Chlorocholine chloride residue distribution in eggs, breast and femur meat of laying hens determined by labelled-^{15}N estimates

Nurhayati [1, 2], J.D. Kabasa[1, 3#], G. Thinggaard[1] and U. ter Meulen[1]

[1]Institute of Animal Physiology and Animal Nutrition, Göttingen University, Kellnerweg 6, D-37077, Germany
[2]Department of Animal Nutrition and Feed Science, Jambi University, Kampus Pinang Masak Mendalo Darat, Jambi 36361 Indonesia
[3]Faculty of Veterinary Medicine, Makerere University, P.O. Box 7062, Kampala, Uganda

Abstract

The distribution of chlorocholine chloride (CCC) residue or its metabolites in the meat and eggs of laying hens was studied using the ^{15}N delta value (δ^{15}N) and ^{15}N atom % derived from ^{15}N-CCC containing diets. In a completely randomised design, 20 laying hens were divided into four groups allocated four different diets namely; 0 mg ^{15}N-CCC /kg feed a control diet (group A); 5 mg ^{15}N-CCC /kg feed (group B), 50 mg ^{15}N-CCC /kg (group C) and 100 mg ^{15}N-CCC /kg (group D) for 11 days. During the seven days that followed, ^{15}N-CCC diets were withdrawn and all hens were restored to feeding on the control diet. The δ^{15}N excess and ^{15}N atom % excess in meat and eggs of hens fed diets containing ^{15}N-CCC, were higher than in the control diet after 11 days of treatment and seven days after withdrawal of ^{15}N-CCC, except for the egg yolk values of hens fed 5 mg ^{15}N-CCC /kg feed. The δ^{15}N excess and ^{15}N atom % excess of meat, egg yolk and egg albumen were dependent on dietary ^{15}N-CCC concentrations and differed significantly between tissues for each of the three ^{15}N-CCC concentrations examined. Femur meat δ^{15}N excess and ^{15}N atom % excess were similar to that of breast meat but differed significantly from that of other tissues. The results show that tissue type is a factor in CCC residue/metabolite accumulation in chicken products suggesting differences in exposure or risk of CCC on consumers.

Keywords: Chlorocholine chloride residues, poultry products, labeled-^{15}N
[#]Corresponding author. E-mail: kabasajd@vetmed.mak.ac.ug

Introduction

Chlorocholine chloride (CCC), commonly known as Chlormequat, is a plant growth retardant widely used in commercial agriculture to improve the shape, size and yield of cereal crops. The CCC residues and its metabolites accumulate in cereal plants and can be detected in different plant products (Blinn, 1967; Bier & Dedek, 1970; 1972; Bohring, 1972; 1982). Although a small quantity of residue of CCC is allowed in foodstuffs, there are concerns that the residue may be harmful to both animals and humans. Consumption of excess CCC tended to increase the incidence of cancers such as leukaemia in rats (National Cancer Institute, 1979). Reports that CCC is completely metabolised into other compounds without leaving any residues in the tissues are not conclusive (Dekhuijzen & Bodlaender, 1973; Dekhuijzen & Vonk, 1974). To protect animals and humans from the intake of CCC residues in food, the European Union has set maximum CCC residue limits of 2 mg/kg for cereals, except for oats (5 mg/kg) and pears (10 mg/kg). Using radioactive-labelled CCC at different concentrations in feed, several workers demonstrated the presence of CCC residues in rats (Blinn, 1967; Romanowski, 1972; Gonzales, 1997), cows (Lampeter & Bier, 1970), oviducts of laying hens (Landazuri, 1992) and ovaries of pigs (Azem, 1996). When different concentrations (5, 50 and 250 mg) of ^{15}N-labelled CCC/kg feed were fed to laying hens, significantly higher ^{15}N concentrations were recorded in the egg yolk and albumen from hens fed the diets containing 250-mg/kg ^{15}N-CCC (Songsang, 2000). The extent of CCC residue distribution in tissues may, however, differ due to differences in environmental factors, genotype, physiological and nutritional state of the individual (Ackermann *et al*., 1975; Sachse, 1977; Torner *et al.*, 1999). High CCC concentrations in tissues increase the exposure risk of individuals consuming food products from such tissues. This study investigated the distribution of CCC residue and its metabolites in the eggs and meat of laying hens fed diets containing varying concentrations of CCC. For the purpose of this investigation the ^{15}N-labelled CCC (^{15}N-CCC) estimating method was used to trace the fate of the compound.

