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Impact of Acidified Sodium Chlorite and Enzyme Treatment on the Microbial Load and Energy Bioavailability of Feedstuffs

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Abstract

The presence of microbial load and high fiber content in various non conventional feedstuffs limit their utilization in poultry feed. In the present study, the feedstuffs were treated with acidified sodium chlorite (ASC) and its impact on the microbial load and metabolizable energy availability was assessed in the chickens. The effect of supplementation of feed grade enzyme was also evaluated either alone or in combination with ASC treatment. ASC was prepared by adding citric acid to an aqueous solution of sodium chlorite (625 g/liter). The sanitizing effect of ASC was assessed at 0, 100, 250 and 500 ppm levels in the meat cum bone meal (MBM) and sunflower meal (SFM), while its impact on the energy bioavailability from SFM and de-oiled rice bran (DORB) was assessed at 0, 100 and 250 ppm levels with (0.3 g/Kg) and without exogenous enzyme supplementation. The results revealed that ASC treatment was effective in reducing the microbial load in MBM and SFM. Regarding the metabolizable energy availability, ASC treatment produced appreciable improvement both in SFM and DORB, while enzyme supplementation was effective only with SFM. It may be concluded that ASC treatment has a sanitizing effect and improves the energy bioavailability from feed ingredients like SFM and DORB in the chicken.

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Introduction

Feed ingredients like sunflower meal (SFM) and meat cum bone meal (MBM) often contain heavy microbial load and thus constitute a potential route of infection or contamination in poultry houses (Williams, 1981; Himathongkham *et al.* 1996). Also ingredients like SFM and de-oiled rice bran (DORB) are high in fiber and lignin content, which constitute a barrier around energy rich cell contents, resulting in low metabolizable energy (Furlan *et al.* 2001). Sodium chlorite (NaClO2, molecular weight 90.44) owing to its lignolytic action has been tried by various workers (Goering *et al.* 1971; Canale *et al.* 1992) to improve the digestibility of various forages in ruminants. Similarly acidified sodium chlorite (ASC) has been also approved by Food and Drug Administration, U.S.A. as a secondary direct food additive permitted in food for human consumption specifically as an antimicrobial intervention treatment for poultry carcasses, poultry carcass parts, red meat carcasses, red meat parts and organs, seafood, and raw agricultural commodities (Warf *et al.* 2001). In the present experiment, because of increasing public concern over the production of safe food from animal origins and the need to look for newer sources of energy or methods to improve utilization of energy from available resources, the effect of acidified sodium chlorite (ASC) treatment on the microbial load of various feedstuffs was analyzed. The impact of ASC treatment, with and without feed grade enzymes supplementations on energy bioavailability was also investigated.

Materials and Methods

Preparation of acidified sodium chlorite

ASC was prepared by adding citric acid (Himedia) in the powder form, to an aqueous solution (625 g/liter) of sodium chlorite (80%, SD Fine Chem. Ltd.) to attain a pH of 3.5. This stock solution had a concentration of 500 ppm/Ml. Immediately before use, the stock solution of ASC was diluted in water to obtain suitable concentrations and then thoroughly mixed with feed ingredients or mixed feed to achieve different concentrations.

Feed microbial load

The standard methods of APHA (1984) for microbiological assays were used to assess the sanitizing effect of ASC in MBM and SFM. These ingredients were sprayed and thoroughly mixed with ASC at different concentrations (0, 100, 250 and 500 ppm). A total of 25 g of each feed ingredient (3 samples of each feedstuff at all treatment levels) was mixed with 225 Ml normal saline solution (NSS) and 1 Ml aliquot of this was further transferred to 9 ml of NSS to make tenfold serial dilution for testing. Then 1 Ml from the serially 10-fold diluted samples was taken into petri plates, followed by addition of plate count agar or MacConkey agar for estimating

total plate count and presumptive coliform count, respectively. The plates were then allowed to solidify and incubated at 37° C for 24 hrs. The visible colonies were counted from each plate and multiplied by the dilution factor and expressed as log CFU (colony forming unit) per g of feedstuff.

