OPEN ACCESS
Journal of Animal Health and Production
Research Article

Performance, Puberty, Pregnancy, and Hemato-bichemical Parameters of Friesian Heifers Orally Administrated with Protected Fat or Glycerol

Wael Mohamed Wafa[1*], Mohammed Mahmoud Hegazy[1], Mohamed Mohamed El-said Ibrahim[1], Hamdy Abdala El-Nagar[1], Rehab Fawzy Ismail[2]

[1]Animal Production Research Institute, Agricultural Research Center, Giza, Egypt; [2]Department of Animal Production, Faculty of Agriculture, Mansoura University, Egypt.

Abstract | The objective of this study was to evaluate the impact of protected fat (PF) or glycerol (GL), oral administration on puberty, some metabolic parameters, and pregnancy rate of Friesian heifers. Eighteen Friesian female calves (14.55±1.15 months of age and 205.0±16.09 kg LBW) were divided into three groups (6 in each) according to age and body weight. All groups were fed same diet; control group was untreated (CG), while those in the 2nd and 3rd groups were orally treated with 300 ml GL/head/twice/week (GG) or daily 25 g PF/100 LBW (PFG) from 14 to 18 month of age. Results showed that PFG achieved the highest (P<0.05) body weights, weight gains, and body measurements at puberty, 1st service, and conception. Values of red and white blood cells, hemoglobin, packed cell volume, plasma total proteins, globulin, glucose, total cholesterol, total lipids, and triglycerides increased (P<0.05), while those of urea-N, creatinine, and aspartate and alanine transaminases decreased (P<0.05) in GG and PFG compared with CG. Ages at puberty, 1st service, and conception were earlier (P<0.05) in GG and PFG than in CG. Pregnancy rate was the highest in GG (100%), followed by PFG (83.3%), and the lowest in CG (66.7%) within a service period of 90-days (P<0.05). Plasma progesterone at puberty and conception was higher (P<0.05) in GG and PFG than in CG. In conclusion, pre-puberty treatment of female calves orally with 300 ml glycerol twice/week/head or protected fat (25 g/100 kg) for 4 months is important for raising breeding heifers for precious puberty, early ages at 1st service and conception, increased pregnancy rate, and better body confirmation.

Keywords | Friesian heifers, Glycerol, Protected fat, Puberty, Health status.

Received | July 05, 2022; **Accepted** | August 17, 2022; **Published** | October 15, 2022
***Correspondence** | Wael Mohamed Wafa, Animal Production Research Institute, Agricultural Research Center, Giza, Egypt; **Email**: drwaelfatoh@hotmail.com
Citation | Wafa WM, Hegazy MM, Ibrahim MS, El-Nagar HA, Ismail RF (2022). Performance, puberty, pregnancy, and hemato-bichemical parameters of friesian heifers orally administrated with protected fat or glycerol. J. Anim. Health Prod. 10(4): 454-465.
DOI | http://dx.doi.org/10.17582/journal.jahp/2022/10.4.454.465
ISSN | 2308-2801

INTRODUCTION

Heifers are future herd of a dairy farm to replace the older and uneconomical females of the farm through voluntary culling (Bhatti, et al., 2007). The cost of rearing replacement heifers is one of the highest investments in the dairy industry. Thus, proper nutritional and management practices must be placed to maximize the return of investment (Jolazadeh et al., 2019).

Precocious puberty allows the heifer to have more estrous cycles before breeding age, improves pregnancy rate following the 1st service (Buskirk et al., 1995), and enhanced animal longevity (Lesmeister et al., 1973). Age at puberty ranged between 10-14 months in different breeds of heifers (Freetly and Cundiff, 1997). Friesian heifers usually attain puberty when they reach about 55-60% (Bayatkouhsar et al., 2013) or 60 to 65% (Endecott et al., 2013) of their adult body weight which may be a standard during

the starting breeding season in heifers. The age at which they attain puberty can be highly variable, ranging from 16 to 30 months (Bayatkouhsar et al., 2013). Therefore, the reproductive efficiency in heifers was determining by age at puberty (Perry, 2016).

Feed additives can improve gut health of the animals, which results in increased digestion rate, better growth performance (Kawakami et al., 2010; Frizzo et al., 2011), and very effective weight gains (Berge et al., 2005). Energy balance (EB) is the principal nutritional factor regulating the reproductive system (Wade et al., 1996). Establishment of normal ovulatory cycles may be related to the time of EB nadir. In this respect, Beam and Butler (1997) observed that only 24% of follicles that matured prior to the EB nadir ovulated, whereas 75% of follicles that matured after the EB nadir ovulated.

One of the most valuable sources of extra-energy supplementation is propionate compounds. Propionate is the major glucose precursor in ruminants that are in positive EB and is anti-ketogenic (Drackley 1999). The dietary energy level increases in fats compared with carbohydrates (Mattos et al., 2000). Protected fats (PF) are fatty acids combined with Ca salts that help these fatty acids to escape from fermentation in the rumen and increase their dietary contents in the small intestine (Jenkins and McGuire 2006; Palmquist and Jenkins, 2017). Therefore, PF are used as an alternative to improve the density of dietary energy in dairy cows (Hammon et al., 2008). The beneficial effects of fatty acids on reproductive performance may involve increased energy density of the diet, affect the follicular development, elevate progesterone level, prevent signals of luteolytic during pregnancy, and improve quality of embryos (Dirandeh et al., 2013). Supplementation of by-pass fat improves the EB of lactating buffaloes (Atkare et al., 2018) and dairy cows (Ballou et al., 2009) during early post-partum period.

Physiologically, impacts of Glucogen (e.g., glycerol) treatment, as oral dose, were due to their absorption by ruminal microflora as intact molecules and metabolite products (Kalyesubula et al., 2019). Glycerol (GL) is another gluconeogenic precursor that might be effective in the treatment of ketosis, and is safe to administer in larger amounts as a drench (Osman et al., 2008). GL is a by-product of biofuel production that is used in cattle feed, with mature cattle being capable of consuming up to one kg GL/day (Südekum, 2007). The energy value of GL is estimated to be 16.2 MJ metabolizable energy (ME) of dry matter for ruminants (Mach et al., 2009). Consumed GL may be fermented in the rumen, absorbed across the rumen epithelium or escape the rumen by outflow through the omasal orifice (Werner Omazic, et al., 2015).