Materials and Methods

Twenty 280-day old Brown-breed laying hens, weighing between 1727 and 2269 g, were housed in individual layer cages and fed for seven days on a control diet consisting of ^{15}N-CCC free wheat, maize, fish meal, a vitamin-mineral premix and feed lime. The diet contained 163.7 g crude protein/kg and 11.48 MJ ME/kg. Thereafter, the hens were divided randomly into four experimental groups. Each group was allocated one of the four experimental diets: a control diet containing 0 mg ^{15}N-CCC/kg (group A) and diets containing 5 mg ^{15}N-CCC/kg (group B), 50 mg ^{15}N-CCC/kg (group C) and 100 mg ^{15}N-CCC/kg (group D). The ^{15}N-CCC (atom ^{15}N content, 99%) (MSD Isotopes, MERCK-FROSST Canada Inc., Montreal, Canada) was mixed in feed to concentrations of 5, 50 and 100 mg/kg in the final diet. The experimental diets were fed for 11 days, after which the diets containing ^{15}N-CCC were withdrawn and the hens were fed the ^{15}N-CCC free diet for seven days. Feed and water were offered *ad libitum* and cage temperatures were maintained at 19 – 21 °C during the experiment.

Eggs were collected daily, and the yolk and albumen portions of the eggs were separated immediately after collection using an egg separator (Labortek, Germany). The eggs from the different treatments were processed in separate rooms with separate equipment to avoid cross contamination with ^{15}N. Meat samples were collected from the breast and femur muscles by biopsies on day 11 of the experimental period and seven days after ^{15}N-CCC withdrawal. All samples were weighed, analysed for dry matter content and stored at -21 °C until further analyses.

The 11 days of experimental feeding were justified because yolk deposition takes place in 10 to 11 days (Etches, 1996; Hartmann, 2001). The seven days withdrawal period adopted was based on the observations of Coffman *et al.* (1999) that at least seven - eight days prior to slaughter are required to keep food derived from hens supplemented with antibiotics and/or drugs safe for human consumption.

The measurement of ^{15}N was done according to the method described by Reineking *et al.* (1993) in the Forschungszentrum Waldökosysteme, Kompetenszentrum Stabile Isotope (KOSI) Göttingen University, Germany. The procedure involved using an elemental analyser (EA, Carlo-Erba 1500 nitrogen analyser) coupled into the Finnigan MAT Continuous Flow interface (ConFlo II^{TM}), which connects to Finnigan MAT Isotope Ratio Mass Spectrometry (IRMS, Finnigan MAT 251). Acetanilide (C_8H_9NO) was the standard material for checking the analytical variability. It had a composition of 71.1% (C), 10.4% (N), 6.7% (H) and 11.8% (O) and contained 0.366943 of ^{15}N atom % and 1.76 ^{o}/oo of δ^{15}N. The relative δ^{15}N was calculated using the formula of Mariotti (1983):

$$\delta^{15}N = \left[\frac{R_{sa}}{R_{wst}} - 1\right] \times 1000$$

$$R_{sa} = \frac{i_{29_{sa}}}{i_{28_{sa}}} \qquad R_{wst} = \frac{i_{29_{wst}}}{i_{28_{wst}}}$$

The ^{15}N atom % was calculated based on the international standard of ^{15}N in air (Mariotti, 1983) as follows:

$$^{15}\text{N atom \%} = \frac{R_{ist}\left(1000 + \delta^{sa}/_{ist}\right) \times 100}{R_{ist}\left(1000 + \delta^{sa}/_{ist}\right) + 1000}$$

where :

R = absolute isotope ratios, measured for the sample and standard. The value of absolute isotope ratios of international standard in Goettingen was 0.003676496.
wst = work standard,
sa = sample,
i = current ion which i_{29} is ion of ^{15}N and ^{14}N, i_{28} is ion of $^{14}N_2$
ist = international standard

Atom % excess (APE) and delta value excess (δ^{15}NE) were calculated by difference between the ^{15}N values of the treatment and that of the control. Similarly, the difference between δ value of the working standard and international standard of –13.468 was obtained.

Results and Discussion

The results of excess δ^{15}N and excess atom % ^{15}N in egg yolk, egg albumen, breast and femur meat of hens fed diets with varying levels of ^{15}N-CCC for eleven days are shown in Table 1 and Figure 1. The 11

days of feeding diets containing ^{15}N-CCC resulted in increased δ^{15}NE and APE in the meats and eggs of hens ($P < 0.05$). The significant increases were more in hens fed higher concentrations of ^{15}N-CCC. The δ^{15}NE and APE values were highest in the egg yolks and decreased in the order: egg yolk > egg albumen > breast meat > femur meat.

The contribution of ^{15}N in the meat N was lower than in the eggs at all doses examined. The results are similar to that of Wilbur (1975) who found that after 24 hours of oral application of 3 mg/kg CCC per laying hen per day, the CCC residue was higher in the yolk than in the albumen. However, residue levels in the muscle were not detectable (<0.01 mg/kg).

