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Interaction of oxidative feed sanitizer with dietary methionine on growth and immune-responsiveness of broiler chickens

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ABSTRACT

Acidified sodium chlorite (ASC), a well known sanitizer, reduces microbial load and improves energy bioavailability from fiber/lignin rich feedstuffs. However, ASC also reacts with sulphur containing amino acid methionine, the first limiting amino acid in maize-soy based diet. So the present experiment was conducted to assess the interaction of ASC with dietary methionine in terms of growth performance, meat quality and immune-competence of broiler chicks. A basal diet was formulated without methionine supplementation (otherwise adequate in all nutrients) and then treated with 3 levels of ASC (0, 100 and 250 ppm). Each ASC treated basal diet was supplemented without or with DL-methionine to meet 100 and 120% of NRC requirement of methionine in a 3 × 3 factorial design. Day-old broiler chicks (288) were divided into 36 groups of 8 chicks each and each dietary treatment was offered to 4 replicated groups. At 6 weeks of age, methionine supplementation produced significant increase in live body weight, weight gain and feed conversion ratio. Among meat quality parameters, thiobarbituric acid reactive substance value increased significantly upon ASC treatment and reduced by methionine supplementation at 120%. Among cut-up parts, neck yield increased significantly with increased level of methionine. Increase in methionine level also produced improvement in humoral and cell mediated immunity. Thus, it may be concluded that ASC treatment deteriorated oxidative stability of meat while methionine supplementation improved broiler performance, meat quality and immunocompetence.

Key words: Acidified sodium chlorite, Broilers performance, Immune-competence, Meat quality, Methionine

Acidified sodium chlorite (ASC) produced by adding citric acid to an aqueous solution of sodium chlorite, was approved by FDA as an antimicrobial intervention treatment for poultry carcasses, poultry carcass parts, red meat carcasses, red meat parts and organs, seafood, and raw agricultural commodities owing to its strong biocidal properties. ASC also reduces microbial load in various feed ingredients and improves the energy bioavailability from fiber/lignin rich feedstuff and maize-soy mixture (Thakur *et al.* 2014) in chicken. But being oxidative in nature, ASC may interact with chemical groups in amino acids, fatty acids, which are susceptible to oxidation thus, may deteriorate quality of feed and ultimately may induce oxidative stress to the consumers. Methionine is the first limiting amino acid in commercial maize-soy based mixed feeds, and need to be supplemented to improve dietary amino acid balance and promotes greater protein build up in poultry (Bunchasak 2009). Schutte and Pack (1995) reported that the total sulphur amino acid (TSAA) requirement was higher for maximum feed efficiency and breast meat yield than for obtaining maximum weight gain. Chlorine dioxide, which has oxidizing properties similar to ASC, also reported to react with amino acids, especially those containing sulphur in their structure (Tan *et al*. 1987). Thakur and Mandal (2015) recorded a small statistically non-significant reduction in methionine digestibility from feedstuffs like soybean meal, meat-cum-bone meal and sunflower meal indicating ASC had a tendency to interact with methionine and in turn rendering it less bioavailable.

So the present experiment was conducted to assess the interaction of acidified sodium chlorite with dietary methionine for growth performance, meat quality and immune-competence of broiler chicks.

MATERIALS AND METHODS

Acidified sodium chlorite was prepared by adding citric acid in powder form, to an aqueous solution (625 g/l) of sodium chlorite (80%) to attain a pH of 3.5. This stock solution had a concentration of 500 ppm/ml and immediately before use, the stock solution of ASC was diluted in water and thoroughly mixed with feed to achieve different concentrations.

To assess interaction of ASC with dietary methionine, dietary treatments were formulated using a basal diet

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(otherwise adequate in all nutrients except methionine) treated with 3 levels of ASC (0, 100 and 250 ppm). Each diet was supplemented without (methionine deficient diet) or with DL-methionine to meet 100 and 120% of methionine requirement as suggested by NRC (1994) in a 3 × 3 factorial design. Day-old broiler chicks (288) were divided into 36 groups of 8 chicks each. Each dietary treatment was offered to 4 replicated groups of chicks during their starting (0–3 week) and finishing (4–6 week of age) growth phases.

