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# TERATOGENIC EFFECT OF SODIUM METABISULPHITE IN THE EMBRYOGENESIS OF *Gallus gallus domesticus*

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## ABSTRACT

The pinnacle of advances in food processing offered tremendous advantage in the preservation of foods with the aid of food additives. One of the commonly used food preservatives is the derivatives of sulfite, Sodium Metabisulfite (SMB). In this study, the teratogenic effect of SMB are evaluated using the Chicken Embryo Test. The 2.5, 40 ppm of SMB were administered into chicken eggs using the standard procedure. The 40 samples of eggs were placed in a commercial incubator. After 5 days incubation period, the eggs were examined for specific developmental anomalies. The results showed that malformations and deformities were observed in chicken embryo and there were even suppressions of growth and development especially in the 40 ppm concentration. Thus, the study has shown that SMB probably effects on the developmental stages in chicken embryo.

**Keywords:** Embryogenesis, Food Preservatives, Sodium Metabisulfite, Teratogenic

## INTRODUCTION

The safety of food storage for a prolonged period is indeed one of the indispensable human needs. This can be achieved by different methods of preservation which has advanced over time. In general, for preservation purposes, various chemical agents such as sulfites are used. Sulfite refers to sulfur dioxide gas; hydrogen sulfites; metabisulfite; and sulfur salts containing potassium, calcium, or sodium. Sulfite additives are used to preserve dried fruit, processed fish, seafood, meats, and some canned goods (Leclercq et al. 2017). Sulfite additives are used primarily for controlling food spoilage, microbial growth, and prevention of browning (Chang et al. 1997). According to the 1958 Food Additives Amendment to the Federal Food, Drug, and Cosmetic Act, several food preservatives including sulfites were declared Generally Regarded as Safe (GRAS) with regulated prescription (Irwin et al. 2017).

However, this claim was not supported by the committee of International Program on Chemical Safety (IPCS). IPCS conducted an evaluation on various toxicological studies and recommended that a suitable alternative method of preservation should be encouraged aside from the use of sulfites that may have led to high levels of acute intake and which have been associated with life-threatening adverse reactions (WHO, 1999).

According to the literature, the two derivatives of Sulfites namely Sodium Metabisulfite (SMB) and Sodium Bisulfite (SB) have been considered as unsafe due to some health concerns. The SMB has been reported to decrease the total number of spermatogonia, primary spermatocyte, spermatids and leydig cells, decrease sperm count, motility, and increased sperm abnormality (Shekarforoush et al. 2015). SB has bactericidal and bacteriostatic effects on common probiotics such as *Lactobacillus* species casei, *plantarum* and *rhamnosus*, and *Streptococcus thermophilus* in the human gut microbiota (Irwin et al. 2017).

on the safety assessment of sodium sulfite, potassium sulfite, ammonium sulfite, sodium bisulfite, ammonium bisulfite, sodium metabisulfite and potassium metabisulfite, undiluted SMB was not found to be an irritant to rabbits following occlusive exposures, but 50% concentrations was irritating to guinea pigs following repeated exposure(Nair and Elmore, 2003). This is a concentration that cannot be found in cosmetics, hair, and other personal care products. It was also found to be negative in mutagenicity studies. The study concluded that SMB is a safe ingredient in cosmetics and personal care products. However, SMB is slightly hazardous in case of skin contact (permeator), of eye contact (irritant) and that it may be toxic to upper respiratory tract, skin, and eyes (Nair and Elmore, 2003).

Further, the effects of SMB on mitosis were investigated. There is a significant reduction of mitotic index at all concentrations and treatment period. SMB has cytotoxic, aneugenic, and genotoxic effects on Allium cepa. There are still few literatures proving that SMB has negative adverse effects on organisms, specifically on its development and/or reproductive parameters, thus, can affect normal mechanism of growth and development (Rencuzogullari et al. 2001).