Therefore, the objective of the present experiment is evaluating the impact of oral administration of protected fat or glycerol for four months (14 to 18 months of age), as a source of energy, on puberty, some metabolic parameters, and pregnancy rate of Friesian heifers.

MATERIALS AND METHODS

The experimental work of this study was conducted at Animal Production Research Institute (APRI), Egypt, in cooperation with Department of Animal Production, Faculty of Agriculture, Mansoura University. The handling and management of animals were conducted according to the Directive 2010/63/EU for animal protection which used for scientific purposes (Official Journal of the European Union, 2010).

Experimental design

A total of 18 healthy Friesian female calves with 14.55±1.15 months of age and 205.00 ± 16.09 kg LBW were divided randomly into three experimental groups according to age and body weight (6 in each). The control animals received a control diet without treatments (CG). Animals in the 2nd and 3rd groups received the control diet with oral-dose of 300 ml Glycerol/head/ twice a week (GG; El-Kasrawy et al., 2020), or daily dose of 25 g protected fat /100 LBW (PFG; Mohd Azmi et al., 2021) for four months from 14 to 18 month of age as a treatment period.

The used glycerol was with purity of ≥99% (Sigma, Sigma Aldrich Chemie Gmbh, Munich, Germany) and the treatment dose was according to El-Kasrawy et al. (2020), while protected fat was MEGALAC® (VOLAC ingredients SDN, BHD Malaysia). MEGALAC is prepared from crude palm oil that contains high levels of Polyunsaturated Fatty Acid (PUFA) and contains 84% oil, 9% calcium, 5% moisture and 32 MJ/ kg DM. It contains a fatty acid profile of 44% palmitic, 40% oleic and 9.5% linoleic acids.

Throughout four reproductive stages, pre-puberty (14 month of age), puberty, first service, and conception, amounts of feeds were adjusted every two weeks according to animal live body weight. All experimental Friesian female calves were individually housed in semi-open sheds and managed under similar environmental and managerial conditions.

Feeding system

Feeding of animals in all groups was achieved individually on a diet containing concentrate feed mixture (CFM), berseem hay (BH), and rice straw (RS) according to APRI requirements of Friesian calves. Diets were given twice (7 a.m. and 4 p.m.), while clean water was available all day time. Chemical analysis of different feedstuffs fed to ani-

mals is shown in Table 1.

Table 1: Chemical analysis of concentrate feed mixture (CFM), berseem hay (BH) and rice straw (RS) in the control diet of the experimental animals.

Item	DM %	Chemical analysis % (on DM basis)					
		OM	CP	EE	CF	NFE	Ash
CFM	89.25	91.52	16.15	3.31	11.35	60.71	8.48
RS	89.63	82.21	3.36	1.27	33.98	43.60	17.79
BH	90.42	87.36	12.73	1.39	29.46	43.78	12.64

DM= Dry matter, OM= Organic matter, CP= Crude protein, CF= Crude fiber, EE= Ether extract, NFE= nitrogen-free extract.

Experimental procedures

Live body weight of heifers pre-puberty and at puberty, first service, and conception was recorded then average daily gain of calves at intervals from pre-puberty-puberty, puberty-1st service, 1st service –conception, and pre-puberty- conception was calculated. Body measurements including body length, barrel girth, wither, hip height, and hook bone width (HBW) were estimated by tap pre-puberty, at puberty, and at conception according to Hoffman (1997).

Heifers were observed for estrous activity by introducing teaser bull twice (6 a.m. and 6 p.m.) starting at 14 month of age. Age at puberty was determined by exhibiting the 1st estrous signs in each calf. At the 1st estrus post-puberty, heifers in heat were naturally served by fertile mature bull, then age at 1st service and conception was recorded.

Reproductive performance parameters, including age at puberty, 1st service, and conceptions as well as pregnancy rate were recorded according to Abd El-Latif (2005). Pregnancy was diagnosed by rectal palpation on day 50 post-services, then pregnancy rate was calculated. Non-pregnant animals were re-served, and the number of services per conception within 90-days service period was recorded. Pregnancy was indicated by determination of P4 level in blood plasma 50-day post-service for pregnant animals. Pregnancy period was calculated within 60 and 90 days of the 1st service.

Blood samples and analytical procedures

Blood samples were taken from all animals in each group pre-puberty (14.47-14.62 months of age), at puberty (1st estrous activity), and at conception. Blood samples were collected by the jugular vein puncture into two heparinized test tubes, the 1st as whole blood and the 2nd for obtaining blood plasma by centrifugation at 4000 rpm for 15 minutes, then plasma was stored at -20°C until performing biochemical analysis.

In the whole blood samples, hemoglobin (Hb) concentration and packed cell volume (PCV%) were directly measured by Mission® Plus kit (REF C132-3031, USA) according to Henry (2001). Count of red blood cells (RBCs) and white blood cells (WBCs) was determined by a veterinary hematology analyzer (Exigo, Boule medical AB., Sweden).

In blood plasma samples, biochemical concentrations of total proteins (Tietz, 1990), albumin (Tietz, 1994), glucose (Trinder, 1969), total cholesterol (Richmond, 1973), triglycerides (McGowan et al., 1983), urea nitrogen (Tietz, 1990), and creatinine (Bartles et al., 1972) were assayed photo-metrically using spectrophotometer (JENWAY 6405 UV/Vis) and commercial kits (Bioassay systems, San Francisco, USA). Concentration of globulin was computed by subtracting concentration of total proteins from albumin.

The liver function was evaluated by measuring activity of ALT and AST enzymes in blood plasma according to Reitman and Frankel (1957). Blood plasma concentrations of P4 were assayed by radioimmunoassay (RIA) commercial kits (Coat-A-Count®-TKT31) by using Automatic Mini-Gamma Counter (LKB-1275; Saunders, 1995).

