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## Metabolic Attributes, and Milk Production of Egyptian Buffalo Cows Supplemented with Protected Glucose during Transition Period

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### ARTICLE INFO

*Key words:*

Negative energy balance; Transition period; Buffalo cows; Rumen protected glucose

### ABSTRACT

The negative energy balance (NEB) is a common issue for dairy buffalo cows during the transition period which is the time around three weeks before and after parturition. The effect of rumen protected glucose (RPG) on metabolic attributes as well as milk production was studied using sixteen clinically healthy buffalo cows with average body weight of 450-500 kg at third parity selected from Mehallet Moussa Experimental Station (Kafr El-Sheikh, North Delta), which belongs to the Animal Production Research Institute, Ministry of agriculture, Egypt. The buffalo cows were divided randomly into two groups (n=8 per group). The first group fed the basal diets without supplementation and served as a control, while the second group was received the same diet supplemented with 250g RPG/ head/ day for 42 days started at three weeks pre-partum and lasted three weeks postpartum. The metabolic profiles of buffalo cows were affected by the dietary treatment. The concentrations of serum TG (triglycerides), TC (total cholesterol), glucose, insulin, and IGF-1 (insulin like growth factor-1) were higher in RPG-treated group than the control group (p<0.05), however GGT (Gamma-glutamyl transferase), AST (aspartate transaminase), ALT (alanine transaminase), NEFAs (non-esterified fatty acids) and BHBA ( $\beta$ -hydroxyl butyric acid) were significantly (p<0.0001) shrunk in RPG-treated groups in relative to the control group. Milk traits including total milk yield, daily milk yield, and lactation period were significantly improved (p<0.05) in RPG-treated group compared to the control, the significant differences were calculated to be +470.25, kg, +1.78 kg, and +28.86 days, respectively. Finally, during the transition period, RPG supplementation increased the concentration of serum TG and TC which were sufficiently used as energy yielding nutrients for improving milk yield and protecting against ketosis.

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## INTRODUCTION

The transition period is considered one of the most challenging stages for the dairy buffalo cow as the animals are more susceptible for negative energy balance (NEB), which is attributed to the drastic rise in energy demand as well as the sharp decline of voluntary feed intake (Agrawal et al., 2017). During this period, the productivity of dairy buffalo cow decreases sharply as a result to the metabolic and endocrine disorders (Janovick and Drackley, 2010). Excessive lipolysis is triggered by the mobilization of the body's energy reserves and low energy intake (Morris et al., 2009). It may happen when lipid mobilization from adipose tissue exceeds the oxidative capacity of the liver, and this may in turn lead to metabolic disorders such as fatty liver disease, increased reactive oxygen species (ROS), reduced paraoxonase activity, and initiation of oxidative stress (Turk et al., 2008).

Transition from a non-lactating stage or pregnancy, to a lactating stage or non-pregnancy requires elegant metabolic adaptations to ensure the availability of glucose needed to start lactation (Grummer et al., 2004; Guo et al., 2007; Selim et al., 2014; Li et al., 2019; McCarthy et al., 2020). Research efforts considered that formulating balanced diets in their energy content have been devoted to mitigating the harmful effects of NEB on dairy animals during the transition period (Guo et al., 2007; Janovick and Drackley, 2010; Van Dorland et al., 2012). Glucose is the precursor for lactose synthesis, and the latter is the primary osmotic regulation of milk production (O'Brien et al., 2008). In addition to the formation of lactogens,

Kvidera et al. (2017) emphasized the importance of glucose in promoting the immune response and thus health status. The direct uptake of protected glucose in the small intestine is more energy-efficient (Moran et al., 2014). Previous study confirmed that supplementation transition dairy cows with rumen-protected glucose (RPG) resulted in a remarkable reduces in the levels of blood pro inflammatory marker and increase milk yield (Li et al., 2019). Zhang et al. (2019) demonstrated that the supplementation of dairy cows with dietary RPG during the transition period can enhance immune homeostasis, and stimulate epithelial metabolism in the ileum. Additionally, Zabuli et al. (2010) suggested that the high circulating glucose concentration is a positive metabolic signal to the reproductive axis. Somchit et al. (2007) enhanced folliculogenesis and thus ovulation rate by short-term dietary addition of high energy inputs during the onset of ovulatory follicle(s).

Even though the maximum productivity, re-productivity, and health necessitate the availableness of glucose, but it is difficult for ruminants to reach the highest carbohydrate level in the post absorptive state because the increase in dietetic soluble carbohydrates may compromise rumen and post-absorptive health (Kleen et al., 2003). That is why the best strategy with early lactating cows is to provide a dietetic source of glucose that can easily reach the small intestine after a minimal digestion in the rumen. The present study has been carried out seeking means to alleviate the harmful impacts of NEB: dietary supplementation with Menoglu Plus as a source of RPG for buffalo cows during the transition period.

## MATERIAL AND METHODS

### A- Animals and housing

Sixteen clinically healthy buffalo cows with average body weight of 450-500 kg at third parity were selected from Mehallet Moussa Experimental Station (Kafr El-Sheikh, North Delta), which belongs to the Animal Production Research Institute, Ministry of agriculture, Egypt. Animals were milked twice daily (at 6 a.m. and 6 p.m.) with hand milking. All animals were identified by ear tags. Animals vaccinated for Anthrax, foot and mouth disease, and blackleg. Young calves were vaccinated recently by Rb51. Additionally; herds were tested for tuberculosis and Brucellosis on a quarterly basis. Buffaloes had free access to open air and housed in the same shed, where 50% of the yard area was sheltered and the other part was opened. Animals were individually fed with basal ration of concentrate feed mixture, Egyptian clover (*Trifolium alexandrinum*) and rice straw to fulfill energy and protein requirements for lactating buffaloes according to Mudgal (1988) with free access to water.

