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Zeolite as a Feed Additive to Reduce *Salmonella* and Improve Production Performance in Broilers

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Abstract: Zeolite (clinopitolites) was added to broiler feed at concentrations of 1.0%, 1.5% or 2.0% and was evaluated for its effectiveness to reduce *Salmonella* in broilers and its effects on production performance. These experiments were conducted both in the summer and winter seasons. It was found that adding zeolite in the broiler diet significantly ($p<0.05$) reduced *Salmonella* levels, as compared to the control, on the chicken body, in the ceca and on the chicken carcass, both in the winter and summer seasons. In addition, it was found that zeolite treatments had a positive effect on the production parameters that were measured, but only in the winter season. This study showed the significance of using zeolite, as a feed additive for broilers, as part of a comprehensive program to control *Salmonella* at the broiler farm.

Key words: Broilers, zeolite, production performance, *Salmonella*

INTRODUCTION

The Kuwait poultry industry is one of the important animal industries and it supplies approximately 50% of poultry meat for consumption in Kuwait (Ministry of Planning, 2003). Contamination of poultry by food-borne pathogens such as *Salmonella* is considered one of the major problems facing the progress of the poultry industry in Kuwait. In recent years, the broiler industry in Kuwait suffered significant economic losses due to a lack of a complete monitoring program to significantly reduce pathogens in poultry. In addition, on two occasions in the year of 2006, the Kuwait Ministry of Public Health banned locally produced broilers from the market, because of the high incidence of *Salmonella* contamination. In turn, this incident caused significant economic loss to the Kuwait broiler industry.

It is clear that *Salmonella* food contamination that is associated with the consumption of poultry products is considered a serious problem for the local poultry industry as well as for the consumers. Therefore, it becomes apparent that controlling *Salmonella* at the farm and at the processing plant are important goals to both the industry and the public health authorities in Kuwait. This control can be done by utilizing different treatments to reduce the levels of *Salmonella* at the farm level to the lowest possible and apply good practices at the processing plants to prevent the risk of cross contamination during processing. Treatments such as competitive exclusion (Seo *et al*., 2000; Stern *et al*., 2001; Nakamura *et al*., 2002) and probiotics (Line *et al*., 1998; Johannsen *et al*., 2004) have been reported. We previously reported on the use of competitive exclusion treatment to reduce *Salmonella* at the farm (Al-Zenki *et al*., 2009). In the current study, we are reporting the effect of adding zeolite in the broiler feed on reducing *Salmonella* in broilers.

Zeolite (clinopitolites - hydrated aluminosilicates), which is a beneficial and inexpensive feed additive, has the ability of binding to nitrogenous cations and leads to lowering ammonia emission. Forms of zeolite have been used to remove ammonium from aqueous solutions (Wen *et al*., 2010) and help in reducing ammonia production by pullets and laying hens (Nakaue and Koelliker, 1981; Wu-Haan *et al*., 2007) and in broiler houses (Cabuk *et al*., 2004). It is important to remove ammonia from poultry houses since it has been reported that high atmospheric ammonia in poultry facilities has been linked to damaged respiratory tract lining, reduced resistance to respiratory diseases, increased ascites and lower performance (Beker *et al*., 2004). Furthermore, natural zeolites are hydrated aluminosilicates that have ion-exchange and adsorption properties and have a large surface area that helps in these adsorption properties (Ji *et al*., 2009; Ruen-ngam *et al*., 2009; Xia *et al*., 2009). In addition, zeolite has been recommended and used effectively in reducing toxic effects of materials such as aflatoxins (Harvey *et al*., 1993; Miazzo *et al*., 2000; Oguz and Kurtoglu, 2000; Jand *et al*., 2005; Ortatatli *et al*., 2005). There is evidence that zeolite can be used as an antimicrobial agent (Haile and Nakhla, 2010). However, studies are needed to investigate the effect of zeolite specifically on reducing

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Salmonella in broilers. Zeolite, as a feed additive, has also shown to improve metabolizable energy in pigs (Shurson *et al*., 1984). It is important to mention that the effect of using zeolite on production performance is significant in determining the influence on production efficiency and in turn on cost of production. It has been shown that zeolite can be used as a cost effective feed additive to improve broiler performance. It was found that natural zeolite significantly improved feed efficiency of broiler chicks (Elliot and Edwards, 1991; Gezen *et al*., 2004; Eleroglu and Yalcin, 2005). However, Cabuk *et al*. (2004) reported that feed conversion of broilers fed zeolite was not affected. Therefore, further studies are needed to determine if zeolite has positive effects on broiler production performance.