Statistical analysis

The obtained data were statistically analyzed according to IBM SPSS analysis program (SPSS, version 25, 2017). Data concerning live body weight, daily gain, body measurements, hemato-biochemical parameters, age at puberty, 1st service, and progesterone profile of animals were statistically analyzed by one-way ANOVA. Duncan Multiple Range Test (Duncan, 1955) was used for detecting the significant differences among groups at $P<0.05$. However, pregnancy rate of calves was tested by using Chi-square test.

RESULTS

Live body weight and gain

Treatments showed significant ($P<0.05$) effect on LBW and average daily gain (ADG) of animals at different reproductive stages (Figs. 1 and 2, respectively). Animals in PFG were the heaviest at puberty, 1st service, and conception (Fig. 1) and the highest ADG at different reproductive intervals (Fig. 2) as compared to CG ($P<0.05$). Heifers in GG were heavier ($P<0.05$) than control (CG) only at conception (Fig. 1), but showed higher ($P<0.05$) ADG than CG only at intervals (pre-puberty-puberty and pre-puberty – conception, Fig. 2).

Body measurements

Treatments showed significant ($P<0.05$) effect on all body measurements studied only at puberty and conception (Table 2). Animals in treatment groups (GG and PFG) had better body measurements at puberty ($P<0.05$) and

Table 2: Effect of treatment on calf's body measurements pre-puberty, at puberty and at conception.

Item	Body measurement (cm)				
	Body length	Barrel girth	Wither	Hip height	HBW≠
Pre-puberty					
CG	84.83±3.97	139.33±8.62	82.00±5.36	84.17±5.60	27.17±1.17
GG	86.00±5.21	138.83±9.76	82.50±7.06	84.33±6.77	27.50±1.38
PFG	85.83±2.78	139.50±6.28	81.67±4.67	83.50±4.84	26.50±1.22
P-value	0.869^{NS}	0.990^{NS}	0.969^{NS}	0.966^{NS}	0.398^{NS}
At puberty					
CG	112.33±4.67^{b}	186.67±3.38^{b}	112.83±1.72^{b}	114.67±1.63^{b}	31.17±2.14^{b}
GG	120.83±5.19^{a}	193.50±3.88^{a}	116.67±3.14^{a}	117.83±3.54^{ab}	36.17±1.94^{a}
PFG	122.83±4.44^{a}	195.67±1.75^{a}	117.50±2.59^{a}	119.00±2.68^{a}	36.83±1.47^{a}
P-value	0.004**	0.000***	0.014*	0.040*	0.000***
At conception					
CG	124.00±8.32	200.67±9.91^{b}	116.50±3.27^{b}	118.83±3.25^{b}	34.83±2.93^{b}
GG	130.83±8.40	214.33±6.62^{a}	120.17±2.93^{a}	122.17±3.60^{ab}	37.83±1.47^{a}
PFG	134.33±4.72	215.47±4.93^{a}	121.67±2.06^{a}	123.50±2.08^{a}	38.50±1.05^{a}
P-value	0.076^{NS}	0.013*	0.017*	0.048*	0.014*

Means with different superscripts within the same column for each stage are significantly different at P<0.05. NS: Not significant. * Significant at P<0.05. ** Significant at P<0.01. *** Significant at P<0.001. ≠ HBW: Hook bone width.

Table 3: Effect of treatment on blood cells count, hemoglobin, and packed cell volume pre-puberty, at puberty and at conception.

Item	Hematological parameter			
	RBC (x10^{6}/mm^{3})	WBC (x10^{3}/mm^{3})	Hb (g/dl)	PCV (%)
Pre-puberty				
CG	7.67±0.28	7.88±0.11	8.85±0.71	32.90±0.69
GG	7.42±0.20	7.95±0.27	9.03±0.34	33.06±1.22
PFG	7.40±0.17	7.91±0.28	8.95±0.29	32.61±0.86
P-value	0.637^{NS}	0.976^{NS}	0.966^{NS}	0.945^{NS}
At puberty				
CG	7.88±0.09^{b}	8.08±0.15^{b}	9.82±0.11^{b}	35.80±1.16^{b}
GG	9.52±0.32^{a}	8.86±0.23^{a}	11.27±0.65^{a}	39.18±1.15^{a}
PFG	9.72±0.07^{a}	8.98±0.26^{a}	11.45±0.43^{a}	40.23±0.67^{a}
P-value	0.000***	0.021*	0.043*	0.020*
At conception				
CG	8.04±0.12^{b}	8.21±0.16^{b}	10.62±0.10^{b}	36.12±1.14^{b}
GG	10.72±0.07^{a}	9.01±0.22^{a}	12.47±0.65^{a}	41.07±0.66^{a}
PFG	10.81±0.32^{a}	9.14±0.26^{a}	12.75±0.42^{a}	42.04±1.16^{a}
P-value	0.000***	0.018*	0.009**	0.009**

Means with different superscripts within the same column for each stage are significantly different at P<0.05. RBC: Red blood cells count. WBC: White blood cells count. Hb: Hemoglobin. PCV: Packed cell volume. NS: Not significant. * Significant at P<0.05. ** Significant at P<0.01. *** Significant at P<0.001.

conception (P≥0.05) than the controls in term of longer body, increased barrel girth, wither, hip height, and hook bone width in comparison with CG. Such measurements are in parallel with the change in LBW and average daily gain of animals in different groups.

Hematological parameters

The significant effect of treatment on hematological parameters revealed remarkable improvements (P<0.05) in GG and PFG regard to count of RBCs and WBCs, Hb concentration, and PCV percentage at puberty and con-

ception. The most effect of treatments was found on RBCs rather than on other hematological parameters studied (Table 3).

Figure 1: Live body weight of animals at different reproductive stages (pre-puberty to conception). (Means at each stage with different superscripts differ significantly at P<0.05).

