CYTOTOXICITY, BIOCHEMICAL EFFECT AND ENVIRONMENTAL IMPACT OF MONENSIN ON DAIRY CATTLE

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ABSTRACT

Received at: 21/4/2015	Rumensin (monensin) is the first ionophore to be approved by the Food and Drug Administration (FDA) for increased milk production efficiency. Monensin treatment
Accepted: 21/6/2015	at dose of 10 gm /ton in total mixed ration lead to increase both milk production and body weight through its effect on increase feed digestibility, microbial digestion and also decrease losses of nitrogen in feces which lead to decrease environmental haz ards of nitrogen on both animal and public health. More over there is no cytotoxicity recorded due to monensin treatment at dose of 10gm/ton. Monensin treated animals showed significant increase in total protein serum level, globulin serum level, urea serum level, and cholesterol serum level level while had no effect on albumin, tota bilrubin and glucose serum levels. Taken together, monensin is safe for use in dairy animals and has an environmental impact role.

Key words: Cytotoxicity, Monensin, Dairy Cattle, Biochemical Effect.

INTRODUCTION

In 2050, we will need to increase the food production by 100% to be enough for population over the world. so we need to increase the production of food especially of animal source through the use of advanced technology. Important one is growth promoting agent and biologcal gene modulation of animal breeds although, European and Egyptian laws refuse completely the use of hormones.

Rumensin (monensin) is the first ionophore to be approved by the Food and Drug Administration (FDA) for increased milk production efficiency (production of marketable 4.0% solids-corrected milk per unit of feed intake) when fed to dairy cows. Monensin is an ionophore widely used in the dairy cattle industry throughout the world end especially in germany (Emmerich *et al.*, 2013). More over, there are many clinical experiments indicated that Monensin explored efficacy for various metabolic, production, and health outcomes of dairy cattle (Duffield *et al.*, 2012) and a number of investigations have demonstrated that monensin increases milk yield when fed to cows offered mixed grain and forage diets (Granzin and Dryden, 1999 and Dubuc *et al.*, 2010).

The dairy industry has improved the efficiency of milk production over the years. Total mixed rations and other factors had led to a more than four fold increase in milk production per cows since 1940. However, the feed required for production of that milk only increased two fold. These changes led to a doubling in the efficiency of milk production. However, milk production efficiency (MPE) as a metric, is not commonly measured like feed conversion is in other livestock enterprises. For example, in the U.S. A. about 95% of the cattle in feedlots fed rumensin in their rations (Raun, 1990).

Sodium monensin, an ionophore antibiotic produced by Streptomyces cinnamonensis had many benefits such as modified the ruminal flora (Hamilton *et al.*, 2010 and McGarvey *et al.*, 2010) and improved the digestive efficiency of cattle. The effects of monensin supplementation include increased ruminal propionate production, reduced in vivo and in vitro production of methane, increased dry matter and starch digestibility, decreased production of bacterial protein in the rumen, increased nitrogen retention and significantly increased flow of amino acids to the duodenum and digestion of amino acids in the duodenum. A decreased ruminal turnover rate and increased rates of ruminal fill have been noted with treatment and monensin can modify the flux of ions across epithelial cells of the intestine and increase the uptake of calcium, selenium, and other cations. The treatment of lactating dairy cows with monensin has resulted in increased plasma glucose concentrations and decreased plasma ketone concentrations. The capacity of monensin to alter metabolism suggests that the effects of monensin treatment on reproduction, health, and production of dairy cows require further investigation (Beckett *et al.*, 1998).

There are a closely link between the amount of feed consumed and amount of milk production (Britt *et al.*, 2004).

More over, Series of several conducted mutagenicity studies was provided (Ames, chromosome aberration test in vitro and a micronucleus test in vivo) that Monensin was negative in the Ames test, was not clastogenic in vitro and did not increase the incidence of micronucleated cells in vivo.

While in the in vitro cytogenetic assay the relevelance diplochromosomes remained unclear (Veterinary medicine and European medicine agency, 2007). The rational of this study to investigate the cytotoxicity and safety of monensin use for dairy cattle.

MATERIALS and METHODS

I- Materials. 1) Rumensin^R.