The concentrate mixture was composed of 21% yellow corn, 20% soybean meal, 30% barley, 25% wheat bran, 1% common salt, 2% di-calcium phosphate, and 1% premix. These rations provided 67% total digestible nutrient (TDN) and 12% crude protein (CP). Herd reproduction was basically by natural mating and the diagnosis of pregnancy was performed between 45 and 60 days after insemination as the fetus can rely on their dams for nutrition during this period via the completed placenta and then repeated by palpating per rectum the uterus and its contents

approximately 2-3 months before expected calving day.

The pregnant buffaloes put into a maternity yard a few days (1-2) before birth for closer monitoring after showing the calving signs. Once the calves are able to stand after parturition, they immediately begin to suckle their dams within the 1 to 6 hours to get the colostrum. Calves separated to a special calf's pen after colostrum feeding which continues for about 4 to 5 days. The calves weaned at 90 kg of body weight or three months of age. Biweekly milk production and lactation period for individual buffalo cow were recorded at the farm data set.

### B- Diet

Menoglu Plus as a source of Rumen protected glucose (RPG) was purchased from B.C. Pharma Company, Meet Ghamer, Dakahlia, Egypt. Before 3 weeks of the expected calving date, the buffalo cows were divided into two homogenous experimental groups. The first group received the basal diet without addition of rumen protected glucose and served as a control while the second group received 250 g RPG/ head/ day. The treatment started three weeks pre-partum and lasted three weeks post-partum.

### C- Blood constituents

At 2 weeks pre-partum, partum, 2 weeks post-partum, blood samples were collected via jugular vein puncture in clean, sterilized tubes and centrifuged at 3000 rpm for 20 minutes by a centrifuge (T32c; Janetzki, Wallhausen, Germany). Serum samples were separated and kept at -20 °C until the biochemical analysis. Blood serum metabolites including triglycerides (TG), total cholesterol (TC), low density lipoprotein (LDL), high density lipoprotein (HDL), glucose, insulin,

Insulin like growth factor-1 (IGF-1), Gamma-glutamyl transferase (GGT), aspartate transaminase (AST), and alanine transaminase (ALT) were determined spectrophotometrically using commercial kits from Biodiagnostic Company (Giza, Egypt). Non-esterified fatty acids (NEFA) were determined using diagnostic kits on photometric systems (DIA Lab, Austria). The level of Beta-hydroxybutyric acid (BHBA) was measured using diagnostic kits (POINTE Scientific INC, USA).

#### **D- Statistical analysis and model procedure**

The Levene and Shapiro–Wilk tests were conducted in order to check for normality and homogeneity of variance (Razali and Wah 2011). The mixed model of statistical analysis system (SAS., 2012 version 8, Cary, NC, USA) was used for assessing lipid profile, liver function, ketosis and energy metabolism indicators, the statistical model includes the effect of RPG, sampling time and their interactions as fixed factors, while buffalo individual as a random factor, Multiple comparisons among means were carried out by the Duncan's Multiple Range Test (DMRT, Steel and Torrie, 1980). The differences between the two groups for milk production were detected by T-test (Proc T-test). Results were expressed as means  $\pm$ SE. statistical significance was accepted at probability less than 0.05.

#### **RESULTS AND DISCUSSION**

The aim of the current study was to assess the efficiency of rumen protected glucose (RPG) during transition period in enhancing energy balance and metabolism and thus subsequent milk traits of Egyptian buffalo cows. Indeed, the periods of

negative energy balance (NEB) such as the prepartum period, the dairy animals overcome the NEB by utilizing the lipid reserve or lipolysis (Gomaa et al., 2021). Lipid profile represented by triglycerides (TG), low density lipoprotein (LDL), and high density lipoprotein (HDL) as well as total cholesterol (TC) for buffalo cows given RPG during transition period are presented in Table 1 and Figure 1A-D. Enriched buffalo cows diets by RPG decreased TG, HDL, and TC significantly ( $p=0.0188$ ,  $0.0025$ , and  $0.0459$ , respectively, however the LDL did not affected significantly by the dietary treatment ( $p=0.8862$ ). Regardless the effect of treatment, the analysis of treatment by sampling time interaction revealed that the concentration of HDL in blood serum of RPG-treated group was significantly higher during prepartum period than post-partum period ( $p<0.05$ ), minimized during the calving period in both of two groups. Meanwhile, the transition period did not affect the concentration of blood serum TC significantly in the control group ( $p>0.05$ ), whereas it was significantly higher during pre-partum period compared to the partum and post-partum periods ( $p<0.05$ ) in the RPG-treated group. The present study corresponded with the findings of Serdaru et al. (2011) who observed that the concentration of total cholesterol was significantly higher in pregnant buffalo ( $79.0\pm 9.56$  mg/dL) than their lactating counterparts ( $59.25\pm 10.14$  mg/dL). Similarly, El-Maghraby and Mahmoud (2016) came to same results; the concentration of TC during pre-partum period was significantly higher than that was during the post-partum period by 10.51 mg/dl. Gomaa et al. (2021) reported non-significant effects of transition period on LDH, while TG concentrations were

significantly higher during late pregnancy period than parturition or early lactation periods. In contrast; the HDL concentration was significantly higher during post-partum period than during pregnancy period. In lactating buffaloes, energy requirements are involved in the changes of plasma TG and TC, which varied before and after parturition (Abd Ellah et al., 2013; Gomaa et al., 2021). Blood lipids play an essential role in the synthesis of milk fatty acids (Kaneko, 2008) and the blood stream is considered the primary source of fatty acids in milk (Tripathi et al., 2010). It is thought that the level of TC in blood serum is a good indicator for evaluating the level of energy in received diet (Kida, 2011). The remarkable decreasing trend in the concentration of TC and TG in this study could be attributed to the animal's urgent need for energy to keep up with the higher milk yield during the postpartum period (Nath et al., 2004; Ashmawy, 2015). In the same context, Karapehlivan et al. (2007) reported that the demand for TG increases until the peak of lactation, as they are used by the