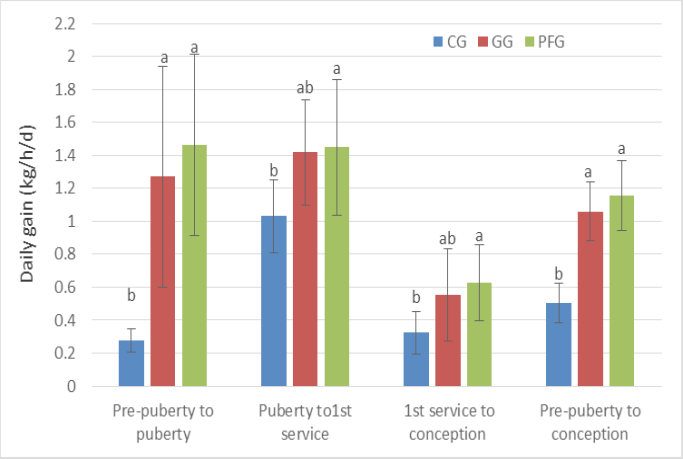

Figure 2: Average daily gain of animals at different reproductive stages (pre-puberty to conception). (Means at each stage with different superscripts differ significantly at P<0.05).

Protein and carbohydrate metabolism

Results in Table 4 showed that GG and PFG had higher (P<0.05) concentration of plasma total proteins, by increasing (P<0.05) globulin level without significant effect on albumin level at puberty and conception. Also, both treatments increased glucose concentration at puberty and conception.

Lipid profile

Data presented in Table 5 revealed a marked increase (P<0.05) in concentration of plasma total cholesterol, total lipids, and triglycerides of claves in PFG as compared to CG at puberty. However, only plasma total lipids concentr-

Table 4: Effect of treatment on protein metabolites in blood plasma pre-puberty, at puberty and at conception.

Item	Blood plasma concentration			
	Total proteins (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Glucose (mg/dl)
Pre-puberty				
CG	6.07±0.20	3.20±0.13	2.87±0.18	52.66±1.48
GG	6.14±0.26	3.25±0.04	2.89±0.24	52.89±1.13
PFG	6.15±0.18	3.23±0.19	2.91±0.21	52.96±1.97
P-value	0.963^{NS}	0.966^{NS}	0.988^{NS}	0.990^{NS}
At puberty				
CG	7.03±0.26^{b}	3.36±0.19	3.66±0.41^{b}	53.41±1.74^{b}
GG	9.21±0.36^{a}	3.91±0.29	5.29±0.32^{a}	59.62±2.15^{a}
PFG	9.59±0.49^{a}	3.97±0.33	5.62±0.43^{a}	62.91±2.04^{a}
P-value	0.011*	0.307^{NS}	0.001***	0.039*
At conception				
CG	7.99±0.18^{b}	3.43±0.18	4.55±0.22^{b}	54.32±1.93^{b}
GG	10.35±0.27^{a}	3.98±0.29	6.37±0.34^{a}	64.76±1.94^{a}
PFG	10.64±0.35^{a}	4.00±0.33	6.64±0.52^{a}	65.86±1.58^{a}
P-value	0.000***	0.307^{NS}	0.003**	0.001***

Means with different superscripts within the same column for each stage are significantly different at P<0.05. NS: Not significant. * Significant at P<0.05. ** Significant at P<0.01. *** Significant at P<0.001.

Table 5: Effect of treatment on lipid profile in blood plasma pre-puberty, at puberty and at conception.

Item	Blood plasma concentration (mg/dl)		
	Total cholesterol	Total lipids	Triglycerides
Pre-puberty			
CG	179.15±5.32	489.00±4.97	20.04±0.89
GG	180.23±5.95	487.53±5.81	18.73±0.78
PFG	178.40±5.06	486.83±7.33	19.25±0.72
P-value	0.965^{NS}	0.948^{NS}	1.000^{NS}
At puberty			
CG	187.36±7.31^{b}	494.66±6.63^{b}	19.22±1.07^{b}
GG	207.04±7.73^{ab}	537.45±5.68^{a}	22.48±1.59^{ab}
PFG	214.03±8.57^{a}	551.72±6.42^{a}	25.69±1.25^{a}
P-value	0.050*	0.000***	0.008**
At conception			
CG	186.36±9.08^{b}	492.21±9.36^{b}	20.37±0.92^{b}
GG	224.47±7.16^{a}	554.71±9.48^{a}	26.83±1.13^{a}
PFG	233.34±8.50^{a}	563.54±10.80^{a}	27.88±1.18^{a}
P-value	0.005**	0.000***	0.000***

Means with different superscripts within the same column for each stage are significantly different at P<0.05. NS: Not significant. * Significant at P<0.05. ** Significant at P<0.01. *** Significant at P<0.001.

Table 6: Effect of treatment on markers of kidney and liver function in blood plasma pre-puberty, at puberty and at conception.

Item	Blood plasma concentration			
	Urea-N (g/dl)	Creatinine (g/dl)	AST (IU/dl)	ALT (IU/dl)
Pre-puberty				
CG	17.29±0.65	1.41±0.06	46.86±1.34	21.03±1.15
GG	17.10±0.88	1.32±0.07	48.32±1.28	20.78±0.88
PFG	17.04±0.92	1.33±0.08	47.18±1.45	20.35±0.99
P-value	0.976^{NS}	0.654^{NS}	0.734^{NS}	0.849^{NS}
At puberty				
CG	16.30±0.61^{a}	1.05±0.12^{a}	46.49±1.07^{a}	20.94±1.18^{a}
GG	10.06±0.52^{b}	0.68±0.10^{b}	39.41±1.28^{b}	16.33±1.01^{b}
PFG	9.33±0.45^{b}	0.66±0.13^{b}	37.40±1.73^{b}	15.09±0.97^{b}
P-value	0.000***	0.050*	0.001***	0.003**
At conception				
CG	15.89±0.68^{a}	0.98±0.13^{a}	46.07±1.89^{a}	20.59±0.96^{a}
GG	8.85±0.56^{b}	0.61±0.07^{b}	35.78±1.86^{b}	14.22±0.58^{b}
PFG	7.82±0.48^{b}	0.58±0.05^{b}	33.77±1.55^{b}	12.75±0.94^{b}
P-value	0.000***	0.013*	0.000***	0.000***

Means with different superscripts within the same column for each stage are significantly different at P<0.05. NS: Not significant. * Significant at P<0.05. ** Significant at P<0.01. *** Significant at P<0.001.

Table 7: Effect of treatment on reproductive performance parameters of heifers.