The objectives of this study was to examine the effects of using different levels of natural zeolite, as a broiler feed additive, on reducing *Salmonella* in broilers and to study whether these treatments would also have an overall beneficial effect on broiler production performance.

MATERIALS AND METHODS

Bird management: The bird management of the current experiments is similar to what was previously reported (Al-Zenki *et al*., 2009). One-day-old broiler chicks (Indian River x Indian River), obtained from one local commercial hatchery, were used in the current study. Our study was conducted on a commercial farm using one part of a house. This part of the house was divided into 36 floor pens that were used in the current experiment as will be described below. These pens were equipped with nipple drinkers, received continuous lighting and wood shavings were used as floor bedding. Water and un-medicated corn/soy-based diets that met the NRC requirements (NRC, 1994) were provided *ad libitum*. The chicks received a pre-starter diet (24.4 CP, 3, 029 kcal ME/kg) from hatch until 7 days-of-age, a starter diet (22.5 CP, 3036 kcal ME/kg) from 8 days to 21 days-of-age and a finisher diet (21.6 CP, 3171 kcal ME/kg) from 22 days to 35 days-of-age when the experiments ended and the birds were slaughtered. It is important to note that all experiments were conducted in a manner that avoided unnecessary discomfort to the animals by the use of proper management and laboratory techniques.

Experimental design: The experimental design of the current study is also similar to that reported previously (Al-Zenki *et al*., 2009). Each of the 36 pens mentioned above was measured at 5 m^2 per pen and housed one hundred and twenty chicks providing 0.042 m^2/bird. The 36 pens were divided into four groups of nine pens each. Each nine pens were assigned to one of the four treatments that will be described below. Data from each pen was considered as one value (one replicate) and thus providing nine replicates per treatment.

This study was repeated both in the summer season, during the months of August and September, the average temperature in the house throughout the period was 28°C and winter season, during the months of December and January and the average temperature in the house throughout the period was 23°C. This was done in order to determine whether or not the effects of zeolite on reducing *Salmonella* contamination and on production performance, is season dependent. In all, a total of 8,640 birds were used for the entire study.

Treatments: Each treatment had nine replicates as mentioned previously. These treatments included the control group with no treatment and a basal diet, as explained under bird management previously, was used (Treatment 1); Zeolite (Iran Zeolite Company, Tahran, Iran), was added to the feed at a concentration of 1.0% (Treatment 2); Zeolite was added to the feed at a concentration of 1.5% (Treatment 3) and Zeolite was added to the feed at a concentration of 2% (Treatment 4). The added zeolite did not replace any other ingredient in the diet.

Sample collection and microbiological analysis: Samples were collected both from the farm and at the processing plant. Farm samples were collected at 7, 21 and 35 days-of-age. While the processing plant samples were collected I) at the evisceration step to obtain the ceca samples and ii) post-chilling step to obtain the carcass samples. All these samples were collected from all thirty-six pens of the different treatments for each of the two seasons.

At the farm, the prevalence of *Salmonella*, which is defined as the occurrence or presence of *Salmonella*, on the chicken body and in the ceca was determined as described previously (Al-Zenki *et al*., 2009). Briefly, five randomly selected chickens were removed at each sampling period from each of the nine pens that were assigned for each treatment and killed by cervical dislocation. Following the detection of *Salmonella* in the collected samples, as will be described below, the percent contamination within each pen was considered as one value (one replicate) thus providing nine replicates from each treatment which were used for the statistical analyses.

The whole bird's body was placed in large sterile stomacher bags containing 400 mL of 0.1% buffered peptone water and rinsed by shaking for two minutes in. The whole bird rinse solution was then poured into sterile containers. The body surface was then rubbed with a sterile cloth containing 70% ethyl alcohol to disinfect the area where the ceca will be removed and then aseptically dissected. Ceca were removed and their contents diluted 1:3 with 0.1% buffered peptone.