Table 1 Excess δ^{15}N in eggs and meat of laying hens eleven days after feeding on diets containing varying concentrations of ^{15}N-CCC

	Excess δ^{15}N			
Chicken product	5 mg ^{15}N-CCC /kg diet	50 mg ^{15}N-CCC /kg diet	100 mg ^{15}N-CCC /kg diet	MSE
Femur meat	0.360^{c}	0.592^{b}	0.885^{a}	0.005
Breast meat	0.547^{c}	0.751^{b}	1.026^{a}	0.001
Egg yolk	2.254^{c}	4.050^{b}	6.754^{a}	0.490
Egg albumen	1.652	1.962	2.381	0.562

$^{a, b, c}$ Row means within the same row and with different superscripts differ ($P < 0.05$)

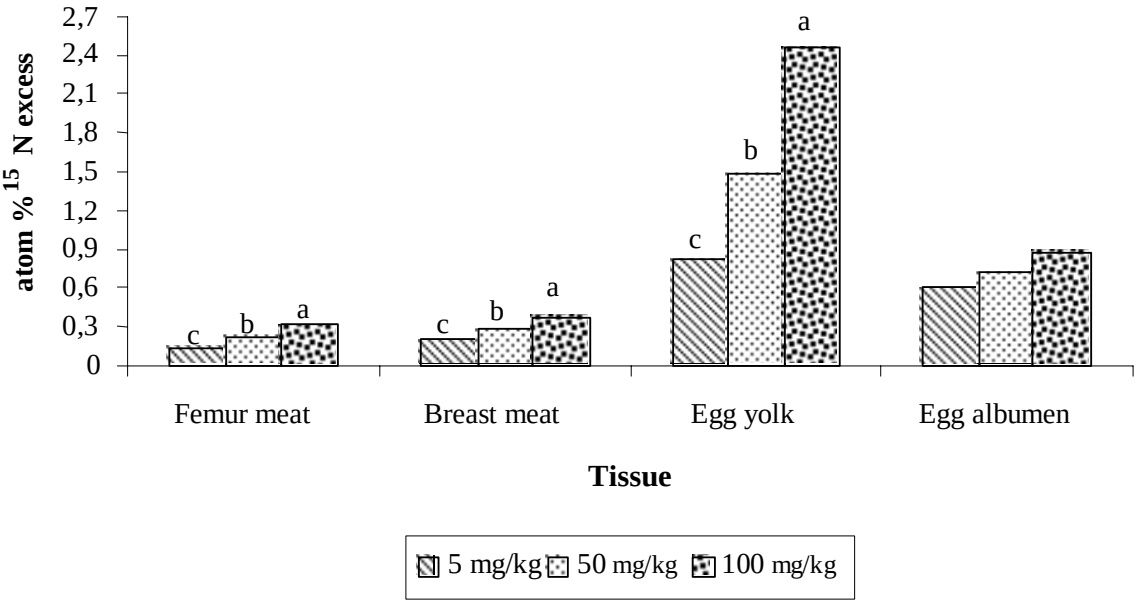

Figure 1 The effect of 11 days of feeding diets containing ^{15}N-CCC on ^{15}N atom % excess in eggs and meat of laying hens

$^{a, b, c}$ Mean values of the same tissue with different superscripts differ ($P < 0.05$)

The pattern of the nitrogen distribution might be due to differences in tissue protein metabolism and flowing rate of nutrients during the production phase that is more in the egg than in muscle tissue. This is similar to reports by Paulson *et al.* (1983) that recorded the lowest pesticide residues concentration in the skeletal muscle of hens. Residue accumulation in the body is affected by several factors including post metabolism persistence of the applied compound and its rate of degradation (Paulson *et al.*, 1983).

The results of excess δ^{15}N value and that of excess atom % ^{15}N (i.e. differences between the treatment and control values) in the eggs and meat of hens seven days after withdrawal of ^{15}N-CCC diets are shown in Table 2 and Figure 2, respectively. Seven days after ^{15}N-CCC diet withdrawal, δ^{15}NE and APE concentrations in the meat of hens fed CCC treated feeds were still higher ($P < 0.05$) than in the control diets. For the eggs (yolk and albumen), the concentrations did not differ ($P < 0.05$) at any treatment level.

However, all values were lower than those observed after 11 days of feeding on treated diets. The δ^{15}NE and APE in meat differed significantly at $P < 0.05$ among treatments, whereas those of egg yolk and albumen were similar during the same time period of eleven days.