Item	Experimental group			*P*-value
	CG	GG	PFG	
Pre-puberty age (Initial age, months)	14.47±1.38	14.23±1.33	14.28±1.53	0.956^{NS}
Age at puberty (months)	20.06±2.86^{a}	16.61±2.27^{b}	16.43±2.19^{b}	0.037*
Age at 1st service (months)	22.98±2.97^{a}	18.69±2.40^{b}	18.29±2.31^{b}	0.011*
Age at conception (months)	25.23±2.82^{a}	20.31±2.45^{b}	20.11±2.74^{b}	0.007**
Service period (day)	67.50±11.75	48.60±6.17	54.50±10.13	0.389^{NS}
Number of services/conception	3.33±0.61	2.33±0.33	2.50±0.50	0.338^{NS}
Pregnancy rate within 60-d service period	16.7^{c}	50^{a}	33.33^{b}	-
Pregnancy rate within 90-d service period	66.7^{b}	100^{a}	83.3^{ab}	-

Means in the same row with different superscripts differ significantly (P<0.05).
NS: Not significant. * Significant at P<0.05. ** Significant at P<0.01.

-ation was increased (P<0.05) in GG as compared to CG at puberty. At conception, both treatments increased (P<0.05) lipid profile as compared to CG. The most effect of treatment was recorded on total lipids and triglycerides at puberty and conception.

Kidney and liver function of calves

Effect of treatment on plasma concentration of urea-N, creatinine, AST and ALT at puberty and conception was significant (Table 6). At both stages, concentration of plasma urea-N and creatinine, as markers of kidney function, and AST and ALT activities, as variables for function of the liver, were lower (P<0.05) in GG and PFG than in CG.

Reproductive performance of heifers

Effect of treatment on age at different reproductive stages was significant (P<0.05, Table 7). Heifers in treatment groups (GG and PFG) showed earlier (P<0.05) ages at puberty, 1st service, and conception than those in CG, being the earliest in PFG. Service period length (SPL) was shorter (P<0.05) and number of services per conception (NSC) was lower (P<0.05) in GG and PFG than in CG, but the differences were not significant. Pregnancy rate (PR) was the highest in GG, followed by PFG, and the lowest in CG (P<0.05) within 60 and 90-day service period (Table 7).

Plasma progesterone profile

Effect of treatment on progesterone (P4) profile in blood plasma of heifers at puberty and conception was significant (P<0.05), but insignificant pre-puberty differences were observed as illustrated in Fig. 3. Heifers in treatment groups (GG and PFG) had higher plasma P4 concentration (P<0.05) at puberty and conception than in CG. At conception, P4 concentration was the highest in PFG, followed by GG, and the lowest values were recorded in CG (P<0.05).

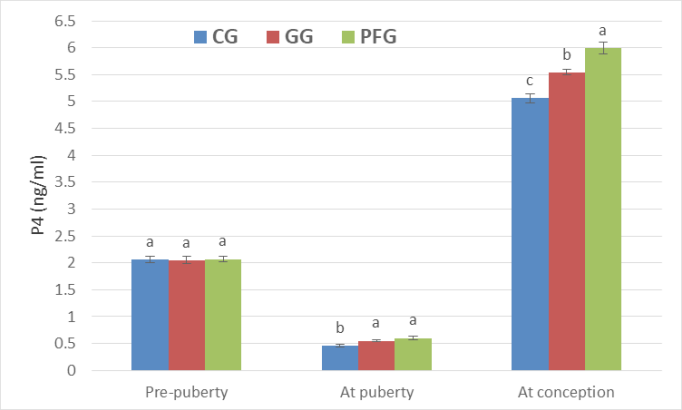

Figure 3: Concentration of plasma P4 of heifers in different groups pre-puberty, at puberty, and at conception. (Means in the same column with different superscripts differ significantly at P<0.05).

DISCUSSION

The current study aimed to evaluate the impact of dietary supplementation of protected fat or glycerol, as a source of energy, on puberty, some metabolic parameters, and pregnancy rate of Friesian heifers. Results concerning the LBW, average daily gain, and body measurements of heifers at puberty, 1st service, and conception indicated that the dietary addition of glycerol or protected fat improved the growth performance of Friesian heifers, but PFG showed the highest performance as compared to the control heifers from pre-puberty up to conception. In similar trend to our results, Putrino et al. (2006) found that feeding Nellore heifers on protected fat (Lacto Plus® produced from vegetable fat soy) increased body weight gain with less dry matter intake. Also, feeding male bulls (14-month-old) on diets with 5% protected fat (LAC-100 Yakult® based on soybean oil complexed with calcium) improved average daily weight gain and feed conversion ratio (Jaeger and Oliveira, 2007). In sheep, body weight of cull ewes fed diet supplemented with ruminal protected fat (RPF) of rice bran oil was improved significantly as compared to control (Bhatt et al., 2013; Bhatt and Sahoo, 2017). On the other hand, lipid sources influence the average daily weight gain (P<0.05), but significant differences were not found in the protein efficiency ratio and feed conversion of heifers (Fiorentini et al., 2012). Supplementation of different types of protected fats was reported to have no effects on animal performance of Dorper sheep (Behan et al., 2019). In our study the highest LBW and average daily gain are in parallel with the maximal values of different body measurements in heifers fed protected fat diet. According to Anderson (2008), body measurements serve as useful reference for growth performance. Inclusion of fat in ruminant diets can improve methanogenesis process and efficiency of energy by lowering the methane production in the rumen and inclusion of long-chain fatty acids in the fat synthesis, metabolically, without potentially requirement for acetate and glucose (Doreau and Chilliard, 1997).