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Rumensin was kindly obtained from Elanco Company for pharmaceutical preparation, Egypt. **Common name.** Monensin **Trade name.** Rumensin **Chemical name.** sodium (2R,3S,4S)-4-[(2R,5R,7S,8R,9S)-2-[(2R,5S)-5-ethyl-5-[(2S,3R,5S)-5-[(2S,3S,5R,6S)-6-hydroxy-6-(hydroxymethyl)-3,5dimethyl-oxan-2-yl]-3-methyl-oxolan-2-yl]oxolan-2yl]-9-hydroxy-2,8-dimethyl-1,6dioxaspiro[4.5]decan-7-yl]-3-methoxy-2-methylpentanoate

2)- Kits.

- 1. Total protein kits (dp international)
- 2. Albumin kits (Dimond Diagnostics).
- 3. Glucose kits (Spinreact)
- 4. Urea kits (Dimond Diagnostics)
- 5. Cholesterol kits (Spinreact)
- 6. Total bilirubin Kits (APC Diagnostics)

3)- Equipments.

Spectrophotometer (Hang Fen 7230, china) Automatic pipettes

4) Experimental animals.

this experiments in his dairy farm on Damita governorate on 20 Holstein dairy cow, after first delivery were fed on total mixed ration (TMR) which consists of silage, corn, soy bean, cotton seed cake, hay, mineral mixtures and vitamins mixtures Total mixed ration (TMR)

	TMR	
Ingredient % of DM		
silage	38.39	
corn	12.79	
Soy bean meal	11.9	
Cotton seed cake	15.3	
hay	21.3	
Mineral mixtures	0.08	
Vitamins mixtures	0.08	

Table 1: Ingredients, composition, of the experimental total mixed ration (TMR) for experimental cows.

Ration was done according nutrient requirements of dairy Cattle animal needs during lactation period especially energy and protein ratio (NRC., 2001)

Methods

Monensin treated animals.

We examined the effects of monensin on feed intake and milk production in 10 Holstein cows fed total mixed ration supported with monensin while the other 10 Holstein cows fed total mixed ration without monensin. Diets were fed for ad libitum intake four times a day and water was also available for ad libitum intake. The experimental period lasted 3 wks and comprised 2 wks of adaptation to monensin, as rec-

ommended by Thornton and Owens (1981), and 3 wks of experimental observations. The daily individual dose of the monensin premix was mixed with 140 g of Soy bean meal at dose of 10g/ ton and then added to the diet of the specific cows. The cows were milked twice daily and milk production of each cows was recorded daily. Blood samples were collected into sterile tubes for separation of serum and isolation of Buffy coat for culturing of lymphocyte. Serum was analyzed for urea, glucose, cholesterol, total bilirubin, total protein and albumin levels.

1. Biochemical analysis.

a- Determination of serum total protein was determined according to the method of Henery (1964).

b- Determination of serum albumin was determined according to the method of Doumnas *et al.* (1971).

c- Determination of serum glucose level.was determined according to the method of Kaplan (1984).

d- Determination of serum bilirubin level was determined according to the method of Jendrassik and Grof (1938).

g- Determination of serum urea level was determined according to the method of Patton and Crouch (1977).

h- Determination of serum cholesterol level was determined according to the method of Naito and Kaplan (1984).

2- Weighting of animals before and after treatment to assess effect of monensin on body weights.

3- Weighting of daily milk yield of each animals to assess effect of monensin on milk yield.

5. Determination of Chromosomal Aberrations in cultured lymphocyte

Blood cells from dairy cows fed on diet enriched by monensin were cultured for 72 h at 38°C in 5 mL TCM-199, 1 mL fetal calf serum and 0.1 mL phytohaemagglutinin (PHA). After incubation, cells were treated with colchicines (0.05%) for 2 h, then with a hypotonic (0.075 M KCl) for 30 min. After fixation in acetic acid. ethanol (1.3) solution, the cells suspensions were dropped on wet slides then flamed to dry. The slides were stained with Giemsa stain and covered with DPX mounting media for chromosomal analysis. Chromosomal abnormalities were recorded in at least 50 metaphase spreads for each animal

4. Statistical analysis.

Data obtained in this study were statistically analyzed for student T-test

RESULTS

1- Biochmical changes due to administration of monensin

Monensin treated animals showed significant increase in total protein serum level, globulin serum level, urea serum level and cholesterol serum level when compared with control group while had no effect on total bilrubin, serum level albumin and glucose serum levels when compared with control group. The present study found that monensin improved the productivity of animal through increase immunity levels, especially high level of globulin and avoid the animal sub-clinical infection. More over, increase the serum protein level is very important for milk production. Additionally, There is no effect of monensin on glucose level and these results could attributed to glucose precursors, primarily propionate and amino acids, become essential for a successful lactation. Most of this glucose is produced by liver, and propionate is the single largest contributor to liver glucose production. Glucose synthesis must increase to meet the needs of lactose synthesis. These results illustrated in table (2) and fig (1)