Metabolizable energy

Two bioassays were conducted to determine the effect of ASC treatment with or without enzyme supplementation on the available energy content of SFM and DORB. The feed ingredients were treated with 0, 100 and 250 ppm of ASC in dry form (moisture 10 g Kg^{-1}), kept in airtight conditions under darkness for 24 hrs and then sun dried for 8 hr. The energy bioassay was conducted through practical diet replacement method (Sibbald and Slinger, 1963) at two substitution levels (20% and 40%) with (0.3 g/Kg) or without supplementation of commercial feed enzyme. The enzyme preparation contained xylanases, 3000; cellulases, 40; beta-glucanases, 150; pectinase, 150; amylase, 2000; proteinases, 600; alpha-galactosidase, 250; beta-galactosidase, 200; lipase, 400; and phytase, 50 mUnits/g. Fifty-six adult (35 week old) cockerels of comparable body weight were used for nitrogen corrected apparent metabolizable energy (AMEn) estimation. All the birds were housed in individual metabolic cages fitted with separate feeders, waterers and dropping trays. The birds had free access to fresh drinking water and feed all the times during the assay period. The birds were adapted for 20 days in cages on conventional grower diets. Thereafter, the reference diet (Table 1.) and test diets containing treated SFM or DORB (with 0, 100 and 250 ppm of ASC), each at two substitution levels (20% and 40%) with and without enzyme supplementation, were offered to the birds for a preliminary period of 10 days. Each diet was offered to four replicated birds. The feed intake during this period was recorded. This was followed by an assay period of four days during which a weighed quantity of feed was offered and the total excreta produced was collected quantitatively. The excreta samples were dried at 65ºC in a hot air oven. The dried feed residue, if any and excreta samples were ground and assayed for gross energy using a ballistic bomb calorimeter (Model CBB 330, Gallenkamp, San Diego). The nitrogen content of feed residue and excreta was also estimated according to AOAC (1990). The AMEn corrected values of the diets were calculated according to Hill and Anderson (1958). The AMEn values of diets were then multiplied by 1.01 since the minerals and vitamin premix were superimposed at 10 g Kg^{1} diet.

Data were subjected to analysis of variance following completely randomized deign (CRD) and the differences among dietary treatments were tested for statistical significance using Duncan’s multiple range test (Duncan, 1955). The correlations between the ASC levels and total plate count (TPC) or presumptive coliform count (PCC) were also drawn (Snedecor and Cochran, 1980).

Table 1. Composition of the reference diet

Ingredients	SFM / DORB (g kg^{-1})
Maize	690
SBM	160
DORB	50
SFM	50
Fish Meal	50
Wheat	0
Trace Min*	1
Vit Premix**	1
Salt	2
Limestone	4
Dicalcium Phosphate	2
Chemical Composition	
Calculated ME (KCal/Kg)	2986
Crude Protein (%)	18.36
Calorie: Crude Protein ratio	162
Crude Fiber (%)	5.20
Ether Extract (%)	2.63
Calcium (%)	0.66
Total Phosphorus (%)	0.25

*Trace mineral premix supplied Mg- 300, Mn- 55, I- 0.4, Fe- 56, Zn-30 and Cu- 4 mg kg^{-1} diet.
**The vitamin premix supplied vitamin A- 8250 IU, vitamin D_3- 1200 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^{-1} diet.

Results

ASC treatment (0, 50, 100, 250 and 500 ppm) of MBM and SFM was effective in reducing the microbial load. There was a dose dependent reduction in both total plate count (TPC) and presumptive coliform count (PCC) in the ingredients (Table 2), as significant ($P<0.01$) negative co-relation existed between the levels of ASC and TPC (r = -0.992 and -0.981 in MBM and SFM, respectively) or PCC (r = -0.973 and 0.999 in MBM and SFM, respectively).