Improving growth performance of heifers in GG and PFG was associated with all hematological parameters studied. The dietary addition of glycerol or protected fat increased count of RBCs and WBCs, Hb concentration, and PCV percent of Friesian heifers at puberty and conception. Moreover, elevation of total proteins, globulin, and glucose concentrations in blood plasma of heifers indicated marked improvement in protein and carbohydrate metabolism as affected by the dietary addition of glycerol or protected fat. Increasing plasma total proteins and its fraction globulin in PFG as compared to control was proved by Zeedan et al. (2010), who found an increase in total serum proteins due to the inclusion of either oil or fat in the diets of small ruminant and buffalos. Ghoniem and Atia, (2020) found that plasma total proteins and their fractions as well as glucose concentrations increased by feeding Suffolk x Ossimi ewes diets supplemented with 4 and 6% fatty acids. Similar findings were reported by Abo-Donia (2003) and Zeedan (2003). Although Porcu et al. (2018) found a decrease in total proteins and urea circulating concentrations as affected by intra-ruminal dosing of a glucogenic mixture on lactating Sarda ewes, Saleem et al. (2018) found that feeding glycerol to lactating buffaloes did not affect serum total protein, globulin, and albumin. These findings are in contrast to the increase in total proteins and globulins observed in our study under the effect of glycerol. This conflict may be attributed to level of glycerol treatment and species and age variations. Glycerol (Gl) are used as gluconeogenic precursors (Rizos, et al., 2008; Piantoni and Allen, 2015). The observed increase in glucose level in GG is in accordance with Osman, et al. (2008), who found increased glucose plasma concentration after daily oral administration of 400 mL pure GL/day compared to the control group. Wang et al. (2009b) observed a linear increase of plasma glucose with increasing GL supplementation (100, 200 and 300 g/day). Furthermore, several studies have shown that GL can elevate plasma glucose levels (Osman et al., 2008; Linke, 2005; Goff and Horst, 2001). The goals of GL treatment are decrease the incidence of ketosis by gluconeogenesis stimulation, increasing plasma glucose, and decreasing lipolysis (Herdt and Emery, 1992). Research in-

dicates GL is rapidly metabolized in the rumen (Garton et al., 1961; Wright, 1969). The increase in insulin might have been from the portion of GL metabolized to propionate or to the increase in plasma glucose, whereas the increase in plasma glucose might have been from the glucose-sparing effect of acetate, a portion of GL being metabolized to propionate, or from partial absorption of GL and increased gluconeogenesis (Piantoni and Allen, 2015).

The remarkable increase in lipid profile of heifers, in term of increasing levels of total cholesterol, total lipids, and triglycerides, was expected firstly for protected fat and secondly for glycerol treatment. This finding was proved only for protected fat at puberty and conception, and for glycerol only at conception. These results agreed with those reported by Jolazadeh et al. (2019), who found that the supplementation of fat increased pre- and post-partum plasma cholesterol (Jolazadeh et al., 2019). In cows, level of plasma cholesterol increased in animals infused post-ruminally with free long-chain fatty acids (Drackley et al., 1992). Dietary inclusion of MEGALAC, as a protected fat, increased the availability of blood free fatty acids; those are essential components of triglycerides (Shahin, 1993). Dietary fatty acids (4 and 6%) supplementation increased total cholesterol, triglycerides and total lipids as compared to controls during late pregnancy period (Ghoniem and Atia, 2020). In Friesian bulls, protected fat levels (4 or 8%) of dietary ration dry matter increased total lipids and triglycerides in blood plasma, while total cholesterol was higher for 8% fat than the control and 4% fat (El-Bedawy et al., 2004). Similar results were obtained by Avila et al. (2000), Zeedan (2003) and Zeedan et al. (2010). The elevation in plasma total lipids of fat supplemented groups may be attributed to large quantity of fatty acids absorbed from fat supplemented diets through the gut and /or the fact that feeding fat is associated with depression in lipogenic enzyme activities by liver and adipose tissues (Storry, 1981).

The reduction in concentration of urea-N and creatinine, as markers of kidney function, and also the decrease in AST and ALT activities, as markers of liver function, was observed in both GG and PFG in comparing with the control. Such results may be good indicators for higher protein utilization, increased feed efficiency, and normal kidney and liver function of heifers, which may allow the save use of protected fat and glycerol in calves feeding. In the same way, Porcu et al. (2018) found a decrease in urea circulating concentrations as affected by intra-ruminal dosing of a glycogenic mixture on lactating Sarda ewes. Also, Ghoniem and Atia (2020) showed that kidney function, as urea and creatinine concentrations, is in the normal case as affected by 4 or 6% fatty acids supplementation. Contrary, some authors found significant increase in the concentration of serum urea observed adherent to the supplementation of cows with MEGALAC (Sallam et al., 2021), linseed oil addition in lactating Holstein cows (Loor et al., 2005), and in Red Sokoto goats supplemented with palm oil (Otaru et al., 2011). Moreover, liver functions, as the activities of AST and ALT, were not significantly affected by diet supplementations with protected fat. Values of AST and ALT are within the normal range, indicating good nutritional status of animals and normal health condition of their livers (Storry, 1981). The incidence of health problems decreased as compared to control cows by feed fat diets (Jolazadeh et al., 2019).

The rise observed in lipid profile and glucose concentration by both treatments in particular at conception may suggest an increase in energy balance for ovarian activity and reproductive ability of heifers during the interval from puberty to conception in parallel with improving metabolism of protein and carbohydrate. Fat supplementation increased plasma cholesterol and cholesterol content in follicular fluid and corpus luteum (Staples et al., 1998). Another potential mechanism for improved reproductive efficiency with fat supplementation may be related to the strategic deposition of fatty acids in reproductive organs such as the endometrium (Mattos et al., 2003, 2004), which may have played a critical role on early resumption of heat and lower embryo death rate. Glucose level is essential for reproductive organs and development of oocytes (Berlinguer, et al., 2012). The incidence of reproductive problems was lower in fat-treated cows than in control cows (Jolazadeh et al., 2019). Replacing corn with Ca-LCFA from palm oil in the ration of dairy cows at calving increased the number of small (2-5 mm), medium (6-9 mm), and large size (>15 mm) follicles within 25 days postpartum which may be due to the high percentage of linoleic acid in MEGALAC (Sallam et al., 2021) and may be likely a direct result of the effects of high levels of linoleic acid in the rumen (Lucy et al., 1991). These results reflected a positive impact of glycerol or protected fat treatment on precocious ages of heifers at puberty, 1st service and conception, and increasing pregnancy rate, with insignificant effect on number of services per conception. More impact seemed to be for glycerol than protected fat treatment on pregnancy of heifers. Jolazadeh et al. (2019) reported that supplemental fat shortened the days at first estrus and first service compared with cows fed control diet. Pregnancy rate was increased in cows treated with fat as compared to control (75.1 vs. 55.7%). They added that the differences in the number of AI per pregnancy among dietary treatments were not significant. Rodney et al. (2018) suggested that increased fatty acids, starch, and metabolizable energy balance intake were positively associated with increasing pregnancy rate of cows.

