EFFECT OF RUMEN-PROTECTED-GLUCOSE SUPPLEMENTATION DURING TRANSITION PEROID ON BLOOD METABOLITIES AND REPRODUCTIVE PERFORMANCE IN DAIRY COWS

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ABSTRACT

Supplementation of rumen protected glucose (RPG) may be a useful dietary strategy to improve energy balance in transition dairy cows. The present study was performed to investigate whether supplementation with RPG could improve metabolic status, milk production and reduce infection during the transition period in cows. Fifty five healthy Holstein dairy cows (4–5 years old; 515 ± 42 kg body weight; 32.1 kg milk production per day) with the second lactation were enrolled in the study. Cows were randomly assigned into two groups: group (1) control group which fed on control diet, group (2) RPG group which fed on control diet supplemented with RPG at rate of 250 g/cow/day. Supplementation was applied from 20 day before parturition to 60 days postpartum. Blood was sampled on 21 postpartum to examine some serum biochemical parameters [glucose (GLU), non-esterified fatty acid (NEFA) - β-hydroxybutyric acid (β-HBA) and insulin (INS]. Results showed that serum glucose level was insignificantly different between RPG supplemented and control cows. On the contrary, a rapid decrease was observed in concentration of NEFA and β-HBA and activity of aspartate aminotransferase (AST) in response to RPG supplementation. Meanwhile, dietary RPG supplementation increased plasma concentration of insulin (INS) relative to control cows.

Keyword:

Dairy cattle - Rumen protected glucose - Blood metabolites -Transition period –Reproductive performance.

INTRODUCTION

There is a variety of situations during lactation in which insufficient glucose justify the time availability may limit productivity mainly in ruminants **(Kvidera, 2017).** Multiple studies on how to increase available glucose to relieve negative energy balance (NEB) in early lactating dairy cows have been operated. Some studies revealed that milk production increased by administering intravenous injection of exogenous glucose to lactating dairy cows **(Brown and Allen, 2013 and Curtis *et al.*, 2018).** Plasma glucose concentration was elevated by infusing glucose into the abomasum **(Nichols e*t al.*,2016 and Gualdrón-Duarte and Allen, 2018),** However, glucose supplementation by intravenous injection or gastrointestinal infusion is not feasible in large commercial dairy farm and providing glucose by feeding additional carbohydrates have a negative effect **(Colman *et al.*, 2013).** Meanwhile, the direct absorption of glucose by the small intestineismore energyefficient **(Moran *et al.*, 2014)**.Therefore, it may be beneficial to find a way to deliver directly glucose in the small intestine, where it can be absorbed and used for milk production **(Zhang *et al.*, 2019)** through feeding rumen protected glucose.

Rumen protected glucose (RPG) is a good source of glucose for early lactation dairy cows to relieve negative energy balance (NEB), encapsulated by hydrogenated fat to escape rumen digestion and it can be released completely after entering the intestine **(Wang *et al.*, 2020a).** More glucose is thus delivered to the small intestine then absorbed directly by the small intestine epithelium, rather than relying on the gluconeogenesis of the liver independently **(Zhang *et al*., 2019).** The form of glucose supplementation can block fat mobilization and gluconeogenesis in dairy cows in the NEB state **(Nichols *et al.*, 2016)** and also large amounts of β-hydroxybutyric acid (BHBA), non-esterified fatty acid (NEFA), acetone and acetoacetic acid, etc. were no longer produced in the liver. Meanwhile, the original high concentration of these substances in the blood are also rapidly metabolized in the body and the fat accumulated in the liver would be transported out of the liver in the form of lipoproteins in time, or be oxidized in liver cells, and the body gradually deposits protein and fat **(Omphalius *et al.*, 2020).** The life -weight therefore returns to normal, so as to achieve the purpose of alleviating negative energy balance (NEB) **(Li e*t al.*, 2019).** In addition, due to the blocking of gluconeogenesis, the blood ammonia production is reduced, which no longer affects the reproduction of dairy cows, and the conception rate during estrus increases **(Wang *et al.*,**

2020a). Studies had found that RPG can increase the milk production **(Li *et al.*, 2019),** relieve the inflammatory response **(Wang *et al.*, 2020b),** participate in ileal epithelium metabolism and regulate genes related to immune homeostasis **(Zhang *et al.*,2019)** in postpartum cows. The present study was performed to investigate whether supplementation with RPG could improve metabolic status, milk production and reduce infection during the transition period in cows.