During the metabolisable energy bioassay of SFM, the daily feed intake of the birds for various groups varied from 63.59 to 73.82 g $bird^{-1}$ (Table 3), which was equivalent to the intake of 166.58 to 191.87 Kcal AMEn and 14.47 to 16.82 g of CP. Enzyme supplementation caused reduction ($P<0.05$) in feed, energy and crude protein (CP) intake compared to non-supplemented group. There was no significant interaction between ASC level and enzyme supplementation for DMM, EM and AMEn of the diets.

Table 2. Effect of ASC treatment on microbial load (log_{10} CFU/g) in MBM and SFM

Ingredient	MBM		SFM	
ASC level (ppm)	TPC	PCC	TPC	PCC
0	5.94[a]	5.28[a]	6.26[a]	5.87[a]
50	5.82[ab]	5.20[a]	6.23[a]	5.74[b]
100	5.64[b]	4.99[b]	6.10[b]	5.67[b]
250	5.42[c]	4.66[c]	5.94[c]	5.41[c]
500	5.01[d]	4.40[d]	5.76[d]	4.99[d]
SEM	0.09	0.09	0.05	0.08

TPC is the total plate count, PCC is the presumptive coliform count.
Values bearing different superscripts within the columns vary significantly (P=0.001).

Table 3. Effect of ASC treatment and enzyme supplementation on intake, metabolizability and apparent metabolizable energy content of diet and test ingredient (sunflower seed meal)

	DMI ($gb^{-1}d^{-1}$)	EI ($Kcalb^{-1}d^{-1}$)	CPI ($gb^{-1}d^{-1}$)	DMM (%)	GEM (%)	AMEn ($Kcalkg^{-1}$)	AMEn Test ($Kcalkg^{-1}$)
Interaction							
ASC_0	69.45	180.60	15.85	60.27	67.25	2832	1635
ASC_0 + E	71.22	184.39	16.23	60.30	66.79	2817	1619
ASC_{100}	72.12	186.77	16.52	60.65	67.23	2835	1658
ASC_{100} + E	63.84	167.15	14.52	60.79	67.30	2847	1721
ASC_{250}	73.81	191.87	16.82	60.17	66.69	2829	1631
ASC_{250} + E	63.59	166.58	14.47	59.96	67.47	2848	1703
Pooled SEM	1.32	3.45	0.33	0.82	0.62	24.62	18.11
ASC level (ppm)							
0	70.34	182.49	16.04	60.28	67.02	2825	1627
100	67.98	176.96	15.52	60.72	67.27	2841	1689
250	68.70	179.22	15.65	60.07	67.08	2837	1667
Enzyme							
0 g Kg^1	71.80[x]	186.41[x]	16.40[x]	60.36	67.06	2832	1641
3g Kg^1	66.22[y]	172.71[y]	15.08[y]	60.35	67.19	2837	1681
Probability							
ASC x E	NS	NS	NS	NS	NS	NS	NS
ASC	NS	NS	NS	NS	NS	NS	NS
Enzyme	P<0.05	P<0.05	P<0.05	NS	NS	NS	NS

Values bearing different superscripts within a column vary significantly.
NS= Non-significant (P>0.05).
Where DMI is dry matter intake, EI is energy intake, CPI is crude protein intake, DMM is dry matter metabolizability, AMEn is nitrogen corrected apparent metabolisable energy and AMEn test is nitrogen corrected apparent metabolisable energy of test ingredient ie sun flower seed meal.

The daily feed intake for various groups varied from 72.09 to 82.60 g bird^{-1} in metabolisable energy bioassay for DORB (Table 4.), which corresponded to 196.80 to 221.12 Kcal AMEn and 12.44 to 14.19 g of CP. ASC treatment produced no significant effect on DMM, EM and AMEn values of diets containing DORB. There was also no significant interaction between ASC level and enzyme supplementation for DMM, EM and AMEn of the diets. ASC treatment increased the AMEn of DORB by 4.7 and 5.8% at 100 and 250 ppm levels, respectively, while enzyme supplementation did not produce any beneficial effects.