Improving pregnancy rate of animals in GG may be due to that glycerol is beneficial for improving energy status (Chung et al., 2007). Previous reports, *in vivo* and *in vitro,* have showed the impact of glycerol on volatile fatty acids (VFA) profiles, with higher proportions of propionate relative to acetate (Wang et al., 2009a; Avila et al., 2011; Carvalho et al., 2011). Glycerol is an energy-rich component, with an estimated value of 16.2 MJ ME/kg of DM for ruminants (Mach et al., 2009), and the glycogenic property of glycerol is well established (Cori and Shine, 1935). Comparing the combined protected fat treatments to control resulted in a higher ($P<0.05$) conception rate to first service for the fat treatments (McNamara, et al., 2003). Dietary addition of fatty acids supported reproductive management (Rodney et al., 2015; Klebaniuk et al., 2017; Bryszak et al., 2019). Besides the increment of energy density, PUFA influence fertility in farm animals by modulating the biosynthesis of prostaglandins, steroids, and the transcriptional regulation of genes involved in the control of fertility (Waters et al., 2012; Marei et al., 2018). Positive energy balance in dairy cows is associated with increasing LH pulse frequency, growth rate and diameter of dominant follicle, weight of the corpus luteum, estradiol, and progesterone (Pryce and Royal, 2004; van Knegsel et al., 2005). This finding is proven in our study by increasing P4 profile with increased fat source, as an intake for heifers. It is well known that cholesterol uptake is the main source for P4 thenthysis in the lureal cells of the corpus luteum of pregnant animals. Lammoglia et al. (2000) found that high-energy diet positively impacted progesterone profile in heifers. In dairy cows, CL function was affected by diets containing PUFA, as a direct effect on production of P4 or an indirect effect on arachidonic acid synthesis (Mattos et al., 2000).

CONCLUSION

Based on the forgoing results, pre-puberty treatment of female heifers orally with 300 ml GL twice/week/head or daily protected fat (25 g/100 kg/d) for 4 months is important for raising breeding female heifers for precious puberty, early ages at 1st service and conception, increased pregnancy rate, and better body confirmation during the 1st parity. Supplementation of heifers with glycerol or protected fat is considered as an accepted strategy for supporting fertility management and improving the animal performance.

ACKNOWLEDGEMENTS

The authors thank the Animal Production Research Institute and Department of Animal Production, Faculty of Agriculture, Mansoura University.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR'S CONTRIBUTION

All authors were contributed to design the experimental work. Wael Mohamed Wafa, Hamdy Abdala El-Nagar conducted the experimental procedures and collected data. Mohammed Mahmoud Hegazy, Mohamed Mohamed El-said Ibrahim and Rehab Fawzy Ismail performed the sample preparations for laboratory analysis. Wael Mohamed Wafa conducted the statistical analyses and critically revised the manuscript.

REFERENCES

Abd El-Latif MA. (2005). Physiological and nutritional studies on reproduction of dairy cattle. Ph. D. Thesis, Fac. of Agric., Mansoura Univ., Egypt.

Abo-Donia FMA. (2003). Using different sources of soap-stock as non-conventional source of energy in sheep rations. Egypt. J. Nutr. Feeds 6 (Special Issue): 1381.

Anderson N. (2008). Dairy Cow Comfort Tie-stall Dimensions. Infosheet January 2008. OMAFRA, Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, ON, Canada.

Atkare VG, Zinjarde RM, Shambharkar AD. (2018). Effect of Feeding of Bypass Fat Mineral Mixture on Productive Performance and Quality of Milk of Buffaloes in Gadchiroli Tahsil. Int. J. Curr. Microbiol. App. Sci., 7(8): 4694-4701. https://doi.org/10.20546/ijcmas.2018.708.493

Avila CD, DePeters EJ, Perez-Monti H, Taylor SJ, Zinn RA. (2000). Influences of saturation ratio of supplemental dietary fat on digestion and milk yield in dairy cows. J. Dairy Sci., 83(7):1505-1519. https://doi.org/10.3168/jds.S0022-0302(00)75023-5. PMID: 10908059.

Avila JS, Chaves AV, Hernandez-Calva M, Beauchemin KA, McGinn SM, Wang Y, Harstad OM, McAllister TA. (2011). Effects of replacing barley grain in feedlot diets with increasing levels of glycerol on in vitro fermentation and methane production. Anim. Feed Sci. Technol., 166-167: 265-268. https://doi.org/10.1016/j.anifeedsci.2011.04.016

Ballou MA, Gomes RC, Juchem SO, DePeters EJ. (2009). Effects of dietary supplemental fish oil during the peripartum period on blood metabolites and hepatic fatty acid compositions and total triacylglycerol concentrations of multiparous Holstein cows. J. Dairy Sci., 92(2): 657-669. https://doi.org/10.3168/jds.2008-1196.

Bartles H, Bohmer M, Heirli C. (1972). Serum creatinine determination without protein preciptation. Clin. Chem. Acta., 37:193-197. ISBN: 0009-8981.

Bayatkouhsar J, Tahmasebi AM, Naserian AA, Mokarram RR, Valizadeh R. (2013). Effects of supplementation of lactic acid bacteria on growth performance, blood metabolites and fecal coliform and lactobacilli of young dairy calves. Anim. Feed Sci. Technol., 186(1): 1-11. https://doi.org/10.1016/j.anifeedsci.2013.04.015.