2. Effect of administration of monensin on both body weights and daily milk yield

There was a significant increase in body weight of dairy cows treated with monensin and this result could be attributed to Monensin shifts the microbial population in the rumen by promoting the growth of more efficient bacteria involved in carbohydrate metabolism. This results in an increase in propionate production in the rumen Thus, more energy is obtained from every pound of feed. Also increase feed efficiency through increase feed digestibility and greater supply of bypass protein to the small intestine and a subsequent increase in the use of absorbed nonessential AA for gluconeogenesis. This would lead to a rise in deamination of these AA and higher concentration of BUN.

From environmental view, monensin supplementation increased ruminal propionate production and subsequently propionate reduced in vivo and in vitro production of methane. So monensin is useful for safety of environment and prevents environmental sharp change. These results illustrated in table (3) and fig (2).

3. Estimation of chromosomal aberration of cultivated lymphocyte

The present study showed that lymphocyte of dairy cattle treated with monensin showed no chromosomal aberration at dose of 10gm/ ton. More over there was no diplochromosomes.

	Total protein g/dl	Albumin g/dl	Globulin g/dl	Urea mg/dl	Glucose mg/dl	Cholesterol mg/dl	Total Bilirubin mg/dl
	T vs C	T vs C	T vs C	T vs C	T vs C	T vs C	T vs C
Mean	31.8 ^a 18.8 ^b	4.41 ^a 4.1 ^a	27.8 ^a 14.7 ^b	50.4 ^a 39.5 ^b	61 ^a 59.9 ^a	239.8 ^a 190 ^b	20.8 ^a 20.7 ^a
S. E.	±1.3±0.28	±0.8±0.34	±3.89±0.98	±3.8±1.5	±1.1 ±0.4	±17.8±11.8	±0.5 ±0.5

Table 2: Showing biochemical changes due to monesin administration at dose of 10 gm/ton for dairy cows.

A, b, c, d. Different letters are significantly different between groups at P \leq 0.01%

Table 3: Showing the effect of monensin administration at dose of 10 gm/ton on body weights and milk yeild of dairy cows

	Body weights		Milk yeild		
	T vs	С	T vs	С	
Mean	565.5ª	482.5 ^b	23.7 ^a	20.65 ^b	
S.E.	±25.4	± 18.9	±1.8	± 1.08	

A, b, c, d. Different letters are significantly different between groups at P \leq 0.01%

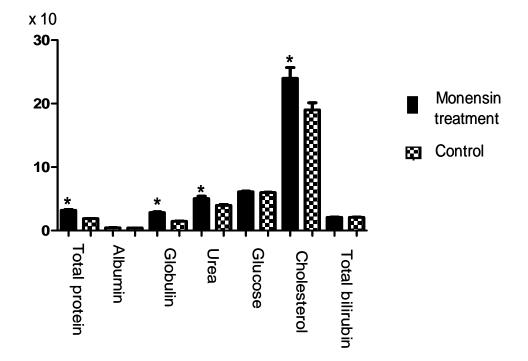


Fig. (1): Showing biochemical changes due to monesin administration at dose of 10 gm/ton for dairy cows

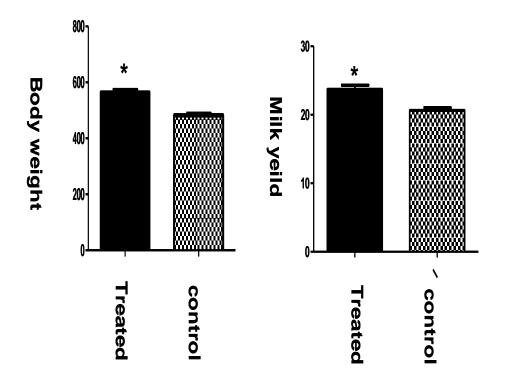


Fig. (2): Showing the effect of monensin administration at dose of 10 gm/ton on body weights and daily milk yeild of dairy cows.

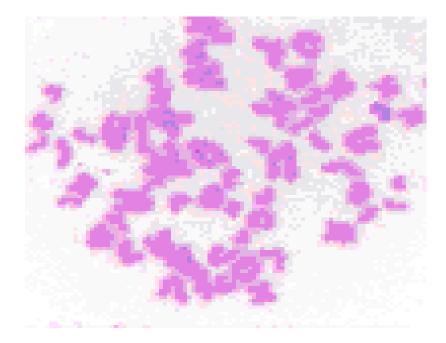


Fig. 3: Lymphocyte of dairy cattle treated with monensin show no change at dose of 10gm/ ton.