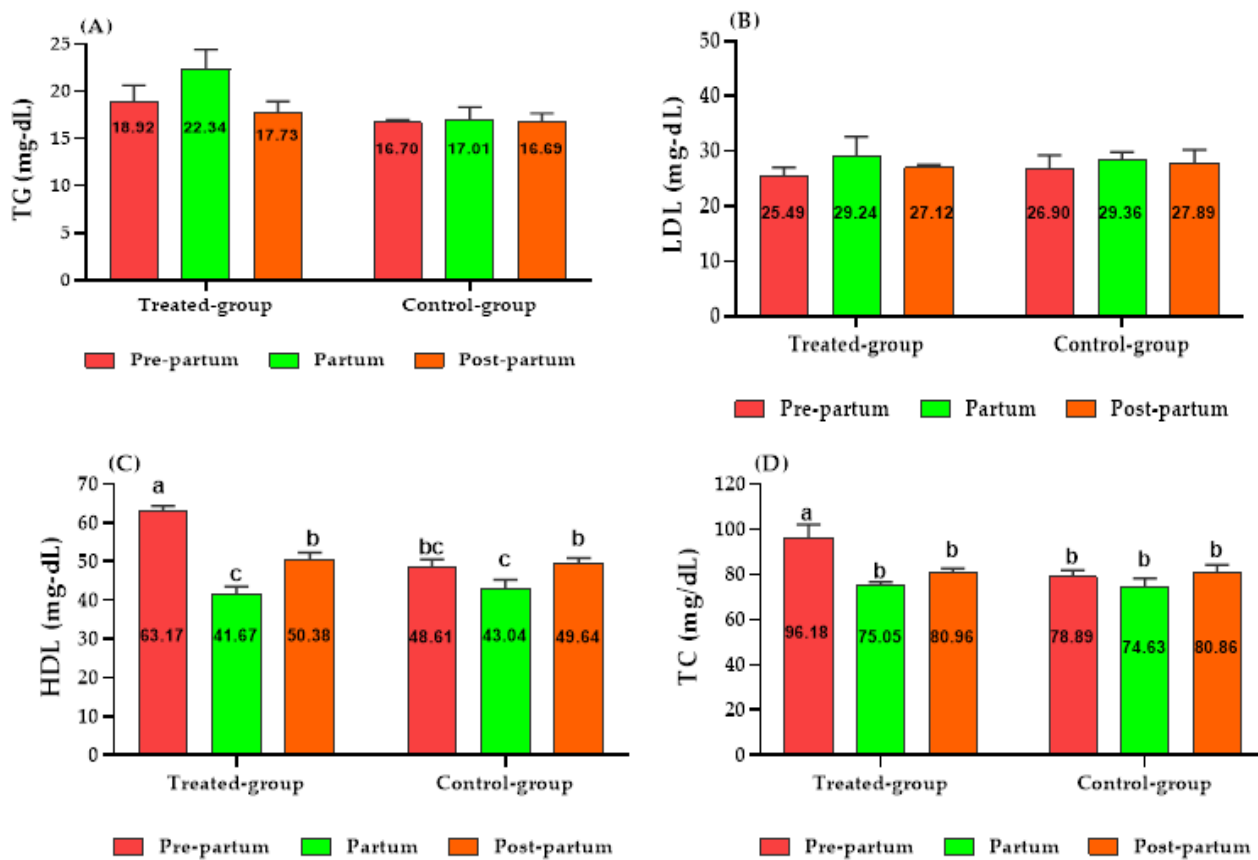
mammary glands to formulate milky fats.

During the early lactation period, there was a NEB which results in increasing the non-estratified fatty acid (NEFA) which could be re-esterified into TG in the liver and exported as very low density lipoproteins (VLDL), an important metabolic response is to mobilize triglycerides (TG's) from adipose tissue to be accumulated in liver (Teama and Gad, 2014; Mohamed et al., 2015; Cincović et al., 2012). Matthew (2016) reported that the lactating cows break down TG in adipose tissue to yield glycerol during lactation period which is a substrate for glucose synthesis in addition to NEFA that can be used for energy. Interestingly, even though the non-significant effect of treatment by time interaction on TG concentration in the present study, the supplementation of buffalo cows with protected glucose increased blood serum TG, being in the normal range (Abd Ellah et al., 2013), which reflect the beneficial effects of RPG in the prevention of increasing the NEFA and thus Dyslipidemia which is known to be elevation of blood TC and/or TG or LDL (Yuan et al., 2021).

**Table 1:** Lipid profile of buffalo cows received protected glucose during transmitting period

Items	Treatment (T)		p -Value		
	Treated-group	Control-group	T	Time (Ti)	T × Ti
TG(mg/dL)	19.31±1.00 <sup>a</sup>	16.50±0.447 <sup>b</sup>	0.0188	0.3304	0.5009
LDL(mg/dL)	27.00±1.629	27.32±1.253	0.8862	0.8455	0.9968
HDL(mg/dL)	51.71±2.716 <sup>a</sup>	46.67±1.367 <sup>b</sup>	0.0025	<0.0001	0.0003
TC (mg/dL)	82.57±2.973 <sup>a</sup>	77.29±1.600 <sup>b</sup>	0.0459	0.0052	0.0255

Values bearing different superscripts (a,b) in the same row are differed significantly ( $p < 0.05$ ); TG, Tri-glycerides; LDL, low density lipoprotein; HDL, high density lipoprotein; TC, total cholesterol.



**Figure 1:** Effects of treatment by time interaction (means  $\pm$  SEM) on Tri-glycerides (A), low density lipoprotein (B), high density lipoprotein (C), and total cholesterol (D) for buffalo cows received protected glucose during transmitting period.

The primary source of glucose in ruminants is gluconeogenesis (85% from the liver and %15 from the kidneys) with very limited net portal uptake (Benson et al., 2002; Brockman, 2005). The level of blood glucose reflects the status of carbohydrate status (Gomaa et al., 2021). Blood glucose concentration is usually under homeostasis control as it is an important nutrient required for normal body function (McArt et al., 2013). Additionally, glucose can regulate the levels of other blood metabolites (Noakes et al., 2001; Lucy, 2019). During the post-partum period, the low blood glucose resulted in several reproductive disorders represented by reduces the conception rate and repeat

breeding (Lucy et al., 2013; Ahmed et al., 2017).

