($P > 0.05$). There were no differences in plasma chloride between all supplemented treatments ($P > 0.05$). No other treatment differences in plasma chemistry values were observed.

Experiment 2: Pullets

Stress measures and plasma chemistry values for pullets are presented in Table 3. Treatment differences were observed for all stress measures. Plasma CORT in SC compared with S4 ($P < 0.05$), while all other treatments were statistically intermediate. H/L ratio was higher in SC compared with NC, S1, and S4 ($P < 0.05$), while S2 and S3 were statistically intermediate. Anti-NDV antibody titer was lower in SC than S4 ($P < 0.05$), while all other treatments were statistically intermediate. Plasma levels of protein differed by treatment: total protein was lowest in SC ($P < 0.05$) and highest in S1 ($P < 0.05$). Plasma protein in S1 did not differ from S2 ($P > 0.05$), and NC did not differ from S2, S3, and S4. Uric acid was higher in SC compared with all other treatments ($P < 0.05$) and was lower in S4 compared with NC, SC, S1, and S2 ($P < 0.05$). Plasma cholesterol was lower in NC compared with S1 and S4 ($P < 0.05$), but NC was not different from SC, S2, and S3 ($P > 0.05$). Plasma cholesterol in SC was lower than S2, but SC was not different from NC, S2, S3, and S4 ($P > 0.05$). No other treatment differences in plasma chemistry values were observed.

Experiment 3: Layers

Stress measures and plasma chemistry values for layers are presented in Table 4. H/L ratio was higher in SC and S3 than NC, S1, and S4 ($P < 0.05$), but SC was not different from S2 and S3 ($P > 0.05$). H/L ratio in NC did not differ from S1 or S4 ($P > 0.05$) and S2 did not differ from S4 ($P > 0.05$). No treatment differences were observed for plasma CORT or NDV titer. Plasma glucose was higher in SC compared with all other treatments ($P < 0.05$). Glucose in NC was lower than SC, S1, and S3 ($P < 0.05$), but did not differ from S2 and S4 ($P > 0.05$). Plasma glucose did not differ among all supplemented treatments ($P > 0.05$). Plasma sodium was higher in NC compared with S1, S2, and S3 ($P < 0.05$), and was lower in S2 compared with NC, SC, and S4 ($P < 0.05$). However, sodium did not differ between NC, SC, and S4 ($P > 0.05$), or between S1, S3, and S4 ($P > 0.05$). Plasma chloride was higher in SC compared with S1 and S2 ($P < 0.05$) but did not differ from NC, S3, and S4 ($P > 0.05$). Plasma chloride did not differ among all supplemented treatments ($P > 0.05$). No other treatment differences in plasma chemistry values were observed.

Discussion

BW gain was not affected by treatment in Experiment 1. Similarly, FCR was only improved in NC compared with SC and supplemented treatments in Experiment 1. This disagrees with previous research showing that supplemental zinc improved FCR and ADG (Saleh et al., 2018) in heat stressed broilers. Although Al-Masad (2012) also found no differences in FCR in heat-stressed broilers given supplemental zinc, an increase in antibody titer was observed. NDV titer did not differ in broilers or layers in this study. However, treatment differences were observed in pullets: NDV titer was lower in SC compared

Table 1. Average individual day 35 BW, feed conversion ratio (days 0 to 35) corrected for mortality, and water consumption (days 0 to 35) in broilers in Experiment 1

	NC	SC	S1	S2	S3	S4	SEM
Average bird weight (kg)	2.37	2.34	2.32	2.24	2.30	2.34	0.02
FCR	1.41 ^b	1.49 ^a	1.47 ^a	1.47 ^a	1.49 ^a	1.46 ^a	0.01
Water consumed/pen (kg)	205.9	208.4	208.3	214.3	203.7	203.4	4.72

^{a,b}Values within a row with different superscripts indicate a significant difference at $P < 0.05$.