Table 4. Effect of ASC treatments and enzyme supplementation on intake, metabolizability and apparent metabolizable energy content of diet and test ingredient (de-oiled rice bran)

	DMI ($g\ b^{-1}d^{-1}$)	EI ($Kcal b^{-1}d^{-1}$)	CPI ($g\ b^{-1}d^{-1}$)	DMM (%)	GEM (%)	AMEn ($Kcal kg^{-1}$)	AMEn test ($Kcal kg^{-1}$)
Interaction							
ASC_0	74.44	201.27	12.81	64.20	69.22	2938	2187
ASC_0 + E	82.60	221.12	14.19	63.94	68.86	2922	2170
ASC_{100}	81.34	221.92	14.01	64.13	70.11	2963	2275
ASC_{100} + E	75.25	204.32	12.94	64.35	70.26	2956	2287
ASC_{250}	72.09	196.80	12.44	63.89	69.96	2969	2294
ASC_{250} + E	72.66	198.40	12.50	63.97	69.85	2965	2316
Pooled SEM	1.36	3.62	0.13	0.31	0.346	14.99	17.44
ASC level (ppm)							
0	78.51	211.20	13.5	64.07	69.04	2929	2179[a]
100	78.30	213.12	13.5	64.24	70.18	2959	2281[b]
250	72.37	197.60	12.44	63.93	69.91	2967	2305[b]
Enzyme							
0 g Kg^1	75.96	206.66	13.06	64.07	69.76	2956	2252
3g Kg^1	76.83	207.64	13.25	64.08	69.66	2948	2258
Probability							
ASC x E	NS	NS	NS	NS	NS	NS	NS
ASC	NS	NS	NS	NS	NS	NS	P<0.01
Enzyme	NS	NS	NS	NS	NS	NS	NS

Values bearing different superscript within a column vary significantly.
NS= Non-significant (P>0.05).
Where DMI is dry matter intake, EI is energy intake, CPI is crude protein intake, DMM is dry matter metabolizability, AMEn is nitrogen corrected apparent metabolisable energy and AMEn test is nitrogen corrected apparent metabolisable energy of test ingredient ie de-oiled rice bran.

Discussion

The total microbial load was relatively higher in SFM and ASC treatment was also apparently less effective. As moisture is one of the major factor influencing microbial growth and survival, this variation might be due to the difference in the

moisture content of these two ingredients. In case of SFM, the reduction in the microbial load was less compared to the one observed for MBM, which might be due to the larger and non-uniform particle size in SFM that might have interfered with thorough mixing of ASC with individual particles.

The present estimate of AMEn of SFM (1627 Kcal/Kg DM) was in close agreement with previous studies wherein 1554 and 1506 $kcalKg^{-1}$ AMEn was reported on air dry/ as fed basis, respectively (NRC, 1994; Mandal *et al.* 2005). ASC treatment had a positive effect at 100 and 250 ppm levels that increased AMEn by 3.8 and 2.4%, respectively, compared to the control. SFM is high in lignin content and ASC a lignolytic agent might have caused perforation in the individual cell walls, making the cell contents more accessible to the digestive enzymes. Previous reports regarding enzyme supplementation on energy availability from SFM showed a positive response. However, no such effect was observed in the control group (ASC 0 ppm), though a cumulative 2.44% increase in AMEn was observed (3.86% in 100 ppm and 2.45 % in 250 ppm) indicating ASC treatment, by degradation of lignin, made the cell wall more porous, enabling better action by enzymes on their respective substrates. This relatively lower (2.44%) improvement might be due to the higher crude fibre (27.65 %) content of SFM used in the present study compared to 24% and 25% as in other studies (NRC, 1994; Mandal *et al.* 2005). Also, the enzyme preparation used in this investigation contained less enzymatic activity (β-glucosidase 35132, b-D-xylanopyranosidase-98446, CM cellulase 1906 and amylase 5773 mIU/g preparation.) than used earlier (Mandal *et al.* 2005).