Body weight changes and feed intake were recorded weekly, while mortality of birds as and when occurred. Humoral immunity and cell mediated immunity were determined as per Vander Zijp (1983) and Corrier and Deloach (1990), respectively. At the end of 42 days of age, 6 birds from each treatment were sacrificed. The bursa, spleen and upper 3 lobes of the thymus from the left side of the neck were dissected out, weighed and expressed as relative (mg/100 g) to live weight. The following carcass parameters, viz. deblooded weight, defeathered weight and cut-up part yields (breast, thigh, drumsticks, back, neck and wing) were recorded and expressed as percent of live weight. The meat quality in terms of pH, water holding capacity (WHC), and oxidative status (in terms of thiobarbituric acid reactive substance level, TBARS) was studied in breast muscles. TBARS, expressed as malonaldehyde is a good index reflecting the degree of oxidation (Lohakare *et al.* 2005). The responses of ASC and methionine were analyzed statistically as per Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Growth performance and feed conversion ratio: The live weight and weight gain at fourth week of age was statistically similar amongst all the treatments. However at sixth week of age, methionine levels 100 and 120% produced significant increase in live body weight (P<0.032) and weight gain (P<0.036) compared to basal methionine level (Table 2).

Methionine is the first limiting amino acid in maize-soy based poultry diets and methionine supplementation is required to meet birds' requirement for optimal growth and development. In this study, the methionine level in basal diet was only 0.35 and 0.33 %, which was far below the NRC (1994) recommended level for starter (0.5) and finisher (0.4%) phases, respectively; indicating that diet with basal level of methionine could not supply enough methionine and resulted in growth depression and supplementation of methionine, provided the limiting amino acid required for better live weight. Hassan *et al.* (2003) also showed that addition of methionine (0.15%) in broiler diets improved the live weight, weight gain of the birds compared to non-supplemented. Supplementation of methionine 20% above NRC (1994) requirement has not produced any improvement beyond 100% level indicating excess amino acid was not utilized by birds for protein synthesis and muscle development. El-Wafa *et al*. (2003), reported that increasing methionine level to 120% of the requirement had no significant improvement on broiler performance, but decreased abdominal fat significantly. Hickling *et al*. (1990) fed broilers 2 methionine levels (100 or 116% of NRC, 1984) and found that increasing methionine significantly improved body weight at 6 week of age but not at 3 weeks of age. This variation might be due to difference in growth potential of the germplasm (synthetic male line) used in this study.

Feed intake was not affected by either ASC treatment or methionine supplementation. However, in feed conversion ratio (FCR) methionine supplementation resulted in significant improvement during 5–6 (P<0.001) and 0–6 week's (P<0.003) growth phases (Table 2). This improvement was due to better growth and weight gain, as the feed intake was almost similar amongst the treatment groups. Hassan *et al.* (2003) showed that addition of methionine (0.15%) in broiler diets improved feed conversion of the birds compared to the basal. Schutte and Pack (1995) after feeding a range of methionine and total sulphur amino acid (TSAA) levels for 14–38 days, estimated a TSAA requirement of 0.84% for weight gain and 0.88% for FCR, this partially justifies the small improvement in FCR in diet with methionine 120% level over 100%. ASC treatment did not affect FCR over any of the growth phase.

Carcass characters and meat quality; Carcass traits and cut-up parts yield expressed as percentage of dressed weight

Table 1. The ingredient (%) and nutrient composition of broiler basal starter and finisher ration

Ingredient	Starter ration (0-4 wk)	Finisher ration (4-6 wk)
Maize	56	57.7
Sunflower	6	6
De-oiled rice bran	4.73	11
Soybean meal	30	22.4
Limestone	1.2	1.5
Dicalcium phosphate	1.4	0.8
Trace minerals*	0.1	0.1
Vitamin premix**	0.1	0.1
Salt	0.3	0.3
Lysine	0.01	0.014
Methionine	-	-
Total	100	100
Metabolisable energy (ME)	2802	2801
Crude protein (CP)	21.8	19.5
ME:CP	128.5	143.9
Lysine	1.19	1.02
Methionine	.35	0.33
Total calcium	1.02	0.93
Available phosphorus	0.46	0.36

*Trace mineral premix supplied Mg- 300, Mn- 55, I- 0.4, Fe- 56, Zn-30 and Cu- 4 mg/kg diet. **The vitamin premix supplied vitamin A- 8,250 IU, vitamin D_3- 1,200 ICU; vitamin K- 1 mg; vitamin E- 40 IU; vitamin B_1- 2 mg, vitamin B_2- 4 mg, vitamin B_{12}-10 mcg; niacin- 60 mg; pantothenic acid-10 mg; choline-500 mg/kg diet.