The embryonic development of*Gallus gallus* test has been demonstrated to be reliable and to afford quantifiable end points for evaluation. The compounds under investigation (SMB) can easily be administered and tested (Maci, 1980; Davey and Tickle, 2007). As a carrier of a complete set of developing morphogenetic system, the chick embryo in ovum manifests an advantage over those *in vitro* systems that employ isolated embryos or embryonic tissues that have only limited survival (Kotwani, 1998). The Chicken embryo Test has been utilizedin the evaluation of the effect of endogenous and exogenous factors on development (Tong et al. 2013), effect of eugenol (Gad El-Hak et al. 2018) and sodium benzoate (Emon et al. 2015). In the present study,embryonic development of *Gallus gallus domesticus* was used in the evaluation of the teratogenic effect of SMB.

There have been no agreements on the assessment of the safety consumption of food with SMB additive, which is a health relevant concern. There have been various studies conducted on the effect of SMB but there is a scarce data on the teratogenic effect of SMB on the chicken embryo. Thus, this study has been carried out to identify specific embryonic responses induced by the administration of SMB. The study documented specific malformations, alterations, and developmental implications triggered by the consumption of SMB.

## MATERIALS AND METHODS

### Sample Collection

The eggs from *Gallus gallus domesticus* were collected in different poultries in Macarat, Abuno Iligan City, Philippines. The gathered eggs were five days old and weighed an average of 45 grams.

### Incubation

The eggs were put in the incubator manufactured by Abellar Manufacturing Incorporated at Izon Poultry and Supply and were incubated under standard conditions with temperature of 37.0 degree Celsius and relative humidity of 55% until 18 days. The eggs were candled on the $5^{th}$day of incubation, wherein unfertilized eggs and those with dead embryos were discarded. On day 6 of incubation, the eggs with living embryos (n=40) were randomly divided into four equal groups; control and three doses of sodium metabisulphite concentrations (1ml of 2.5 ppm, 1 ml of 10 ppm and 1 ml of 40 ppm of sodium metabisulphite were used as experimental group. There were 10 eggs used in each set up namely the control without the application of sodium metabisulfite, 2.5ppm, 10 ppm, and 40 ppm SMB.

### Injection of Sodium Metabisulfite (SMB)

Sodium metabisulfite has chemical formula $NA_2S_2O_5$ with molecular weight of 190.11 and has CAS no. 7681-57-4.The experimental group *ovum was i*njected with 1 ml SMB. The administered dose of SMB was calculated based on the allowable content of SMB as preservatives in the Philippine food industry. Ten (10) pieces of eggs as control

were not treated with SMB while the eggs of the 3 experimental groups were injected with 1 ml of 2.5 ppm, 1 ml of 10 ppm, and 1 ml of 40 ppm of SMB. The injections were administered at the small end of the egg, deeply into the albumin.

**Examination of Egg**

The age, malformations, and malposition of the embryo were estimated at the moment of death. The incubated eggs were candled to determine viable egg after 18days. The incubated egg was candled to classify the viable and nonviable eggs. The eggs were then examined of any developmental deformities. The mortality were statistically analysed by *linear correlation*and regression.