There was a significant effect of adding RPG to buffalo cows diets during the transition period on blood glucose ( $p < 0.0001$ ), the significant difference was estimated to be 11.76 mg/dL in favor of RPG-treated group (Table 2). The analysis of treatment by sampling time interaction showed significant increase in blood glucose of RPG-treated group during the periods of pre-partum, partum, and post-partum compared to the corresponded periods in the control group ( $p < 0.05$ ; Figure 2-A). The present outcomes were coincide with the previous results (Piccioli-Cappelli et al., 2014; Kida, 2011) they showed

significant increase in blood glucose of dairy cows received diets with high energy levels during early, middle, and late lactation period. Also, Rulquin and Delaby (1997) reported significant increase in blood glucose during the early lactation period of lactating cows received diets with higher amount of net energy. Furthermore, Caldeira et al. (2007) in a study on non-pregnant sheep, they reported that there was a positive association between the diet energy value and blood serum glucose.

The volatile fatty acids produced in the rumen are the main source for meeting the energy needs of ruminants. The higher production of ketone bodies trigger by the increased lipolysis as a result of decreased blood serum glucose when the diet can't meet the animal's energy needs ( Fiore et al. 2015; İçil et al., 2020). Interestingly, the present results showed significant decrease in Beta Hydroxy Butyric Acid (BHBA) and a significant increase in blood serum glucose of protected glucose group, which reflects the lower production of ketone bodies and thus improved the general health status and improved both of productive and reproductive performance of buffalo cows during the transition period.

The significant decrease ( $p < 0.05$ ) of blood serum glucose during the early lactation period in the present study could be explained by the fact that a large amount of blood glucose is drawn up by the mammary glands to produce milk lactose (Ashmawy, 2015; Nale, 2003). Contradictory results were obtained by Abdulkareem (2013) who reported that the concentration of blood plasma glucose remained steady during calving and early lactation periods. The higher blood glucose concentration during partum period in the present

study agreed with the results of El-Maghrabyand and Mahmoud (2016). The current results may be attributed to the gluconeogenic effect of epinephrine and cortisol as a result to the stress and excitement due to parturition (Gerardo et al., 2009).

Ultimately, glucose may directly control both circulating insulin and insulin like growth factor (IGF-1) production from the liver throughout insulin-stimulated IGF1 synthesis and secretion (Matthew, 2016). As long as blood glucose concentration remains low, both of insulin and IGF-1 remain low, and during the lactation period the cow remains in a catabolic state or tissue-losing (Lucy, 2008). In the case of an increase in the concentration of insulin in the blood, the animal divides glucose towards the muscles and body fat tissues. Moreover, in ruminants, the signaling axis of IGF-1 is responsible for the mediation of growth hormone action on the mammary gland (Etherton, 2004).

The present results showed significant increase in the concentration of blood serum insulin and IGF-1 in the buffalo cows received RPG during transition period compared to the control ( $p < 0.05$ ; Table 2). From the analysis of treatment by sampling time interaction (Figures 2B-C), the values of aforementioned two parameters were significantly higher in RPG-treated group during the calving period than those estimated during the late pregnancy and early lactation periods ( $p < 0.05$ ). Meanwhile, non-significant differences were detected during the three stages of transition period for buffalo cows received the control diet ( $p > 0.05$ ). The present results corresponded with the results of Accorsi et al. (2005); they reported that the insulin concentration during the early

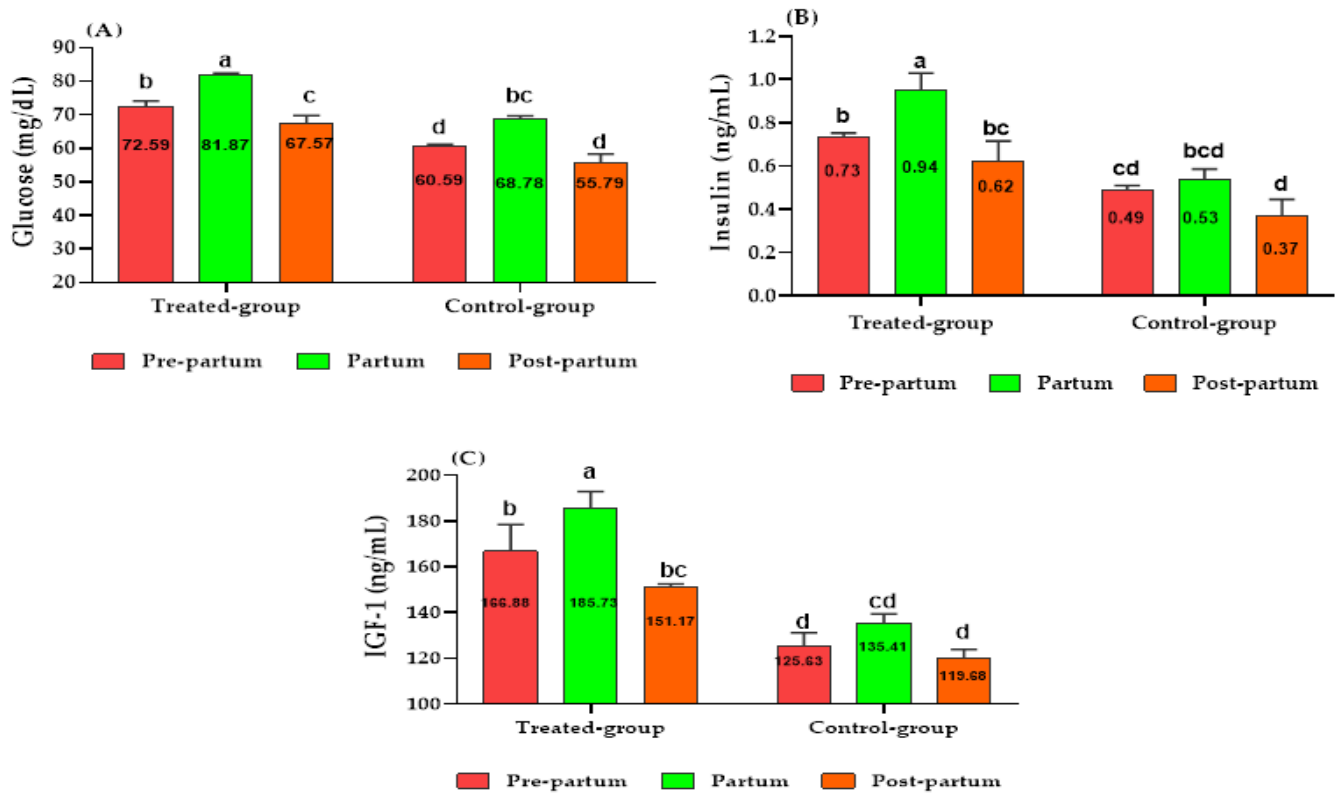
lactation period was low compared to the early lactation period ( $p < 0.05$ ). Also, Ashmawy (2015) reported that the concentration of IGF-1 was significantly