Table 2. Effect of ASC treatment and methionine levels of diet on live weight (g), weight gain (g), feed intake (g) and feed conversion ratio at different growth phases

Treatment		Live wt 4th wk	Live wt 6th wk	Gain 0-4 wk	Gain 4-6 wk	Gain 0-6 wk	FI/b 0-4 wk	FI/b 4-6 wk	FI/b 0-6 wk	FCR 0-4 wk	FCR 4-6 wk	FCR 0-6 wk
Basal	ASC_0	651	1233	603	583	1186	839	1490	2330	2.02	2.78	2.37
	ASC_{100}	656	1221	610	564	1174	829	1502	2331	2.08	2.64	2.34
	ASC_{250}	653	1265	606	612	1218	827	1524	2351	1.99	2.51	2.23
100%	ASC_0	661	1273	614	612	126	833	1505	2338	2.09	2.34	2.21
	ASC_{100}	670	1317	621	647	1269	822	1499	2321	1.93	2.43	2.16
	ASC_{250}	670	1292	620	622	1244	820	1501	2321	2.02	2.35	2.18
120%	ASC_0	669	1299	620	630	1250	823	1518	2342	1.99	2.38	2.18
	ASC_{100}	666	1303	619	637	1256	823	1521	2344	2.01	2.35	2.17
	ASC_{250}	668	1295	621	627	1248	832	1517	2350	2.04	2.33	2.18
	SEM	4.42	10.12	4.42	11.34	10.12	3.04	3.92	5.28	0.02	0.03	0.02
ASC												
ASC_0		659	1274	611	615	1226	832	1504	2336	2.03	2.50	2.25
ASC_{100}		666	1275	618	610	1228	825	1507	2332	2.01	2.47	2.22
ASC_{250}		664	1284	616	620	1237	827	1514	2341	2.02	2.40	2.20
Methionine												
Basal		653	1240^{x}	606	587	1193^{x}	832	1505	2337	2.03	2.65^{x}	2.31^{x}
100%		667	1294^{y}	619	627	1246^{y}	825	1501	2326	2.01	2.37^{y}	2.18^{y}
120%		668	1299^{y}	620	631	1251^{y}	826	1519	2345	2.01	2.35^{y}	2.18^{y}
Probability												
ASC		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Met		NS	P<0.032	NS	NS	P<0.036	NS	NS	NS	NS	P<0.001	P<0.003
ASC × Met		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

^{xy}(Met level), Values carrying different superscript in a column vary significantly; NS, nonsignificant.

Table 3. Effect of ASC treatment and methionine levels of diet on carcass weight, organ weight (% live weight) and meat quality at 6th week of age

Treatment		De-feathered	Dressed	Breast	Back	Thigh	Drumstick	Wings	Neck	pH	TBARS	WHC
Basal	ASC_0	87.44	62.84	24.35	24.35	15.83	15.27	13.49	6.7	5.80	0.154	19.25
	ASC_{100}	87.45	62.82	25.02	24.14	15.55	15.05	13.25	6.98	5.82	0.189	18.75
	ASC_{250}	87.95	64.57	24.64	24.94	15.10	14.85	1.40	7.06	5.78	0.244	19.00
100%	ASC_0	88.02	62.40	25.16	24.86	14.91	14.43	13.43	7.42	5.80	0.146	19.00
	ASC_{100}	87.36	62.12	24.86	24.45	15.16	14.50	13.46	7.57	5.77	0.176	19.00
	ASC_{250}	86.39	62.91	24.72	24.27	15.70	14.15	13.29	7.87	5.80	0.254	19.25
120%	ASC_0	87.83	65.58	25.19	23.94	15.81	14.27	12.76	8.03	5.82	0.179	20.00
	ASC_{100}	86.13	63.43	23.66	24.27	15.16	14.69	14.17	8.05	5.78	0.273	19.75
	ASC_{250}	87.93	61.94	24.88	23.76	15.79	14.58	13.57	7.41	5.78	0.261	19.50
	SEM	0.24	0.44	0.21	0.21	0.16	0.12	0.11	0.15	0.005	0.15	0.15
ASC												
ASC_0		87.76	63.60	24.90	24.38	15.52	14.66	13.22	7.39	5.81	0.159^{l}	19.42
ASC_{100}		86.98	62.79	24.51	24.28	15.29	14.75	13.63	7.54	5.79	0.213^{m}	19.17
ASC_{250}		87.42	63.14	24.75	24.32	15.53	14.53	13.42	7.45	5.79	0.253^{n}	19.25
Methionine												
Basal		87.61	63.41	24.67	24.47	15.49	15.06	13.38	6.92^{x}	5.80	0.196^{x}	19.00
100%		87.26	62.48	24.91	24.52	15.26	14.36	13.39	7.62^{xy}	5.79	0.192^{x}	19.08
120%		87.29	63.65	24.57	23.99	15.59	14.52	13.50	7.83^{y}	5.79	0.238^{y}	19.75
Probability												
ASC		NS	NS	NS	NS	NS	NS	NS	NS	NS	P<0.001	NS
Met		NS	NS	NS	NS	NS	NS	NS	P<0.036	NS	P<0.036	NS
ASC × Met		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

^{lmn}(ASC level) ^{xy}(Met level), Values carrying different superscript in a column vary significantly; NS, nonsignificant (TBA, mg malonaldehyde/kg meat sample).