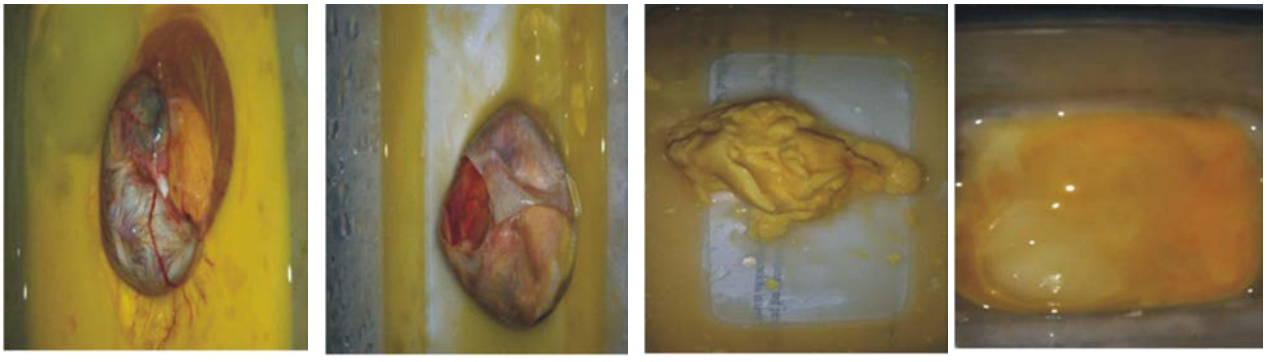
**Data Analysis**

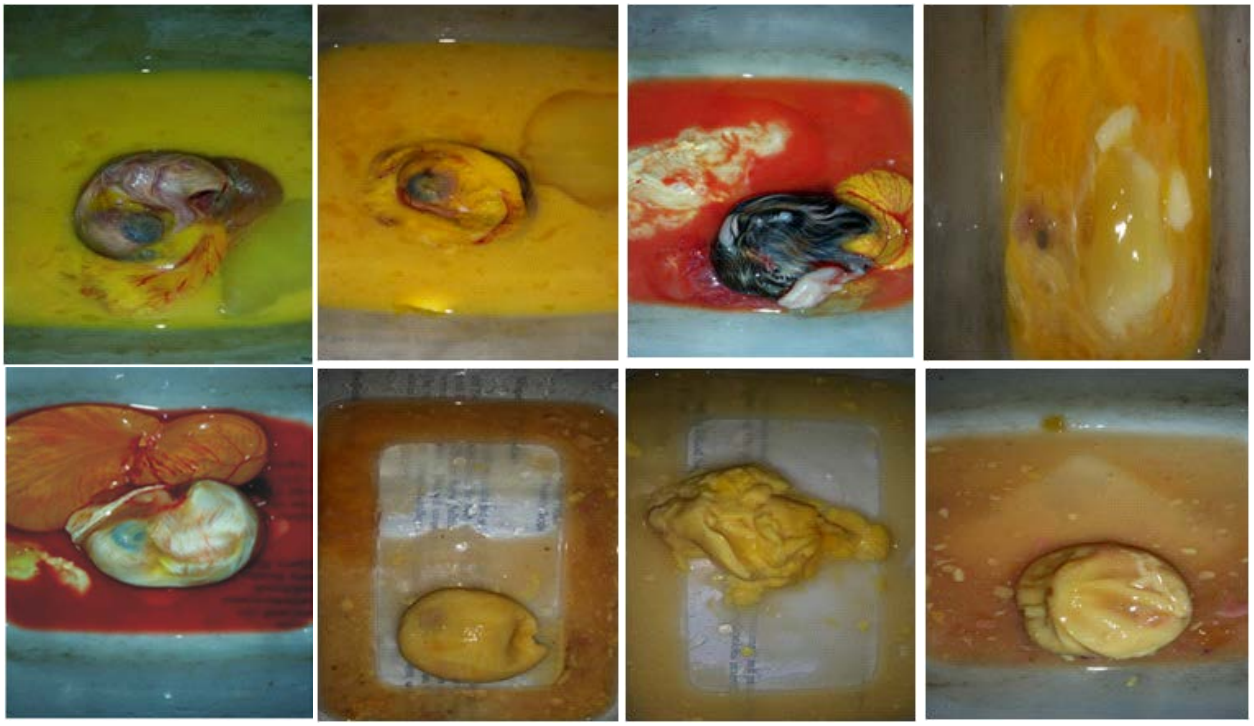
The Linear correlation and regression analysis was used to assess the obtained results. The statistical analyses were performed using Sigma-Stat (SPSS Science Software Ltd., USA).The results were presented as means ± SEM. and considered significant at $P<0.05$ and highly significant at $P<0.01$.

## RESULTS AND DISCUSSION

The use of chicken embryo test in a teratogenic study has been an indispensable tool in the determination of growth and developmental responses. Figure 1 shows the teratogenic effect of the different concentrations of sodium metabisulfite on the chick embryonic development. The 5 days incubation of the egg belong to the stages 26-28 of development based on the detailed series of normal stages in the development of chick embryo. The evaluation of the stages of development were done based on the work of Hamburger and Hamilton (1992).The control set up (Figure 1A) shows that the embryo grows rapidly and assumed to have a hatching position with the head under the right wing and beak toward the air cell. Remaining yolk sac begins entering the body cavity. The images shows the normal formation of the body parts of the chicken expected on its 5th day incubation period, the appearance of the potential basic body plans such as the head, wings, and the visibility of the beak. There is no observed anomaly or malformations on the 5th day of incubation period. The expressions of basic phenotypes are highly probable.