higher in pregnant buffalo cows than their counterparts lactating ( $p < 0.05$ ).

**Table 2:** Blood glucose, insulin, and Insulin like growth factor-1 of buffalo cows received protected glucose during transmitting period

Items	Treatment (T)		p -Value		
	Treated-group	Control- group	T	Time (Ti)	T × Ti
Glu. (mg/dL)	73.87±2.085 <sup>a</sup>	62.11±1.783 <sup>b</sup>	<0.0001	<0.0001	<0.0001
Insulin (ng/mL)	0.78±0.050 <sup>a</sup>	0.47±0.035 <sup>b</sup>	<0.0001	0.0325	0.0002
IGF (ng/mL)	165.34±5.408 <sup>a</sup>	125.95±3.294 <sup>b</sup>	<0.0001	0.0075	0.0001

Values bearing different superscripts (a,b) in the same row are differed significantly ( $p < 0.05$ ); Glu, glucose; IGF, Insulin like growth factor



**Figure 2:** Effects of treatment by time interaction (means ± SEM) on Glucose (A), Insulin (B), and Insulin like growth factor (C) for buffalo cows received protected glucose during transmitting period.

In ruminants; Meyer and Harve, (1998) showed that the ketosis in addition to depression of feed intake and fat cow syndrome are often associated with the activities of Aspartate-Aminotransferase (AST) and Gamma

Glutamyl-transferase (GGT). Increasing the activity of blood serum AST is considered a sensitive biomarker for liver damage (Meyer and Harve, 1998). Mobilization of a large amount of body fat causes fatty liver syndrome resulting



the hypoglycemia in pregnancy toxemia (Sargison, 2007). Between the most reasons of increasing the activity of AST is the reduction of energy in animal diet (Abdel Rahman et al., 2002). On the other side, increasing the levels of GGT causes the cholestasis in addition to a defect in hepatobiliar circulation (Sahinduran et al., 2010). Moreover, GGT could be used as an oxidative stress indicator (Kataria and Kataria, 2012) due to its antioxidant effect inside and outside the cell (Celi, 2010).

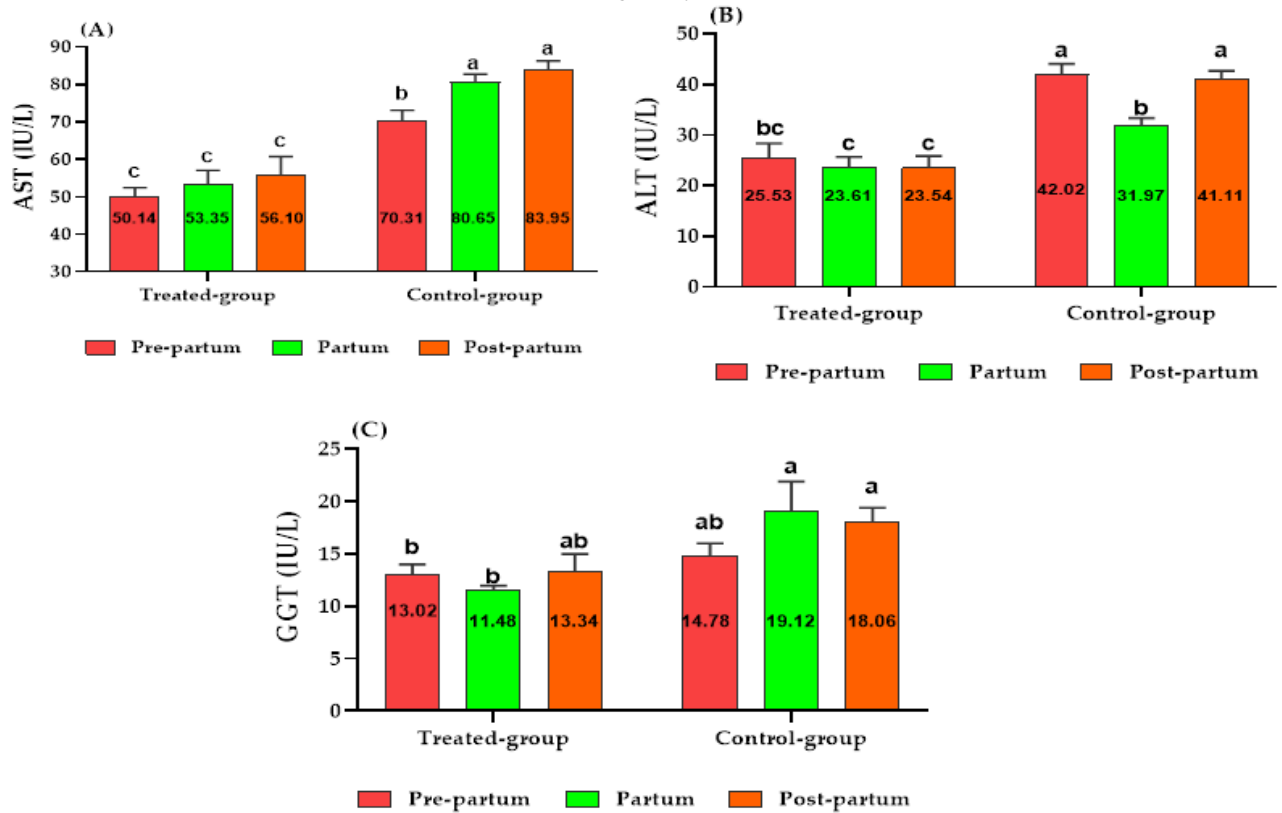
In the present study, supplementation of buffalo cow diets with RPG resulted in significant decrease in liver enzymes (AST and ALT) as well as GGT ( $p < 0.05$ ; Table 3). The results of treatment by time interaction (Figure 3A-C) revealed that there was non-significant differences in all aforementioned liver function indicators in the group received dietary RPG during the periods from late pregnancy to early lactation period ( $p > 0.05$ ). Moreover, both the concentrations of AST and ALT were significantly lower during all considered periods in RPG-treated group than the control group ( $p < 0.05$ ), as a reflection of the remarkable improvements of liver functions in treated group. The present results corresponded with the findings of İçil et al. (2020), who reported that increasing the levels of diet energy resulted in significant decrease in the activities of AST and GGT. Also, in a study conducted on Sheep, Abdel Rahman et al. (2002) reported that