MATERIAL AND METHODS

Animals:

This study was conducted in lactating dairy farm in Kilo 59 Alexandria, Cairo desert, all cows were housed in free stalls, fed twice daily with a total mixed ration meeting the requirement for milk productions with ad-Librium access to water. Fifty fife healthy Holstein dairy cows (4-5 years old; 515 ± 42 kg body weight; 32.1 kg milk production per day) with the second lactation were enrolled in the study. Cows were randomly assigned into two groups: group (1) (n=25) control group which fed on control diet, group (2) (n=30) RPG group which fed on control diet supplemented with RPG at rate of 250g/cow/day (Gluco-Go-60) as follow in Table (1). The RPG used in this study adopted the latest international rumen evolving technology, and the rumen protection level of the product was 54.03 % **(Wang *et al.*, 2020c)**. It was prepared with 45 % glucose as the core material, 45 % hydrogenated fat by mass as the coating, and 10 % water to make it **(Li *et al.*, 2019)**. The RPG addition is granular and the diameters of the particles were 0.6-0.85 mm.All cows were fed from (20day before parturition to 60 day postpartum.

Table (1): Ingredients and nutrient composition of the diets.

Ingredient (% of DM)	20 day before parturition	60 day Postpartum
Corn	2.200	6.200
Soybean meal (46%)	2.200	6.00
Max pro.	0.500	2.00
Fat	0.050	0.200
Easy buffer	0.00	0.180
Magnesium oxide	0.00	0.040
Selenium	0.002	0.003
Cu, Zn, Mn, Co.	0.004	0.006
Biological antitoxic	0.012	0.005
Chemical antitoxic	0.006	0.040
Common salt	0.040	0.040
Mineral mix	0.030	0.050
Vitamin	0.015	0.025
Gluco-Go-60	0.250	0.250
Corn silage 30%	22.500	40.00

Nutrient composition of the diet:

Chemical analysis, (% of DM)	20 day before parturition	60 day postpartum
CP	11.6	14.6
Fat	2.0	2.1
Starch	10.3	14.8
NDF	53.6	45.2
ADF	31.3	25.5
Ash	6.8	6.0
NE_L [2] Mcal/kg	1.30	1.37

Blood Sampling:

Blood samples were collected from the jugular vein of each cow on the +twenty-first day postpartum. Blood samples were centrifuged at 3000 rpm for 15 min to obtain serum; then it was stored at (-20 °C) until analysis.

Blood Biochemical Parameters:

Measurement of serum glucose level according to **Trinder (1969)**, β-hydroxybutyric acid (β-BHBA) quantitative UV assay on serum with BS-300 analyzer according to **Young (2000),** NEFA-HR (2) ACS-ACOD method for the quantitative determination of non-esterified fatty acids (NEFA) in serum according to **Rogiers (1978)**. The measurements were performed on a spectrophotometer type SPV-72, Federal Republic of Germany. The enzymatic activity of aspartate aminotransferase (AST) was carried out according to **Reitman and Frankel (1957).**

Some Hormonal Parameters:

The insulin (INS) levels were measured using a commercial bovine ELISA kit (Moonblind Inc. Lakeforest, CA92630.USA) according to **Gerich (1988).**

Statistical Analysis:

Data were presented as mean ± SEM (standard error of the mean). Independent-samples T test was carried out for the obtained data using SPSS program version 16.0 and $P \leq 0.05$ was considered as statistically significant.

RESULTS

Serum glucose level was insignificantly different between RPG supplemented cows and control cows. On the contrary, a decrease ($P \leq 0.05$) was observed in NEFA, BHBA and AST activity in response to RPG supplementation. Dietary RPG supplementation significantly increases plasma concentrations of insulin ($P \leq 0.05$) relative to control cows.

Table (2): Effect of rumen protected glucose supplementation (RPG) on some serum biochemical parameters of dairy cows during the transition period.

Items	Control group	RPG supplemented
Glucose mg/dl	63.24 ± 2.32^{a}	66.27 ± 3.34^{a}
NEFA mmol/L	1.83 ± 0.01^{a}	0.95 ± 0.02^{b}
B-HBA mmol/L	0.36 ± 0.01^{a}	0.18 ± 0.00^{b}
AST U/L	88.20 ± 5.27^{a}	70.50 ± 6.19^{b}
Insulin ng/ml	1.58 ± 0.03^{b}	2.42 ± 0.01^{a}

RPG= Rumen protected glucose, NEFA=non-esterified fatty acid, β-HBA=Beta-hydroxybutyric acid; Means within a row with different superscript letters differ at ($P \leq 0.05$).

Dietary supplementation with RPG in the transition period showed significant increase in milk yield in supplemented group than in control dairy cows. Health disorders during transition period in RPG group were less than in control group which include retained fetal membranes and metritis.

Table (3): Effect of rumen-protected glucose supplementation (RPG) on some reproductive performance in dairy cows during the transition period.

Parameters	Control group	Supplemented group
First heat	38	36
Ins.no	4.5	4
Prev(-1) open day	121	123
open days	118	106
Prev (-1) lact. Yield	10145	10180
Lact. Avg	33.7	34.6
Preg %	88%	96%
R.F.M	4	2
Metritis	4	1

Ins.no: insemination number; prey (1) open day: previous open day; prey (1) lact. Yiel: previous lactation yield; preg: pregnancy; R.F.M.=retained fetal membrane;Difference between control and RPG supplementation in dairy cows.