On the embryo administered with 2.5 ppm of SMB (Figure 1B), it shows that there are beak and limb deformities, scanty feathers, and the wings did not develop. However, the eyes are developed but head fails to form and there is clumping of potential circulatory system and presumptive brain. There were beak and limb deformities, scanty feathers, and failure of some vital organs were observed. Moreover, the embryo was still well covered with amniotic yolk sac and basic body plan is about to be developed. The growth of the embryo assumed a hatching position with the head under the right wing and yolk sac begins entering body cavity. While Figure 1C shows the chicken embryos injected with 10 ppm SMB. There is no development that took place, instead, there is a clumping of the blastoderm and basic body plan did not develop. The differentiation of basic body plan was suppressed. Also, Figure 1D shows the total inhibition of the embryonic development in eggs injected with 40 ppm of SMB, there is clumping and no cell differentiation that took place. The clumping of blastoderm, stunted development, and differentiation of basic body plan is suppressed.





A. control set up B. 2.5 ppm of SMB C. 10 ppm of SMB D. 40 ppm of SMB

**Figure 1. Teratogenic effect of the different SMB concentrations on the embryonic development of *Gallus gallus domesticus* after 5 days incubation.**

Moreover, to assess the general effect of SMB, the number of viable and nonviable eggs were counted and recorded. Accordingly, Table 1 shows that as the concentration increases, the number of mortality increases too. There are 20% of chick embryo that survived and 80% on 2.5 ppm concentration did not survive. While the effects of 10 and 40 ppm of SMB rendered the 100 % of the egg samples become nonviable. The numbers of nonviable eggs were strongly correlated to the concentration of SMB.

**Table 1. The effect of Sodium Metabisulfite (SMB) on the viability of chicken embryo.**

| Concentration(ppm) | # of Mortality | Survivor | % Mortality |
|---|---|---|---|
| Control | 1 | 9 | 10 |
| 2.5 | 8 | 2 | 80 |
| 10 | 10 | 0 | 100 |
| 40 | 10 | 0 | 100 |

Meanwhile, **Table 2** presented the statistical tool of linear correlation coefficient of 0.591 and regression coefficient 0.349. It suggested the positive relationship between concentration and mortality of chick embryo. The P value which is 1.37(P=0.01) is highly significant. This shows that the increased concentration of SMB contributes to the mortality of the test organism.

**Table 2. The linear correlation and regression of different concentrations of Sodium Metabisulfite (SMB)onthe mortality of chicken embryo.**

| r | $r^2$ | Slope | Y-Intercept | Std. Err. Of Estimate |
|---|---|---|---|---|
| 0.591 | 0.349 | 1.37 | 5.4516 | 4.2221 |
| t | df | P | One-tailed | 0.2047 |
| 1.035 | 2 | | Two-tailed | 0.4094 |

Note: 0.95 and 0.99 Confidence Intervals of rho

In addition, **Figure 2** shows the scatter plot graph of chick embryo. It shows the linear relationship of SMB concentration and the number of mortality. This result will suggest that there is a direct proportionality between SMB and chick mortality, that is, an increase in concentration causes an increase in mortality.