providing 50% and 100% of energy requirements coupled by values of 82.34 and 55.11 u/l, respectively for AST enzyme, while the concentrations of GGT were found to be 38.23 and 32.57u/l, respectively. In buffaloes, Abdulkareem (2013) found that the activity of AST enzyme was significantly higher during early lactation periods ( $102.61 \pm 11.62$  U/L) than during the parturition period ( $90.06 \pm 8.04$  U/L) indicating that the tissue demolition was more pronounced and the hepatic metabolism might be more stressed during this period (Antunovic et al., 2004). Similarly in Holstein dairy cows, Reist et al. (2003) reported that the AST activity was lower pre-partum and began to increase, reaching its peak during early lactation period (the first week post-partum). Lactation period is one of the physiological conditions, which linked to high variability in the activities of blood serum enzymes (Pizzuti and Salvatori 1993). In ruminants, both of serum AST and ALT are used as a marker of muscle integrity. During the early lactation period, De Rosa et al. (2001) reported that the values of ALT in blood serum value varied between 83 and 116 U/l, which are higher than the values obtained in the current study, which confirms the ability of RPG to enhance liver function during this critical period.

**Table 3:** Liver function indicators of buffalo cows received protected glucose during transmitting period

Items	Treatment (T)		p -Value		
	Treated-group	Control-group	T	Time (Ti)	T × Ti
AST(IU/L)	53.41±2.027 <sup>b</sup>	77.64±2.345 <sup>a</sup>	<0.0001	0.0051	0.0254
ALT(IU/L)	25.35±1.195 <sup>b</sup>	38.28±1.730 <sup>a</sup>	<0.0001	0.0261	0.0197
GGT (IU/L)	13.03±0.687 <sup>b</sup>	17.03±1.033 <sup>a</sup>	0.0236	0.3025	0.0483

Values bearing different superscripts (a,b) in the same row are differed significantly ( $p < 0.05$ ); AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, Gamma-glutamyl transferase.



**Figure 3:** Effects of treatment by time interaction (means  $\pm$  SEM) on aspartate aminotransferase (A), alanine aminotransferase (B), and Gamma glutamyl transferase (C) for buffalo cows received protected glucose during transmitting period.

Typically, adipose tissue mobilization begins during pre-partum as result to intake depression likely to occur during the late pregnancy period. The levels of BHBA and NEFA showed opposite trends with regarding the values of body condition score. In particular, NEFA values in buffaloes increased from 1 day pre-parturition to 1 day post-parturition, which suggested that fat mobilization starts toward the end of pregnancy (Fiore et al., 2017). In ruminant animals, both of NEFA and BHBA are considered the major blood indicators of lipid mobilization (Cincović et al., 2012).

Dairy animals break down glycogen in liver and muscle to release glucose during the early postpartum

period. Also, TG are broken down in adipose tissue to yield glycerol that considered the mainly substrate for glucose synthesis and NEFA that can be used for energy (Holcomb et al. 2001; Fiore et al. 2015). The circulating NEFA and BHBA could be rapidly decreased as long as there were increases in the concentration of glucose by increasing the supplementation of glucose and/or decreasing demand (Matthew, 2016). This is because glucose can cause the release of insulin which will antagonize lipolysis and promote lipogenesis. Glucose also provides substrate to the tricarboxylic acid cycle so that BHBA can be fully metabolized (White, 2015). Bani Ismail et al. (2008) observed only a significant negative association between

the concentration of glucose and BHBA when the level of BHBA is higher than 0.86 mmol / l. The present results indicated that there were significant decreases in the concentration of BHBA and NEFAs in blood serum of buffalo cows received RPG in their diets during transition period compared to the control group ( $p < 0.05$ ; Table 4).

Results of treatment by sampling time interaction (Figure 4A-B) indicated that there were non-significant differences in the concentrations of BHBH and NEFAS between pre-partum and postpartum periods ( $p > 0.05$ ) for RPG-treated group, however the significant difference in the concentration of BHBH was observed in the untreated group between the pregnancy and early lactation periods ( $p < 0.05$ ) in favor of early lactation period but remain significantly higher than the corresponding periods in the treated group ( $p < 0.05$ ). In contrast; non-significant differences ( $p > 0.05$ ) between the same aforementioned periods were detected for NEFAs concentrations, minimized during the calving period.

The present results reflected the capability of glucose to regulate the secretion of BHBA and NEFA, protect against the lipolysis, and promote lipogenesis (Matthew, 2016). İçil et al.