DISCUSSION

Rumensin (monensin) is the first ionophore to be approved by the Food and Drug Administration (FDA) for increased milk production efficiency (production of marketable 4.0% solids-corrected milk per unit of feed intake) when fed to dairy cows.

There are a closely link between the amount of feed consumed and amount of milk production (Britt *et al.*, 2004).

The current study supports earlier trials, which established that the inclusion of monensin in dairy cows diets would increase milk yield (Lean and Wade, 1997). The likely mechanism of action to support additional milk yield is that monensin increased the supply of glucogenic precursors resulting from changes in pattern of rumen fermentation.

Increases in blood urea nitrogen due to monensin in dairy cows have been reported previously (Duffield et al., 2012). These studies had much larger sample sizes, which could explain why they obtained significant increases, whereas only numeric increases were observed in our study. Duffield et al. (2012) suggests that this increase is due to a greater supply of bypass protein to the small intestine and a subsequent increase in the use of absorbed nonessential AA for gluconeogenesis. This would lead to a rise in deamination of these AA and higher concentration of BUN. The significant increase in apparent digestibility postcalving and the numeric increase in this digestibility precalving found in our study supports this theory. Also this result agreed with Haimoud et al. (1995) who investigated the effect of monensin (33ppm) on nitrogen, starch and fibre digestion in the lactating and dried dairy cows and found that compared with control cows, monensin reduced rumen degradation of protein allowing greater flow of amino acids to the small intestine. In the same hand, increased milk and blood urea concentration resulted because both parallel dietary Crud Protein content (Broderick and Clayton, 1997).

There is no effect of monensin on glucose level in our work and these results could attributed to glucose precursors, primarily propionate and amino acids, become essential for a successful lactation. Most of this glucose is produced by liver, and propionate is the single largest contributor to liver glucose production. Glucose synthesis must increase to meet the needs of lactose synthesis. Lactose concentration is fairly constant in milk. Glucose is also used to generate reducing equivalents for the synthesis of milk fat (Angel, 2005). Glucose concentrations were not significantly affected by monensin in the current study. This result agree with Mullins *et al.* (2012) who found that monensin supplementation had no effect on plasma glucose and insulin level dairy cows.

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However, numerical trends support previous studies. There may have been a lack of power to illustrate significant effects in the current project. Stephenson *et al.* (1997) disagreed with our study and reported that monensin treated-cows had significantly lower glucose values in the immediate precalving period. Other researchers have reported significantly higher glucose concentrations in monensin treated cows postcalving (Duffield *et al.*, 2012).

The higher cholesterol values suggest that there is greater lipoprotein export from the liver (Gerloff et al., 1986; Kaneene et al., 1997). The data are consistent with Green et al. (1999) who reported a tendency for lower serum β -hydroxybutyrate concentrations during the last 2 wks precalving in cows treated with a monensin at 3 wks before expected calving compared with placebo treated-cows. The data are also supported by Stephenson et al. (1997), who reported that monensin treated-cows had significantly lower β -hydroxybutyrate and non esterified fatty acids values precalving. However, those data were generated in only 24 cows from two dairy farms and they were managed under a pasture feeding system. This finding was attributed to improved liver function through reduced liver fat deposition. In the current study the results reflect less fat transported to the liver (lower nonesterified fatty acids precalving) combined with greater fat export from the liver (higher cholesterol) which supports the hypothesis that monensin inhibits accumulation of triglycerides in the liver of peripartum dairy cows.

There was a significant increase in body weight of dairy cows treated with monensin and these result could be attributed to Monensin shifts the microbial population in the rumen by promoting the growth of more efficient bacteria involved in carbohydrate metabolism. This results in an increase in propionate production in the rumen (Russell, 1989). Thus, more energy is obtained from every pound of feed. Also increase feed efficiency through increase feed digestibility and this agreed with Duffield et al. (2012) suggests that this increase is due to a greater supply of bypass protein to the small intestine and a subsequent increase in the use of absorbed nonessential AA for gluconeogenesis. This would lead to a rise in deamination of these AA and higher concentration of BUN. The significant increase in apparent digestibility postcalving and the numeric increase in this digestibility precalving found in our study supports this theory. In contrast, Rumensin has been shown to reduce ammonia production and microbial populations in vitro; thus, it would be assumed to reduce ruminal ammonia production and subsequent urea production and consequently affect urea recycling (Recktenwald et al., 2013).