At the processing plant, the prevalence of *Salmonella* on the chicken carcass and in the ceca was also determined as described previously (Al-Zenki *et al*., 2009). Numbered leg bands were placed on the chicken as a means of identification. The birds were slaughtered as it is commonly done in the processing plant. The ceca of five randomly selected chickens, representing each pen from each treatment were then collected during the evisceration step. Additionally, the same post-eviscerated carcasses (five samples) were collected after air chilling. Sample preparation for the post-chilled carcass and post-eviscerated ceca samples was carried out as described earlier. Again the percent contamination of the samples within each pen was considered as one value (one replicate) thus providing nine replicates from each treatment which were used for the statistical analyses.

Salmonella detection was carried out as outlined previously (Al-Zenki *et al*., 2009) using the FDA Bacteriological Analytical Manual (BAM) *Salmonella* isolation procedure (US FDA, 1995) after some modification where buffered peptone water replaced lactose broth for pre-enrichment. All samples were pre-enriched at 37°C for 24 h in 0.1% buffered peptone water. After incubation, 1 mL of the pre-enriched samples were transferred to 9 mL of tetrathionate broth and selenite cysteine broth, respectively and incubated for 24 h at 37°C. Following incubation, samples were then streaked onto Xylose Lysine Deoxycholate (XLD) and Bismuth Sulphite Agar (BSA). Suspected *Salmonella* colonies were stabbed into Triple Sugar Iron Agar (TSI) and Lysine Iron Agar (LIA) slants and presumptive *Salmonella* were confirmed by serotyping. Prevalence of *Salmonella* from the five birds that were tested from each pen provided the percent *Salmonella* contamination in each pen. The percentages for the nine pens for each treatment provided the nine replicates and these replicates were used for the statistical analyses.

Production performance measured: Twenty randomly selected birds from each pen in each treatment at each time were weighed at 7, 21 and 35 days-of-age and average weight per bird was calculated. Feed consumption for each pen was determined at 7 days, 21 days and 35 days-of-age and feed consumption per bird and FCR were calculated. Mortality was recorded daily and weekly mortality was calculated. The data obtained from each pen was considered as one value (one replicate) providing a total of nine replicates for each treatment and these replicates were used for the statistical analyses.

Data analysis: Data were analyzed using a one-way analysis of variance utilizing the S-plus statistical program (Crawley, 2002). Treatments were the main effect. Means were separated using Tukey's test and significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Effects of treatments on *Salmonella* reduction: The effects of adding different levels of zeolite in the broiler feed on the prevalence of *Salmonella* on the exterior body and in the ceca, at different ages during the summer season, are shown in Fig. 1 and 2, respectively. Our results show that adding zeolite, at least at the higher dose (2%), significantly ($p<0.05$) reduced *Salmonella* contamination on the exterior body at 7 and 35 days-of-age. The reduction was over 50% of the control group. It is important to note that an average reduction of more than 50% achieved by the 2% zeolite treatment could contribute to the safety of poultry. The antimicrobial effect of zeolite on *Salmonella* reduction has been previously reported (Ricke *et al*., 1995). In addition, Cho *et al*. (2005) reported that zeolite reduced colony forming units of other enteric bacteria. It is possible that the effects of zeolite could be due an intrinsic mechanism in the body that could lead to litter moisture reduction. Adding 2% zeolite in the broiler feed, reduced litter moisture (Gezen *et al*., 2004) and decreased the organic content of the litter and improved its quality (Karamanlis *et al*., 2008). The resulted dried litter leads to the destruction of different microorganisms