(Table 3) was not influenced either by ASC treatment or methionine supplementation except in case of neck yield, which increased significantly (P<0.036) and in dose dependent manner with increased level of methionine. Methionine is often reported to promote breast yield in broiler chicken (Jankowski *et al.* 2014) but no such change was evident in the present trial.

Among the meat quality parameters, pH and WHC values were almost comparable among all the dietary treatments, while TBARS value was influenced by both ASC and methionine level (Table 3). ASC treatment significantly (P<0.001) increased TBARS value in a dose dependent manner, while methionine level 120% produced a significant (P<0.036) reduction in TBARS value. One of the main factors determining the oxidative stability of meat is the level of antioxidants deposited during birds' lifetime (Ahmad and Abbas 2011). As ASC is highly pro-oxidant, it would have induced some degree of oxidative stress, which in turn consumed most of the antioxidants provided by feed, leaving little for deposition in meat, which can retard oxidative rancidity post-slaughter. Methionine on the other hand have antioxidant sparing effect, thus would have resulted in larger deposits of antioxidants like vitamin E, which in turn slowed down the development of meat rancidity. Lohakare *et al.* (2005) reported that providing methionine 20% above the NRC recommended level lower the TBARS value of breast meat. In addition, Rostango *et al.* (1995) observed that supplementation of methionine in poultry diet tend to reduce fat deposition, hence fewer substrates were available for development of rancidity.

Table 4. Effect of ASC treatment and methionine levels of diet on relative weight of immune organs (mg/100g live weight) and immune status

Treatment		Thymus	Bursa	Spleen	HA	PHAP
Basal	ASC_0	168.8	107.6	228.2	9.00	28.67
	ASC_{100}	222.3	99.4	184.7	9.17	28.67
	ASC_{250}	169.8	98.4	185.0	8.17	30.50
100%	ASC_0	167.9	100.3	160.5	8.83	33.83
	ASC_{100}	189.1	79.0	159.0	9.17	34.00
	ASC_{250}	175.2	77.5	215.0	9.17	31.00
120%	ASC_0	156.0	121.9	170.6	10.00	32.17
	ASC_{100}	191.3	97.0	221.2	9.83	33.50
	ASC_{250}	194.7	95.7	195.7	9.33	33.67
	SEM	0.006	0.004	0.007	0.18	0.97
ASC						
ASC_0		164.2	109.9	186.5	9.28	31.56
ASC_{100}		136.3	91.8	188.3	9.39	32.06
ASC_{250}		134.2	90.5	198.6	8.89	31.72
Methionine						
Basal		186.9	101.8	199.3	8.78	29.28
100%		177.4	85.6	178.2	9.06	32.94
120%		180.7	104.9	195.8	9.72	33.11
Probability						
ASC		NS	NS	NS	NS	NS
Met		NS	NS	NS	NS	NS
ASC × Met		NS	NS	NS	NS	NS

NS, Nonsignificant.

Immune organs and immune status: Statistically, no significant variation was observed in either weight of immune organs or immune status (Table 4). But the increase in methionine level produced some appreciable improvement in both humoral (HA-SRBC) and cell mediated immunity determined in terms of PHAP response. Methionine is the most extensively studied amino acid and it may be required for immunity functions that are distinct from transmethylation and trans-sulphuration metabolism, so its requirement for immunity appeared to be greater than the levels required for growth (Cook 1991). Similarly, Bunchasak (2009) also reported that the amount of methionine required to support the immune system seems to be high because it has to be used not only for protein synthesis but also to produce some antioxidants. Methionine supplementation also resulted in significant dose related increase in total antibody, immunoglobulin G level and response to PHAP, suggesting that methionine was required for some components of the antibody response and might be required for thymus derived (T) helper cell function. Similarly, Swain and Johri (2000) reported that methionine supplementation improved leukocyte migration inhibition value and enhanced antibody titer of new castle disease virus. Thakur and Mandal (2015) reported that when feedstuffs like meat-cum-bone meal, sunflower meal and soybean meal were treated with 80 ppm of ASC a small statistically non-significant reduction in methionine digestibility was observed for nearly all the feedstuffs tested, indicating ASC had a tendency to interact with methionine and in turn rendering it less bioavailable. The reduction might be induced by the action of chlorous acid, active component of ASC, on the readily oxidisable functional group in methionine rendering it unavailable for absorption. In the present study, ASC was used at 100 and 250 ppm level, which may have further reduced the methionine bioavailability leading to only a numerical improvement in immunity parameters observed.

It is concluded that feeding ASC treated feed to broiler chickens deteriorated the oxidative stability of meat. However, methionine supplementation improved broiler performance, meat quality and immune-competence and there is no apparent interaction between ASC and dietary methionine levels in broiler feed.

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