274.5kg milk in lactation
1921.5 milk price
12 days in open days

DISCUSSION

Glucose is a key nutrient for the maintenance of basic functions of body tissues and the synthesis of milk during lactation. Low blood glucose concentrations after calving are associated with infertility in postpartum dairy cows perhaps because glucose is a master regulator of hormones and metabolites that control reproductive processes **(Lucy *et al.*, 2014).** Glucose metabolism imbalance exacerbates gluconeogenesis and lipid mobilization, which not only hinders milk quality and milk yield, but also limits the health and reproductive function in dairy cows **(Sauls-Hiesterman *et al.*, 2018).** Concentrations of GLU, β-HBA, NEFA, and activity of AST are important indicators for evaluating the energy balance of cows **(Song *et al.*, 2019).**

The serum glucose concentration is the most important factor affecting the secretion of insulin. In the present study, there were no difference in blood glucose concentration between RPG supplemented group and the control group, and these results agree with **Sauls - Hiesterman *et al.*, 2018; Zhang *et al.*, 2019 and McCarthy *et al.*,2019; 2020.** The increased serum glucose concentration after RPG supplementation was consistent with previous studies **(Brown and Allen, 2013 and Wang *et al.*, 2020a)**. The increased glucose concentration in RPG supplemented group may be related to the dose of 0, 200, 350 and 500 g of RPG supplementation and its own metabolic adaptation **(Wang *et al.*, 2020a).** Meanwhile, **Li *et al.*, (2019**) found that, dietary RPG supplementation tended to decrease the plasma glucose concentration, lesser blood glucose may affect the reproduction through ovarian activity which restored via glucose itself or glucose-mediated actions on circulating concentrations of insulin, IGF1, and / or lipid metabolites (**Green *et al.*, 2012 and Garverick *et al.*, 2013)**. In the current study, supplementation with dietary RPG increased the concentration of insulin in the blood and this result agrees with previous work **Wagner and Schimek, 2010** and McCarthy et al., 2020**.** Meanwhile, **Sauls-Hiesterman *et al.*, (2018)** said that, changes in insulin concentration were greater for the control cows compared with the cows received the dose of RPG (2.2, 4.4, 8.8 lb /head/day). Insulin decreases circulating NEFA concentration through its capacity to stimulate lipogenesis and inhibit lipolysis in adipose tissue **(Hayirli, 2006).** Insulin also has anti-ketogenic properties because it increases peripheral ketone utilization and decreases hepatic ketone production **(Hayirli, 2006).** The concentrations of β-HBA and NEFA were reduced after glucose supplementation. This is similar to a previous report **(McCarthy *et al.*, 2020 and Wang *et al.*, 2020a).** On the contrary, **Li *et al.*, (2019)** found that plasma NEFA concentration increased in RPG supplemented group, this result agreed with supplement amount of RPG at 200 g/d may not be sufficient for increased milk production and therefore more body fat will be mobilized to meet the energy requirements for milk production.

AST activity in RPG supplemented group was significantly decreased than in control group. This is similar to a previous report **(Wang *et al.*, 2020a).** Meanwhile, **Li *et al.*, 2019** found that no significant differences were observed in the plasma activity of AST between the RPG supplemented group and control group of cows during the transition period.

Dietary supplementation with RPG in the transition period showed significant increase in milk yield in supplemented group than in control dairy cows. Some studies found that duodenal infusion of glucose could increase milk yield and milk fat yield **(Li *et al.*, 2019 and Zhang *et al.*, 2019).** In contrast, some other studies showed that infusion of glucose through abdomen or rumen-protected did not affect milk yield **(Larsen and Kristensen, 2009 and Sauls-Hiesterman *et al.*, 2018)**, but reduced milk fat yield **(Larsen and Kristensen 2009)**.The opposite results may be due to the supplementary source, dosage and form of glucose, and the parity, basal diet composition, and physiological state of test animals.

Health disorders during transition period in RPG supplemented group were less than in control groups which include retained fetal membranes (4%, after a normal calving) and metritis. These result are similar to **(Hooshman Dabbasi *et al.*, 2018)** retained fetal membrane, metritis **(Santos *et al.*, 2015)**, these disorders have adverse effects on animal welfare, milk production, reproduction, and farm profitability **(Neves *et al*., 2018).**

CONCLUSION

The key findings of the study were that supplementation with RPG in transition cows improved the postpartum lactation performance (increased milk yield), decreased the degree of negative energy balance (NEB) since it decreased NEFA, β-HBA and increased insulin concentrations, and presumably reduced the incidence of reproductive disorders.

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