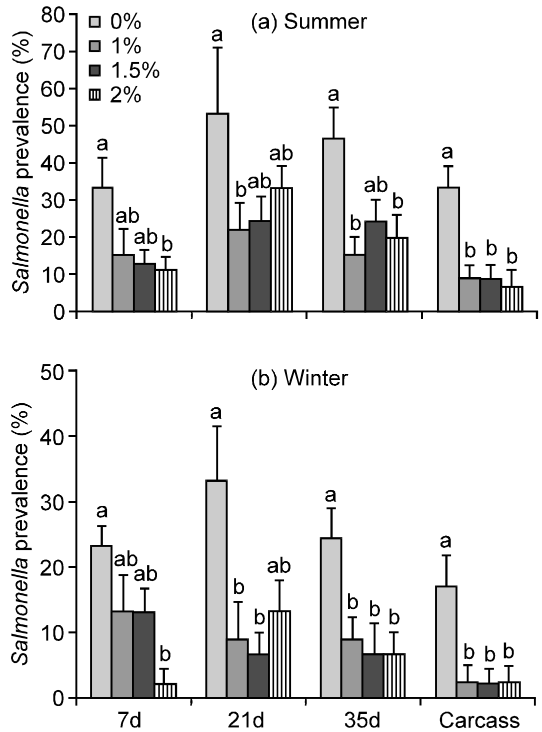

Fig. 1: Effects of different treatments on *Salmonella* prevalence (% *Salmonella* positive per pen), on whole body rinses at 7, 21 and 35 days and post-chill carcass rinses in the summer and winter seasons. (a) Summer, (b) Winter. Means within each age and within carcass with no common superscripts differ significantly ($p<0.05$). Values are expressed as means + SEM (n = 9 replicate pens per treatment group, each replicate value represents the percent prevalence per pen)

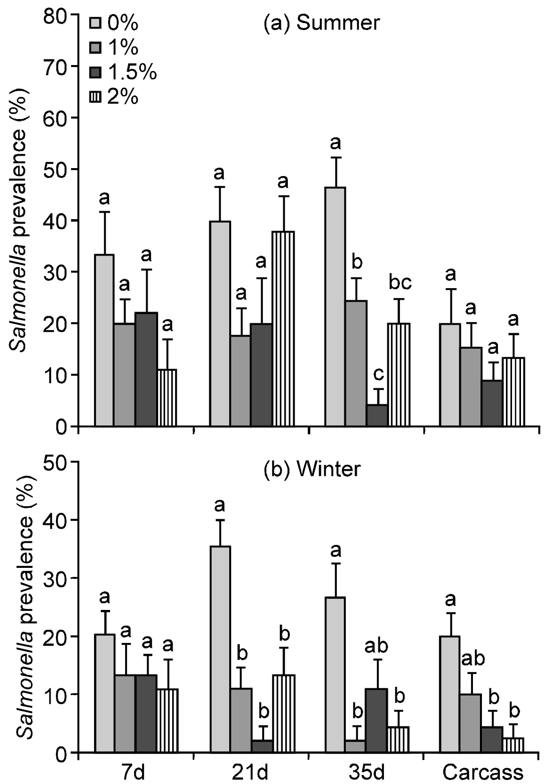

Fig. 2: Effects of different treatments on *Salmonella* prevalence (% *Salmonella* positive per pen), in broiler ceca at 7, 21 and 35 days and ceca of post-chill carcass rinses in the summer and winter seasons. (a) Summer, (b) Winter. Means within each age and within carcass with no common superscripts differ significantly ($p<0.05$). Values are expressed as means + SEM (n = 9 replicate pens per treatment group, each replicate value represents the percent prevalence per pen)

(Himathongkham and Riemann, 1999) including *Salmonella* (Hayes *et al*., 2000). Furthermore, it has been reported that zeolite in the feed decreased broiler serum ammonia (CaiMei and AnGuo, 1999) and helped in reducing ammonia production in pullets and laying hens (Nakaue and Koelliker, 1981) and in broiler houses (Cabuk *et al*., 2004). This effect could be due to the fact that zeolite has a large surface area that helps in enhancing its adsorption properties in the body (Ji *et al*., 2009; Ruen-ngam *et al*., 2009; Xia *et al*., 2009). It should be mentioned that it has been reported that using electrostatic space charge system to reduce ammonia and other particles in the broiler house (Ritz *et al*., 2006) results in reducing airborne *Salmonella* contamination in the broiler house (Holt *et al*., 1999).