Table 2. Stress measures and plasma chemistry values (day 35) for broilers in Experiment 1

	Units	NC	SC	S1	S2	S3	S4	SEM
CORT	pg/mL	108.8 ^b	222.5 ^a	91.7 ^b	92.1 ^b	81.8 ^b	91.2 ^b	37.5
H/L ratio		0.33 ^b	0.51 ^a	0.40 ^b	0.38 ^b	0.36 ^b	0.40 ^b	0.03
NDV titer			6.2	5.1	6.0	5.5	5.5	1.3
Protein	g/dL	3.3	3.5	3.4	3.4	3.4	3.5	0.1
Calcium	mg/dL	11.3	11.8	11.6	11.4	11.6	11.7	0.2
Phosphorus	mg/dL	6.5	6.6	6.5	7.0	7.1	6.6	0.2
Glucose	mg/dL	234.8 ^c	239.9 ^c	264.7 ^a	253.5 ^b	255.7 ^{ab}	252.1 ^b	3.9
ALP	U/L	2772	3006	3619	3004	2656	2790	256
CK	U/L	9135	9904	8589	9487	9385	9424	330
AST/GOT	U/L	277.4	351.4	293.2	312.5	337.3	362.4	22.2
Uric acid	mg/dL	6.0	5.5	6.6	7.2	5.7	6.6	0.5
Cholesterol	mg/dL	129.1 ^{ab}	120.4 ^b	135.5 ^a	119.9 ^b	123.6 ^b	125.0 ^{ab}	4.0
Sodium	mEq/L	157.6 ^b	153.0 ^{ac}	156.5 ^{ab}	156.6 ^{ab}	157.4 ^b	152.5 ^c	1.3
Potassium	mEq/L	5.6	5.3	6.0	6.1	5.9	5.7	0.2
Chloride	mEq/L	113.9 ^b	109.2 ^c	112.3 ^{ab}	113.0 ^{ab}	112.9 ^{ab}	110.9 ^{ac}	0.9
Na/K ratio		28.6	29.5	26.4	27.1	27.3	27.5	0.9

^{a,b,c}Values within a row with different superscripts indicate a significant difference at $P < 0.05$.

Table 3. Stress measures and plasma chemistry values (day 35) for 11-wk-old pullets in Experiment 2.

	Units	NC	SC	S1	S2	S3	S4	SEM
CORT	pg/mL	2976 ^{ab}	4179 ^a	2973 ^{ab}	3376 ^{ab}	3280 ^{ab}	2100 ^b	261
H/L ratio		0.12 ^b	0.19 ^a	0.13 ^b	0.14 ^{ab}	0.15 ^{ab}	0.10 ^b	0.01
NDV titer		11126 ^{ab}	6938 ^b	9456 ^{ab}	8668 ^{ab}	8376 ^{ab}	12011 ^a	651
Protein	g/dL	4.4 ^b	4.0 ^c	5.0 ^a	4.7 ^{ab}	4.6 ^b	4.6 ^b	0.1
Calcium	mg/dL	13.7	14.4	12.9	14.2	13.9	13.5	0.2
Phosphorus	mg/dL	4.4	4.2	5.0	4.5	4.5	4.6	0.1
Glucose	mg/dL	239.1	223.9	240.5	242.4	233.3	232.3	3.8
ALP	U/L	1786.8	1708.5	1757.8	1491.7	1269.3	1473.0	92.2
AST/GOT	U/L	209.0	218.2	220.2	233.5	198.6	216.6	6.7
Uric acid	mg/dL	4.0 ^b	7.0 ^a	4.1 ^b	4.4 ^b	3.6 ^{bc}	3.0 ^c	0.2
Cholesterol	mg/dL	102.4 ^c	109.8 ^{bc}	131.1 ^a	108.9 ^{bc}	112.2 ^{bc}	117.5 ^b	2.2
Sodium	mEq/L	149.4	147.8	150.1	152.1	151.0	151.6	0.7
Potassium	mEq/L	7.8	7.7	7.4	7.6	7.5	7.6	0.1
Chloride	mEq/L	115.6	113.2	115.9	175.0	116.8	174.8	13.9
Na/K ratio		19.4	19.5	20.4	19.7	20.3	19.7	0.2