Notably, monensin had stronger antimethanogenic effects in beef steers than dairy cows, but the effects

in dairy cows could potentially be improved by dietary compos tion modifications and increasing the monensin dose (Grainger *et al.*, 2010 and Appuhamy *et al.*, 2013)

Finally, the present study showed that lymphocyte of dairy cattle treated with monensin show no chromosomal aberration at dose of 10gm/ ton. This result was agree with report of Veterinary medicine and European medicine agency, 2007 for inspection of products for veterinary use.

Author contributions

1) All Authors make substantial contributions to conception and design, and/or acquisition of data, and/or analysis and interpretation of data;

2) Elalfy mahmoud who participate in drafting the article or revising it critically for important intellectual content

3) Elalfy mahmoud who give final approval of the version to be submitted and any revised version.

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CONCLUSION

Any improvement in the conversion of feed to milk has a direct impact on the profit margin of the dairy farm. Our study found that monensin treatment at dose of 10 gm /ton in total mixed ration lead to increase both milk production and body weight through its effect on increase feed digestibility, microbial digestion and also decrease losses of nitrogen in feces which lead to decrease environmental hazards of nitrogen on both animal and public health. More over there is no cytotoxicity recorded due to monensin treatment.

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التاثير السمى الخلوي والكيمائى والبيئ لمركب المونانسن في الأبقار الحلوب

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إن احتياج الإنسان للبروتين الحيواني بصفة مستمرة وذلك لبناء الجسم السليم ما زال يمثل مشكلة كبيرة بسبب الزيادة المستمرة في تعداد السكان في مصر بنسبة عالية لا تتناسب مع زيادة الإنتاج الحيواني من الألبان واللحوم التي تكفى القوى البشرية داخل الوطن. من أجل هذا كان من الضروري الإتجاه الى طرق بحثية جديدة هدفها توفير كمية من الألبان واللحّوم مع استهلاك عليقة اقتصادية بسيطة مع العمل من أجل الحصُّولُ على أعلى انتاج بأقل تكلفة ممكنة وبدرحه امان اعلى. من هذا الهدف استخدم لهذه الدر اسة مركب المونانسن الذي يعتبر من المركبات التي سمح باستخدامها (عالميا بواسطة المنظمة العالَّمية للدواء والغذاء) كاضافات للعلائق المختلفة بهدف الحصول على معدل زيادة كبير في انتاج اللحوم والألبان. هذا بالإضافة الي كونة يعمل علي زيادة تحويل النيتر وجين في الأمعاء وتغيير الكائنات الصغيرة التي تساعد علي الهضم في الكرش وبالتالي ادي آلي زيادة نسبة البروتين وتقليل نسبة الفاقد من مخرجات الحيوانات. اما من ناحية تاثير المونانسن على البيئة المحيطة فانَّة أدَّي الَّي تقليل انتاج غاز الميثان وايضا المركبات النيتروجينية التي تؤدى الى تلوث الماء والهواء. مما أدى التي تقليل الأضرار الناتجة عنَّ زيادة نسبة المركبات النيتروجينية بالنسبة لصحة الإنسان والحيوان. أتضح من هذة الدر اسات السابقة على مركب المونانسن ان اضافتة الى العلائق المختلفة يؤدي الى زيادة انتاج الألبان اليومي وزيادة معدل النمو في الأبقار وايضا معدل التشافي في ماشية انتاج اللحم. بالأضافة الي انه يساهم في تقلبل نسبة الإصابة بمرض الكيتوزس الذي يحدث بعد الولادة مباشرة في الحيوان انه يقال التعرض لللإصابة بالإنتفاخ المستمر وكذلك يقال من احتمالات تغير وضع الكرش. لقت خلصت هذة الدراسة الي أن اعطاء المونانسن في علائق الحيوانات ادي الي زيادة معنوية في نسبة البروتين الكلي واليوريا والجلوبيولين وكذلك نسبة الكولستيرول في مصل الدم. أما نسبة الألبيومين والجلوكور والبيلير وبين ألكلي لم تكن هناك اي زيادات معنوية. أيضا توصلت هذة الدراسة الي ان اعطاء المونانسن في علائق الحيوانات ادي الي زيادة معنوية في اوزان الأبقار وكذلك اوزان اللبن اليوميز واخيرا وجد ان مركب المونانسن ليس له تأثير سام على الكر وموسَّومات و التي تم در استَّها في الخلايا الليمفاويه.