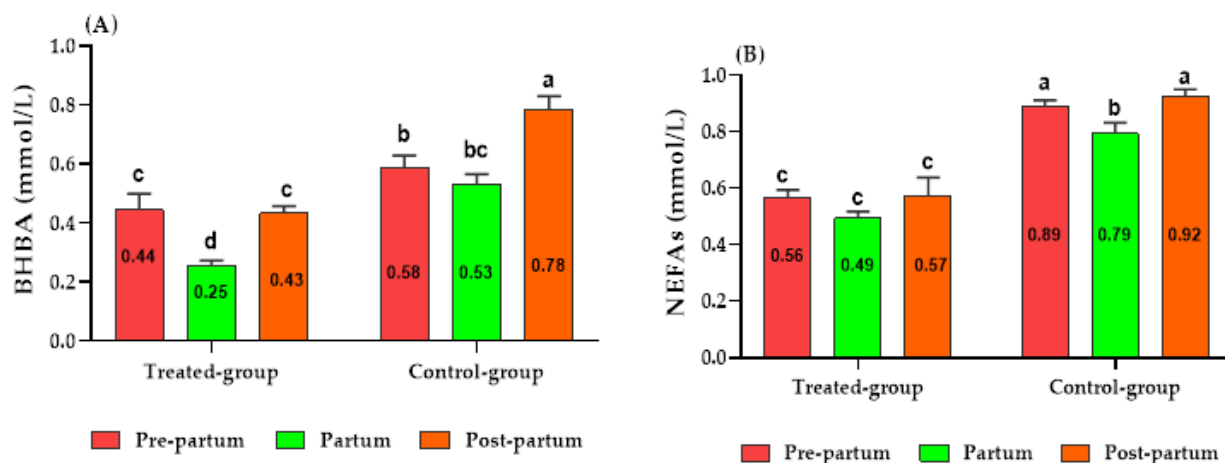
(2020) reported that increasing the energy levels in sheep diets coupled with a significant decrease in BHBH concentration, what in general agreement with the current study. In a former study carried out on buffalo cows, Grasso et al. (2004) showed a significant increase in the level of NEFA at 20–30 days postpartum, which could be a result of the high energy needs during this earlier stage of lactation.

Collectively, the values of NEFA measured on buffalo cow in this study showed the same trend of BHBA during transition period. The values of BHBA obtained from each animal were within the normal and physiological range established for buffalo, which indicated an appropriate fat metabolism (Nozad et al. 2012). During the postpartum period, the low level of glucose is associated with the overproduction of ketones, especially the level of blood BHBA (Fiore et al. 2015). The present study indicated that the level of blood glucose remained stable, indicating lack of changes in the absolute rate of glycogenolysis and gluconeogenesis (Fiore et al., 2017). Bertoni et al. (1994) reported that blood glucose is the only parameter among those involved in energetic metabolism in buffaloes that was not influenced by lactation.

**Table 4:** Ketosis indicators (beta-hydroxybutyric acid, and non-esterified fatty acids) of buffalo cows received protected glucose during transmitting period

Items	Treatment (T)		p -Value		
	Treated-group	Control- group	T	Time (Ti)	T × Ti
BHBA (mmol/L)	0.37±0.030 <sup>b</sup>	0.63±0.040 <sup>a</sup>	<0.0001	0.0003	0.0072
NEFAs (mmol/L)	0.53±0.025 <sup>b</sup>	0.86±0.022 <sup>a</sup>	<0.0001	0.0391	0.0131

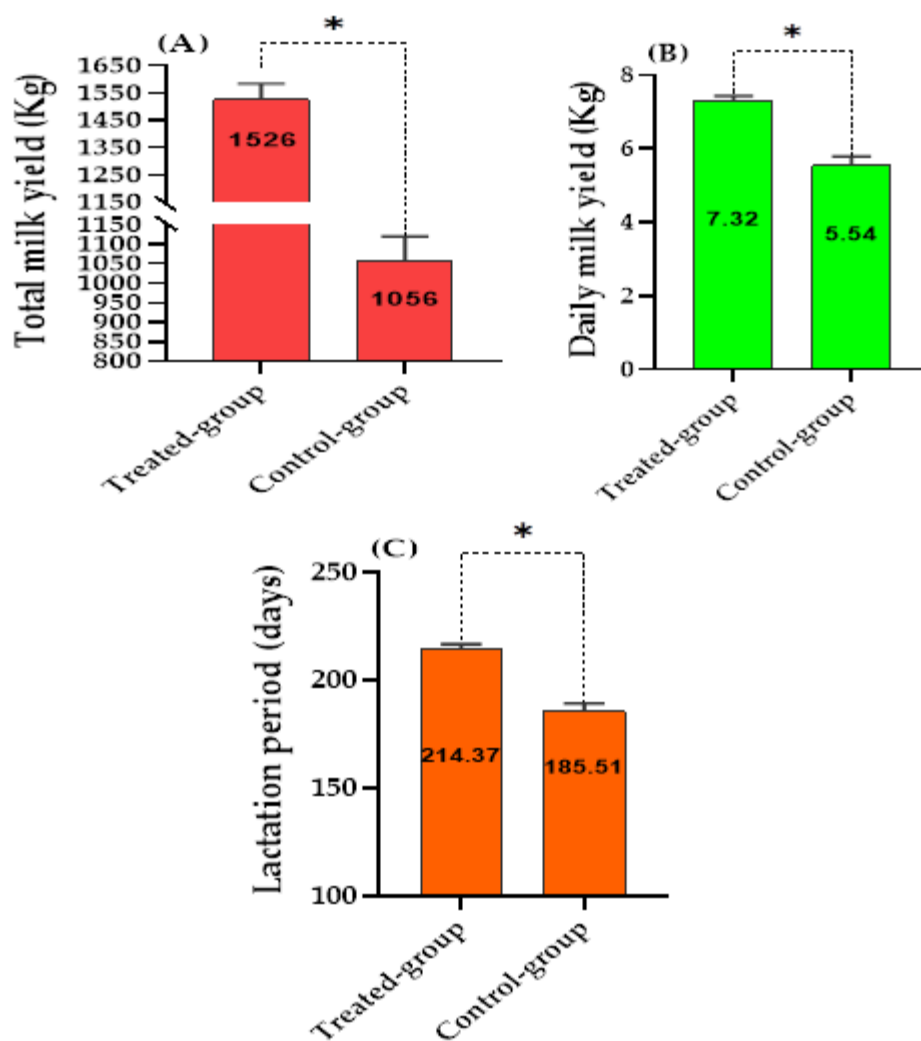
Values bearing different superscripts (a,b) in the same row are differed significantly ( $p < 0.05$ ); BHBA, beta-hydroxybutyric acid; NEFAs, non-esterified fatty acids .