^{a,b,c}Values within a row with different superscripts indicate a significant difference at $P < 0.05$.

with S4, although all treatments were statistically intermediate. This is surprising, since S4 involved the shortest duration (5 d) of supplementation leading up to the stressor. That SC did not differ from any other treatment in antibody titer also differs from [Bartlett and Smith \(2003\)](#), who found that supplemental dietary zinc increased several measures of immune function in broilers, including primary and secondary antibody titer and macrophage number. However, these results were obtained using 181 mg/kg dietary zinc ([Bartlett and Smith, 2003](#)), which may not be comparable to levels present in Zinpro-LQ. Although complexing zinc with AA is believed to improve its absorption in the small intestine ([Burrell et al., 2004](#)), it is possible that the levels of zinc-AA complex introduced in this study were not high enough to meet the requirements during the stress response. Thus, with the exception of increased antibody titer in S4 compared with SC in Experiment 2, adding zinc-AA complex to the drinking water for any duration prior to a stressor may have been insufficient to counteract the negative effects of stress on feed efficiency ([Siegel, 1995](#)) and humoral immune function ([Shini et al., 2010](#)).

Plasma corticosterone and H/L ratio are commonly used to measure stress in poultry, and have been shown to increase in

response to feed deprivation and heat stress ([Shini et al., 2008](#)). Although plasma CORT did not differ among treatments in Experiment 3, birds in the SC treatment had higher plasma CORT and H/L ratio compared with all other treatments in Experiment 1. Thus, adding zinc-AA complex to the drinking water for any duration resulted in stress measures similar to birds in the NC treatment in broilers. In Experiment 2, plasma CORT was lower in S4 compared with SC but no other treatment differences were observed. However, H/L ratio in SC was similar to S2 and S3, and was lower in NC, S1, and S4 compared with SC. Therefore, pullets responded to supplementation differently than broilers, and S4 was the only treatment to reduce both measures of stress compared with SC in pullets.

Mixed effects were observed for plasma glucose, which was higher in all supplemented treatments compared with NC and SC in Experiment 1. There were no treatment differences in plasma glucose in Experiment 2. In Experiment 3, glucose was higher in SC compared with all other treatments, and supplemented treatments did not differ from each other. Corticosteroids are known to alter metabolic processes toward catabolism and increase plasma levels of glucose ([Zhao et al., 2009](#)). This was observed in layers in Experiment 3, but not in broilers in Experiment 1. This is surprising

Table 4. Stress measures and plasma chemistry values (day 35) for 55-wk-old layers in Experiment 3

	Units	NC	SC	S1	S2	S3	S4	SEM
CORT	pg/mL	18789	21044	19398	17266	20395	16984	1259
H/L ratio		0.21 ^c	0.39 ^a	0.18 ^c	0.34 ^{ab}	0.37 ^a	0.22 ^{bc}	0.02
NDV titer		11697	11667	11548	11686	15120	13106	577
Protein	g/dL	5.6	5.2	5.3	5.2	5.3	5.6	0.1
Calcium	mg/dL	39.1	23.6	25.6	26.2	25.4	27.1	2.4
Phosphorus	mg/dL	6.4	5.7	6.1	6.1	6.2	6.6	0.1
Glucose	mg/dL	54.7 ^c	119.8 ^a	86.8 ^b	82.1 ^{bc}	87.7 ^b	71.1 ^{bc}	4.9
ALP	U/L	701.5	870.6	730.9	761.9	943.0	854.5	29.4
CK	U/L	990.3	1452.5	1426.9	1120.4	1297.6	1179.0	58.2
AST/GOT	U/L	185.7	194.6	187.9	168.9	184.7	184.0	2.9
GLDH	U/L	4.1	5.7	4.2	3.8	5.1	4.4	0.3
Uric acid	mg/dL	4.8	3.7	3.4	4.1	4.1	3.5	0.3
Cholesterol	mg/dL	125.3	131.2	106.4	94.5	102.5	129.2	8.5
Sodium	mEq/L	162.1 ^a	159.6 ^{ab}	155.0 ^{cd}	154.3 ^d	157.1 ^{bcd}	158.7 ^{abc}	0.7
Potassium	mEq/L	5.2	5.7	5.7	5.1	5.3	5.5	0.1
Chloride	mEq/L	122.2 ^{ab}	122.9 ^a	118.9 ^{bc}	117.7 ^c	119.2 ^{abc}	120.8 ^{abc}	0.5
Na/K ratio		32.1	29.4	28.8	31.5	30.6	30.4	0.5