As to the effect of adding zeolite in the broiler feed on the prevalence of *Salmonella* in the ceca, during the summer season, it was found that all levels of zeolite reduced *Salmonella* contamination significantly ($p<0.05$) at 35 days-of-age. Our results indicate that adding zeolite in the broiler feed is beneficiary in reducing *Salmonella* contamination on both the body and in the ceca, during the summer season, at the farm level.

The effect of adding zeolite in the broiler feed on the prevalence of *Salmonella* in the ceca from eviscerated chicken and post-chilled carcasses at the processing plant during the summer season are also shown in Fig. 1 and 2, respectively. All levels of zeolite significantly ($p<0.05$) reduced *Salmonella* contamination on the carcasses. *Salmonella* contamination reduction in the ceca from eviscerated chicken was not significant ($p>0.05$). It is important to mention that the *Salmonella* contamination reductions on the carcass after processing, using the current treatments, are the most important results of our current investigation as the reduction is obviously reflected in reducing the levels of *Salmonella* present on the broilers prior to marketing. The percent contamination for the control birds (33.3%) in the current study is above the 20% margin level adopted by Kuwaiti regulatory agencies and of the zero tolerance for *Salmonella* adopted world wide on uncooked carcasses. Furthermore, a *Salmonella* contamination reduction to 8.9% by using 1 and 1.5% zeolite and to only 6.7% by using 2% zeolite in the broiler feed, could contribute greatly to the safety of poultry prior to marketing, as mentioned previously. In addition, this is the first study that shows the direct effect of adding zeolite in the broiler diets on reducing *Salmonella* contamination both at the farm level and in the carcasses from the processing plant.

The effect of adding zeolite in the broiler feed on the prevalence of *Salmonella* on the exterior body and in the ceca at different ages during the winter season are shown in Fig. 1 and 2, respectively. The results for the winter season were similar to that found in the summer season. In the winter season, it was found that adding all the different levels of zeolite in the broiler feed significantly ($p<0.05$) reduced *Salmonella* contamination on the exterior body at 21 and 35 days-of-age and numerical, but not significant reduction ($p>0.05$) at day 7. It is important to note that even though the *Salmonella* contamination on the exterior body of the control group at different ages was more in the summer than in winter, the contamination reduction due to the treatments was more pronounced in the winter than in the summer season. This could be due to the fact that levels of moisture and ammonia in the broiler houses are more in the winter season than in the summer season. It has been reported that ammonia levels in the broiler house are higher during the winter season than during the summer season (Gates *et al*., 2004) which could lead to an increase in *Salmonella* contamination. Therefore, since zeolite has proven effects on reducing ammonia

Table 1: Effects of using zeolite in broiler diet on production performance in the summer and winter seasons

	Summer				Winter			
	Percentage zeolite				Percentage zeolite			
Parameter	0	1	1.5	2	0	1	1.5	2
Mean final BW (g/bird)	$1307^{a,1}\pm1$	$1284^{b}\pm1$	$1285^{b}\pm6$	$1309^{a}\pm1$	$1223^{b,1}\pm11$	$1270^{a}\pm7$	$1313^{a}\pm12$	$1307^{a}\pm11$
TFC (g/bird)	$2469^{a}\pm31$	$2463^{a}\pm27$	$2456^{a}\pm24$	$2460^{a}\pm25$	$2570^{a}\pm27$	$2561^{a}\pm22$	$2539^{a}\pm30$	$2552^{a}\pm30$
Overall FCR	$1.90^{a}\pm0.04$	$1.94^{a}\pm0.02$	$1.92^{a}\pm0.03$	$1.89^{a}\pm0.03$	$2.07^{a}\pm0.07$	$2.04^{ab}\pm0.00$	$1.91^{b}\pm0.03$	$1.93^{b}\pm0.05$
Total mortality (%)	$7.45^{a}\pm0.53$	$7.96^{a}\pm0.43$	$8.34^{a}\pm0.25$	$8.04^{a}\pm0.61$	$2.77^{a}\pm0.58$	$2.30^{a}\pm0.19$	$2.24^{a}\pm0.35$	$2.00^{a}\pm0.53$

Means for each parameter within a season in a raw with no common superscripts differ significantly ($p<0.05$).