**Figure 4:** Effects of treatment by time interaction (means  $\pm$  SEM) on Beta-hydroxybutyric acid (A), and Non-esterified fatty acids (B) for buffalo cows received protected glucose during transmitting period.

During lactation period, the dairy animals require dietary glucose for milk synthesis. The degradation of starches and soluble sugars into volatile fatty acids in the rumen may limit the amount of glucose that can be absorbed in the small intestine, which may limit milk synthesis (Li et al., 2019). Herein, the dietary supplemental of RPG resulted in enhancing the metabolites of buffalo cows either by providing additional energy sources as TC and TG or by increasing the concentration of blood insulin and IGF-1 which acts as signals for improved metabolic status (Scaramuzzi et al., 2006). The current changes in blood metabolism of buffalo cows displayed the importance of using the available energy-yielding metabolites in various productive processes as denoted by the improved

milk traits including total milk yield, daily milk yield and lactation period (Figure 5A-C) in buffalo cows given dietary protected glucose compared with their counterparts in the control group. The increased milk production in the present study was associated with elevated levels of blood serum TC and TG. In lactating sheep, Chiofalo et al. (2005) demonstrated that there was a significant decrease in the concentration of serum TG, which may triggered by the utilization of this metabolite by the mammary glands. Moreover, the significant improvements in milk production of dairy animals fed dietary energy sources have been reported in several previous literatures (Hashem and El-Zarkoun 2017).



**Figure 5:** Effects of treatment by protected glucose during transmitting period on total milk yield (A), daily milk yield (B), and lactation period (C) for buffalo cows received protected glucose during transmitting period.

## CONCLUSION

In conclusion, our results revealed that rumen protected-glucose in the form of Menoglu Plus could be used as an influential energy source for promoting the metabolic attributes and milk production of buffalo cows during the transition period.

## FUNDING:

This research did not receive any external funding

## CONFLICTS OF INTEREST:

The authors declare no conflict of interest

## AUTHORS CONTRIBUTIONS:

Conceptualization, A.A.E. M.M.H. H.A.A. and M.M.E.; methodology, A.A.E., M.M.E., M.M.H., H.A.A. and M.F.A.; software, A.A.E.; validation, A.A.E., M.M.E. H.A.A. and M.M.H.; formal analysis, A.A.E.; resources, A.A.E., M.M.E., M.M.H. H.A.A. and M.F.A.; data curation, A.A.E, M.M.H., H.A.A. M.F.A.; writing—original draft preparation, A.A.E. and M.F.A.; writing—review and editing, A.A.E.;

visualization, A.A.E. and M.M.H.; supervision, A.A.E.; project administration, M.M.H. All authors

have read and agreed to the published version of the manuscript.

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## تأثير إضافة الجلوكوز المحمي على خصائص الدم الميتابوليزمية وإنتاج اللبن في الجاموس المصري

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يعتبر توازن الطاقة السلبية مشكلة شائعة في الجاموس المصري خلال المرحلة الإنتقالية والتي تبدأ من الثلاثة أسابيع الأخير من الحمل وتنتهي في الثلاثة أسابيع الأولى بعد الولادة. تم دراسة تأثير إضافة الجلوكوز المحمي من التكرس في الكرش على الخصائص الميتابوليزمية للدم وإنتاج اللبن على عدد 16 جاموسة بمتوسط وزن 450 الى 550 كجم بمحطة بحوث محلة موسى التابعة لمعهد بحوث الإنتاج الحيواني ، مركز البحوث الزراعية. تم تقسيم الحيوانات إلى مجموعتين (٨ حيوانات في كل مجموعة). تناولت المجموعة الأولى العليقة الأساسية بدون إضافات كمجموعة كنترول بينما تناولت المجموعة الثانية العليقة الأساسية مضاف إليها ٢٥٠ جرام جلوكوز محمي لكل رأس يومياً ولمدة ٤٢ يوم. تم ملاحظة تأثير للمعاملة على الخصائص الميتابوليزمية للدم فكان أعلى تركيز للجليسيريدات الثلاثية والكوليسترول والجلوكوز والأنسولين في المجموعة المعاملة بالجلوكوز مقارنة بالمجموعة الكنترول ، فيما انخفض تركيز كلاً من الأحماض الدهنية الغير مشبعة وحامض البيداتا هيدروكسي بيوتريك وانزيمات الكبد و جاما جلوتاميل ترانسفيراز في المجموعة المعاملة مقارنة بالكنترول. لوحظ تحسن في صفات الحليب المتمثلة في إنتاج اللبن الكلي وإنتاج اللبن اليومي وطول موسم الحليب في المجموعة المعاملة بالجلوكوز المحمي مقارنة بالكنترول وكان الفارق المعنوي في حدود ٤٥٠.٢٥ كجم و ١.٧٨ كجم و ٢٨.٨٦ يوم بالنسبة لإنتاج اللبن الكلي واليومي وطول موسم الحليب ، على التوالي. في النهاية تعتبر الزيادة الحادثة في تركيز كلاً من الجليسيرات الثلاثية والكوليسترول نتيجة لإضافة الجلوكوز المحمي في العليقة كدليل لحالة الطاقة في الجسم مؤشر جيد لتحسن ميزان الطاقة السالب في الجاموس الحلاب خلال المرحلة الإنتقالية وبالتالي زيادة إنتاج اللبن والحماية من الكيتوزيس.

الكلمات المفتاحية: ميزان الطاقة السالب ، الفترة الإنتقالية ، الجاموس المصري ، الجلوكوز المحمي