Table 2 Excess δ^{15}N of eggs and meat of laying hens seven days after withdrawal of diets containing varying amounts of ^{15}N-CCC

Chicken product	Excess δ^{15}N			MSE
	5 mg ^{15}N-CCC / kg diet	50 mg ^{15}N-CCC / kg diet	100 mg ^{15}N-CCC / kg diet	
Femur meat	0.089[c]	0.301[b]	0.560[a]	0.001
Breast meat	0.161[c]	0.431[b]	0.660[a]	0.009
Egg yolk	0.626	1.117	1.240	0.556
Egg albumen	0.102	0.752	1.011	12.300

[a, b, c] Row means within the same row with different superscripts differ ($P < 0.05$)

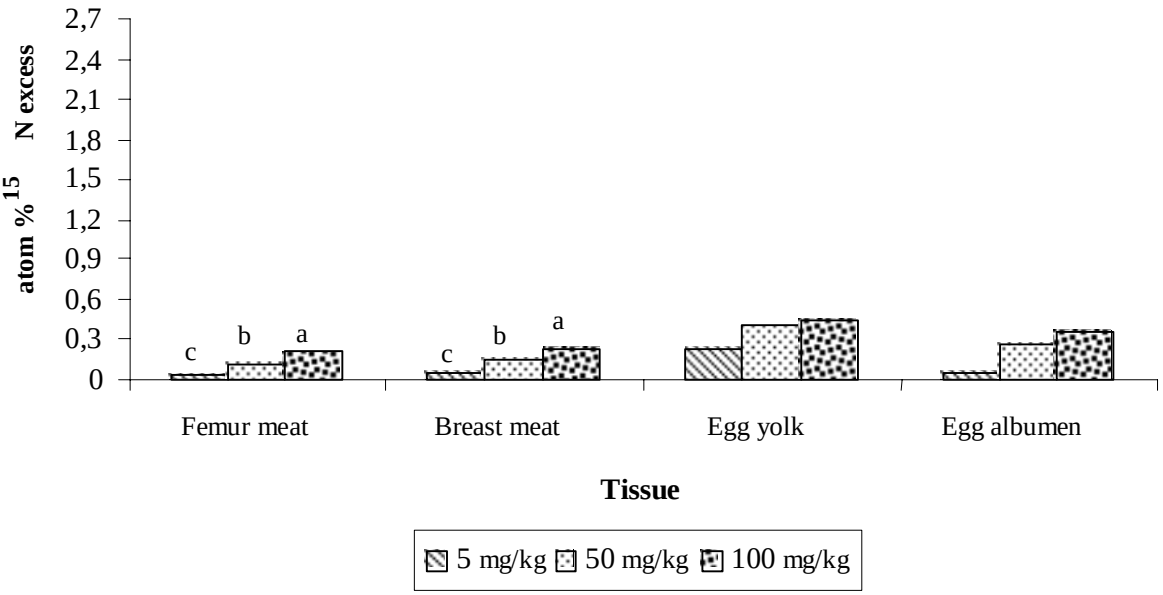

Figure 2 The ^{15}N atom % of the eggs and meat of laying hens seven days after withdrawal of diets containing ^{15}N-CCC

[a, b, c] Means for the same tissue with different superscripts indicate differences ($P < 0.05$) between groups

Differences in tissue metabolism could explain these observations. Calsamiglia *et al*. (1996) observed differences in ^{15}N levels and attributed their findings to variation in metabolism. The low mobility of ^{15}N-CCC could also be a factor (Bohring, 1972; 1982) as it promotes tissue retention of the compound without any metabolism to the other compounds (Bier & Dedek, 1970; 1972). Other authors (Katz, *et al.*, 1973; Beale *et al.*, 1990; Shaikh & Chu, 2000; Furusawa *et al*., 2002) reported varying levels of drug residues in tissues, organs and eggs of poultry at different days after drug withdrawal. The residue levels were attributed to the body fat content that affects drug release back into the blood.

This study investigated the potential distribution of CCC residues in tissues of laying hens. Labelled ^{15}N was used because it is a heavy isotope with a natural abundance of < 1% that is relatively constant in air. This abundance was approximately 0.366% at the time of the study. Since the CCC is a polar compound with low volatility, and the ^{15}N was not degraded in the animal body under the experimental conditions, using ^{15}N as a label of CCC was beneficial. The application of ^{15}N in experimental diets enabled the quantification of the potential distribution of the dietary CCC and its metabolites in the chickens.

Conclusion

The results suggest that the CCC residue and/or its metabolites are distributed in chicken meat and eggs in varying concentrations. The ^{15}N content was highest in egg yolk, followed by egg albumen and breast and lowest in femur meat. The nature of CCC metabolites in the chicken needs clarification.

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