[1]Values are expressed as means ± SEM (n = 9 replicate pens per treatment group, each replicate value is the average for each pen)

TFC = Total Feed Consumption

levels in the broiler houses, as mentioned previously, its effect could be more in the winter than the summer. Thus, it should be expected that in our current study zeolite effect would be more pronounced in the winter than in the summer season as was found. The effects of adding zeolite in the broiler diets on the prevalence of *Salmonella* in the ceca, during winter season, were as expected similar to those found on the exterior body. Again our results indicate that adding zeolite in the broiler feed is beneficial in reducing *Salmonella* contamination on both the body and in the ceca, also during the winter season, at the farm level.

The effect of various treatments on the prevalence of *Salmonella* in the ceca from post-eviscerated chickens and the post-chilled carcasses during the winter season are also shown in Fig. 1 and 2, respectively. Our results clearly indicate that essentially all levels of zeolite that were added to the broiler feed significantly ($p<0.05$) reduced *Salmonella* contamination both on post-chilled carcasses and in the ceca from post eviscerated chickens. Again the effect was more profound in the winter season than in the summer season signifying the importance of the zeolite adsorption property that leads to reducing the level of both ammonia and moisture, as mentioned and explained previously. Therefore, it can be concluded that adding zeolite in the broiler feed could contribute greatly to the safety of poultry prior to marketing, as mentioned previously. In addition, it should be reemphasized that the current study could be the first one to show the direct effect of adding zeolite in the broiler feed on reducing *Salmonella* contamination both at the farm level and on the carcasses from the processing plant.

Effects of treatments on production performance: Data on effect of treatments on production performance including final body weight, total feed consumption, overall feed efficiency and total percent mortality, both in the summer and winter seasons, are shown in Table 1. It is important to note that since the major important goal of the present research is to study the effects of using different levels of natural zeolite in the broiler feed on reducing *Salmonella* and additionally to investigate whether these treatments would have an overall beneficial effect on broiler production performance, only the data on overall performance is reported.

Our results show that adding zeolite in the broiler feed, in general, improved feed conversion, however, the improvement was significant ($p<0.05$) only in the winter season. These results agree with previous reports (Elliot and Edwards, 1991; Gezen *et al*., 2004; Eleroglu and Yalcin, 2005) in which it was found that feed efficiency of broiler chicks was also improved significantly when zeolite was added in the broiler feed. It is important to indicate that, in the current study, the effect of zeolite on improving the feed efficiency was mainly due to an increase in body weight in birds that received zeolite in their feed and to an increase in growth rate. It has been reported previously that zeolite added in the broiler feed increased body weight (Eleroglu and Yalcin, 2005) and increased growth rate (Karamanlis *et al*., 2008). This positive effect of zeolite on production performance could be due to its ability to reduce toxic effects of materials such as aflatoxins as has been reported in several studies (Harvey *et al*., 1993; Miazzo *et al*., 2000; Oguz and Kurtoglu, 2000; Jand *et al*., 2005; Ortatatli *et al*., 2005). Furthermore, it has been indicated that adding zeolite in the broiler feed resulted in reducing abdominal fat (Christaki *et al*., 2006) as well as lowering breast fat content (Prvulovic *et al*., 2008). This could be considered as an additional advantage to using zeolite in the broiler feed.

Our results showed that regardless of the treatment, percent mortality was higher in the summer than in the winter season. This difference could be due to the exposure of the birds to more stress in the summer than in the winter, since house temperature was higher. Conditions that are associated with stress have shown to increase shedding of *Salmonella* Enteritidis (Holt, 1992; Holt, 1993). Finally, it can be mentioned that adding zeolite in the broiler feed did not only reduced *Salmonella* contamination both at the farm level and on the carcasses from the processing plant, but also improved production performance. It should also be indicated that the use of zeolite as a feed additive to broilers is cost effective. Adding zeoilte in the broiler feed could add only 5% to the diet cost. Even though the 5% increase in the cost of production due to the use of zeolite in the broiler diet might appear significant to the total cost of production, the savings due to the reduction of *Salmonella* contamination of broilers in Kuwait, which would lead to reduction in the broiler condemnation is much more significant.

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