The AMEn content of DORB (2187 $KcalKg^{1}$) estimated in the present study was consistent with the previous reports (Zombade and Ichhponani, 1983; Mandal and Pathak, 1996). DORB used in the present study contained 11% lignin and its ASC treatment caused a significant increase in AMEn content. Increased utilization of energy might be due to the high susceptibility of DORB lignin to the lignolytic action of ASC, making the cell contents more accessible to the action of digestive juices and enzymes. It was reported by Goering *et al.* (1971) that $NaClO_2$ decreased lignin content of wheat straw more efficiently than groundnut husk, suggesting that lignin from different sources had a variable susceptibility to the lignolytic action of $NaClO_2$. Enzyme supplementation did not produce any appreciable variations in AMEn content, indicating that the enzyme preparation used was not specific to the chemical nature of the substrate present in the feed ingredients. Choct (2006) has pointed that the current feed grade enzymes are not able/designed to degrade non starch polysaccharide to simple sugars within the food transit time of the chicken.

References

APHA (American Public Health Association). 1984. Compendium of methods for the microbiological examination of foods. 2nd Ed. 800 I Street, NW Washington, DC. 914 Pages.

AOAC (Association of Official Analytical Chemists). 1990. Official method of analysis. 14th Ed. The William Byrd Press. Richmond, Virginia, USA. 1230 Pages.

Canale CJ, Glenn BP & Reeves JB. 1992. Chemically treated alfalfa: lignin composition and in-situ disappearance of neutral detergent fibre components. Journal of Dairy Science, 75: 1543-1554.

Choct, M. 2006. Enzymes for the feed industry: past, present and future. World's Poultry Science Journal, 62: 5-15.

Duncan DB. 1955. Multiple range and F tests. Biometrics, 11: 1-42.

Furlan AC, Mantovani C, Murakami AE, Moreira I, Scapinello C, & Martins EM. 2001. Use of Sunflower Meal in Broiler Chicks Feeding. Revista Brasileira de Zootecnia, 30: 158-164.

Goering HK, Smith LW, Van Soest PJ & Gordon CH. 1971. Digestibility of roughage materials ensiled with sodium chlorite. Journal of Dairy Science, 56: 233-240.

Himathongkham S, Pereira MG & Riemann H. 1996. Heat destruction of salmonella in poultry feed: effect of time, temperature and moisture. Avian Disease, 40: 72-77.

Hill FW & Anderson DL. 1958. Comparison of metabolizable energy and productive energy determinations with growing chicks. Journal of Nutrition, 64: 587-603.

Mandal AB & Pathak NN. 1996. Comparative evaluation of metabolizable energy values of feedstuffs in chicken and guinea fowl. Indian Journal of Animal Science,
66: 930-934.

Mandal AB, Elangovan AV, Tyagi PK, Tyagi PK, Johri AK & Kaur S. 2005. Effect of enzyme supplementation on the metabolisable energy content of solvent-extracted rapeseed and sunflower seed meals for chicken, guinea fowl and quail. British Poultry Science, 46: 75-79.

NRC (National Research Council). 1994. Nutrient requirements of poultry. 9th Rev. Ed. National Academy Press. Washington, DC. 176 Pages.

Sibbald IR & Slinger SJ. 1963. A biological assay for Metabolizable energy in poultry feed ingredients together with findings which demonstrate some of problems associated with evaluation of fats. Poultry Science, 42: 313-325.

Snedecor GW & Cochran WG. 1980. Statistical Methods. 8th Ed. Iowa State University Press. Ames, Iowa. 503 Pages.

Williams JE. 1981. Salmonella in poultry feed – a world wide view - Part I. World's Poultry Science Journal, 36: 6-19.

Warf CC Jr. 2001. The chemistry and mode of action of acidified sodium chlorite. IFT Annual Meeting, New Orleans, Louisiana. Page, 1-91.

Zombade SS & Ichhponani JS. 1983. Nutritive value of raw, parboiled, stabilised and deoiled rice bran for growing chicks. Journal of the Science of Food and Agriculture, 34: 783-788.