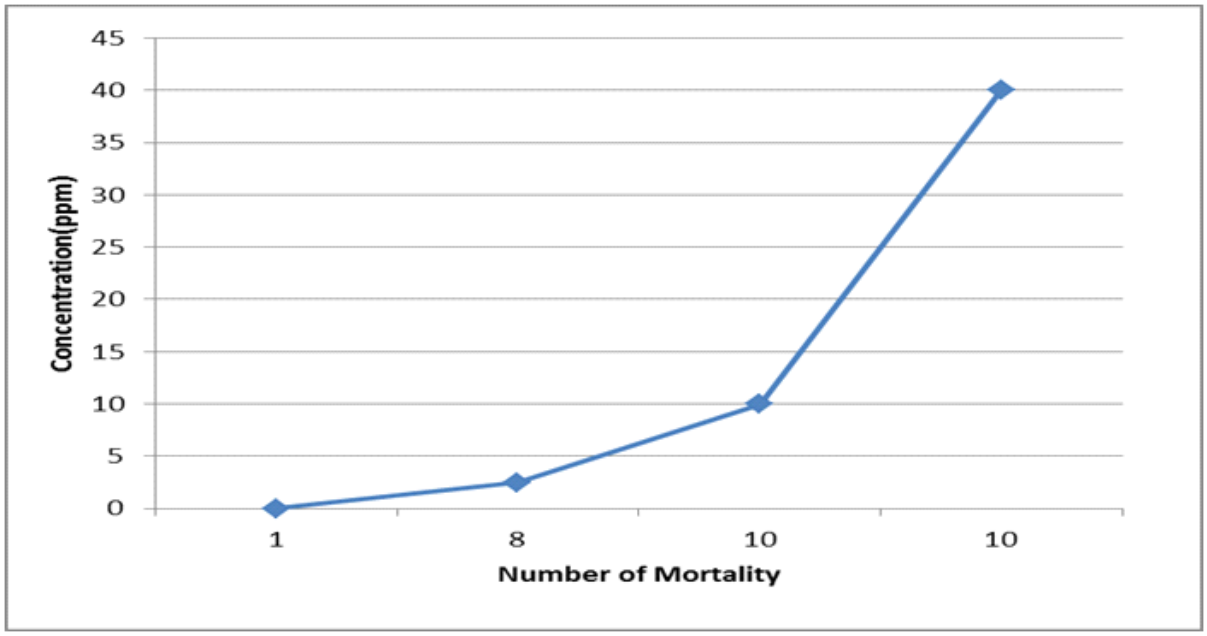



**Figure 2. The scatter plot of the effect of different concentration(y) of SMB on the mortality(x) of chicken embryo.**

The teratogenic responses of the chicken embryo have been dose-dependent. The higher the concentration of the administered SMB, there is a higher severity observed in morphological malformations. It also has suppressive effects on the developing structure of embryo. The concentration dependent effects of SMB were also reported by Shekarforoush et al. (2015), whereas, as the concentration of SMB increases, there is also a decrease total number of spermatogonia, primary spermatocyte and Leydig cells, decreased sperm count, decrease epididymal tubule diameter, and Testosterone. Also, there was a significant decrease in the Mitotic Index of the *Allium cepa* cells, the mitotic cells decreased as the concentrations and duration of treatment increased. Some chromosomal mutations were observed such as chromosome clumping, chromosome bridge and chromosome fragmentation (Rencuzouullari et al. 2001). The reduction in the mitotic activity could be due to inhibition of DNA synthesis which might be caused by the reduction ATP production, which is essential in the stages of mitosis. A decrease in mitotic index could be attributed to the blockage at the G2-phase of the cell cycle, preventing the cell from entering mitosis (Onyemaobi et al. 2012).

The neurotoxic effect of SMB is due to the release of sulphur and oxygen that can damage the development of tissues including the central nervous system. SMB renders toxic effects on the embryonic development (El Kadi et al. 2014).Furthermore, sulfites exerts toxic effects on neuronal cells grown directly or in combination with peroxynitrite. Sulphite oxidase is the key enzyme in the metabolism of sulphites in the body that causes oxidation to sulphate, thus develop severe abnormalities and early death (Reist et al. 1998).In addition SMB has been shown to induce chromosomal aberration, sister chromatic exchange and micronuclei and decrease mitotic index in human lymphocytes (Meng and Zhang, 1999). Bisulfite causes deamination of cytosine in both DNA and RNA (Pagano et al. 1990).The deamination of cytosine causes base-pair substitution mutations. Bisulfite has been shown to cause deamination in cytosine and

adenine. Thus, studies in chicks suggested that the limb abnormalities are most probably caused by transient inhibition of cell division rather than cell death (Brewton and Mac Cabe, 1990).

## CONCLUSION

The administered concentration of SMB triggered major alterations and suppressions in the development of chicken embryo after the 5$^{th}$ day of incubation. Moreover, as the SMB concentration increased, the egg mortality and severity of malformations also increased. The results led to considerations on the evaluation of the allowed amount of SMB in food preservatives for safe human consumption. Furthermore, the researchers recommended that an in-depth study must be conducted about the effects of the usage of SMB preservative in pregnancy and children to understand the consequences of this preservative in early growth and development.

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