^{a,b,c}Values within a row with different superscripts indicate a significant difference at $P < 0.05$.

since supplemental zinc has been shown to reduce plasma glucose (Uyanik et al., 2001), even during heat stress (Kucuk et al., 2003; Bozakova, 2010). Cholesterol did not differ among treatments in Experiment 3. However, cholesterol was higher in S1 compared with SC, S2, and S3 in Experiment 1, and higher in S1 compared with all other treatments in Experiment 2. Corticosterone increases plasma cholesterol (Puvadolpirod and Thaxton, 2000). On the other hand, dietary zinc supplementation has been shown to reduce plasma cholesterol in heat-stressed turkey breeder hens (Bozakova, 2010) and in broilers (Hosseini-Mansoub et al., 2010). However, in this study, plasma cholesterol in SC was lower than S1 in both broilers and pullets. Additionally, cholesterol levels were similar between SC and all other treatments, whether supplemented or not, in both broilers and pullets. It is unclear whether the difference in cholesterol in S1 is due to duration of zinc-AA supplementation or another factor, particularly considering that S1 involved supplementation for an intermediate duration between S2 and S4, and because cholesterol did not differ between SC and the other supplemented treatments.

Uric acid concentrations did not differ in broilers or layers. In pullets, however, uric acid was higher in SC than all other treatments, and lower in S4 compared with NC, S1, and S2. Plasma uric acid has been shown to decrease during feed withdrawal (Delezie et al., 2007). Uric acid increases in response to corticosterone (Lin et al., 2004) and indicate increased protein degradation (Swennen et al., 2006). The S4 treatment had significantly lower CORT and uric acid than SC, indicating that S4 was more effective than other treatments in reducing stress and stress-induced metabolic changes in broilers exposed to random feed withdrawal.

There were differences in plasma concentrations of some electrolytes. In layers, sodium was lower in S1 and S2 compared with SC, and chloride was lower in S1 and S2 compared with both NC and SC. Birds tend to increase excretion of electrolytes due to the action of corticosterone (Olanrewaju et al., 2006) or dehydration such as may occur during heat stress (Iheukwumere and Herbert, 2003). There were no treatment differences in CORT in layers. In pullets, CORT was affected by treatment, but plasma electrolytes were not. Some of the stressors applied to pullets (beak trimming) and layers (heat stress) were different and therefore could have elicited different magnitudes in the

stress response. In addition, because layers were heat stressed and pullets were not, differences in plasma electrolytes in layers may be due to dehydration, despite there being no treatment differences in CORT. In broilers, however, both CORT and H/L ratio were higher in SC than all other treatments. Plasma sodium in SC was lower than NC and S3, and higher than S4, whereas plasma chloride was lower in SC compared with NC, S3, and S4: this suggests that the S3 treatment was more effective in maintaining plasma electrolyte levels in stressed broilers.

In conclusion, adding zinc-AA complex to the drinking water for various durations prior to a stressor produced different effects on physiological parameters in different types of poultry. The S4 treatment reduced both plasma CORT and H/L ratio and increased NDV antibody titer in stressed pullets compared with SC. Supplementation for any duration produced CORT and H/L ratio that was similar to NC and lower than SC in stressed broilers.

Conflict of Interest Statement

Zinpro Corporation provided Zinpro-LQ used in this study and was involved in study design, editing of the manuscript and the decision to publish the results.

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