Beam SW, Butler WR. (1997). Energy balance and ovarian follicle development prior to the first ovulation postpartum in dairy cows receiving three levels of dietary fat. Biol. Reprod.,

56(1): 133-142. https//doi.org/10.1095/biolreprod56.1.133.
Behan AA, Loh TC, Fakurazi S, Kaka U, Kaka A, Samsudin AA. (2019). Effects of Supplementation of Rumen Protected Fats on Rumen Ecology and Digestibility of Nutrients in Sheep. Animals (Basel)., 30; 9(7): 400. http//doi.org/10.3390/ani9070400.
Berge AC, Lindeque P, Moore DA, Sischo WM. (2005). A clinical trial evaluating prophylactic and therapeutic antibiotic use on health and performance of preweaned calves. J. Dairy Sci., 88(6): 2166-2177. https//doi.org/10.3168/jds.S0022-0302(05)72892-7.
Berlinguer F, Gonzalez-Bulnes A, Contreras-Solis I, Spezzigu A, Torres-Rovira L, Succu S, Naitana S, Leoni GG. (2012). Glucogenic supply increases oocyte developmental competence in sheep. Reprod. Fertil. Dev., 24(8): 1055-1062. https://doi.org/10.1071/RD11299.
Bhatt RS, Sahoo A. (2017). Effect of feeding complete feed block containing rumen protected protein, non-protein nitrogen and rumen protected fat on improving body condition and carcass traits of cull ewes. J. Anim. Physiol. Anim. Nutr. (Berl), 101(6): 1147-1158. https://doi.org/10.1111/jpn.12628.
Bhatt RS, Sahoo A, Shinde AK, Karim SA. (2013). Change in body condition and carcass characteristics of cull ewes fed diets supplemented with rumen bypass fat. Livest. Sci., 157, 132–140. https://doi.org/10.1016/j.livsci.2013.06.025.
Bhatti SA, Sarwar MA, Khan MS, Hussain SM. (2007). Reducing the age at first calving through nutritional manipulations in dairy buffaloes and cows: A review. Pakistan Veterinary Journal, 27: 42-47. ISSN : 0253-8318.
Bryszak M, Szumacher-Strabel M, El-Sherbiny M, Stochmal A, Oleszek W, Roj E, Patra AK, Cieslak A. (2019). Effects of berry seed residues on ruminal fermentation, methane concentration, milk production, and fatty acid proportions in the rumen and milk of dairy cows. J Dairy Sci., 102(2): 1257-1273. https://doi.org/10.3168/jds.2018-15322.
Buskirk DD, Faulkner DB, Ireland FA. (1995). Increased postweaning gain of beef heifers enhances fertility and milk production. J. Anim. Sci., 73(4): 937-946. https://doi.org/10.2527/1995.734937x.
Carvalho ER, Schmelz-Roberts NS, White HM, Doane PH, Donkin SS. (2011). Replacing corn with glycerol in diets for transition dairy cows. Journal of dairy science, 94(2): 908-916. https://doi.org/10.3168/jds.2010-3581.
Chung YH, Rico DE, Martinez CM, Cassidy TW, Noirot V, Ames A, Varga GA. (2007). Effects of feeding dry glycerin to early postpartum Holstein dairy cows on lactational performance and metabolic profiles. J. Dairy Sci., 90(12): 5682-5691. https://doi.org/10.3168/jds.2007-0426.
Cori CF, Shine WM. (1935). The formation of carbohydrate from glycerophosphate in the liver of the rat. American Association for the Advancement of Science. Science, 82: 134-135. https://www.jstor.org/stable/i297442.
Dirandeh E, Towhidi A, Zeinoaldini S, Ganjkhanlou M, Ansari Pirsaraei Z, Fouladi-Nashta A. (2013). Effects of different polyunsaturated fatty acid supplementations during the postpartum periods of early lactating dairy cows on milk yield, metabolic responses, and reproductive performances. J. Anim. Sci. 91: 713-721. https://doi.org/10.2527/jas.2012-5359.
Doreau M, Chilliard Y. (1997). Digestion and metabolism of dietary fat in farm animals. Br. J. Nutr. 78, S15–S35. https://doi.org/10.1079/bjn19970132.
Drackley JK. (1999). ADSA Foundation Scholar Award. Biology of dairy cows during the transition period: the final frontier? J Dairy Sci. Nov;82(11):2259-73. https://doi.org/10.3168/jds.s0022-0302(99)75474-3.
Drackley JK, Klusmeyer TH, Trusk AM, Clark JH. (1992). Infusion of long-chain fatty acids varying in saturation and chain length into the abomasum of lactating dairy cows. J Dairy Sci., 75(6): 1517-1526. https://doi.org/10.3168/jds.S0022-0302(92)77908-9.
Duncan DB. (1955). "Multiple range and multiple F tests". Biometrics., 11: 1-42. https://doi.org/ HYPERLINK "https://doi.org/10.2307%2F3001478"10.2307/3001478.
El-Bedawy TM, Gomrnaa IA, Sabbah M Allam, Abu-Donia, FM. (2004). Production of calcium salts of fatty acid from soap-stock on semi industrial scale and its use in finishing rations of Friesian bulls. Egyptian J. Anim. Prod., 41(1): 19-30. (https://ejap.journals.ekb.eg/article_107197_2da0b3eefc2eae1990eecdcaea4d1957.pdf).
El-Kasrawy NI, Swelum AA, Abdel-Latif MA, Alsenosy AEA, Beder NA, Alkahtani S, Abdel-Daim MM, Abd El-Aziz AH. (2020). Efficacy of Different Drenching Regimens of Gluconeogenic Precursors during Transition Period on Body Condition Score, Production, Reproductive Performance, Subclinical Ketosis and Economics of Dairy Cows. Animals (Basel)., 10(6): 937. https://doi.org/10.3390/ani10060937.
Endecott RL, Funston RN, Mulliniks JT, Roberts AJ. (2013). Joint Alpharma-Beef Species Symposium: implications of beef heifer development systems and lifetime productivity. J Anim Sci., 91(3): 1329-1335. https://doi.org/10.2527/jas.2012-5704.
Fiorentini G, Santana MCA, Sampaio AAM, Reis RA, Ribeiro AF, Berchielli TT. (2012). Intake and performance of confined crossbred heifers fed different lipid sources. R. Bras. Zootec., 41(6): 1490-1498. https://doi.org/10.1590/S1516-35982012000600025.
Freetly HC, Cundiff LV. (1997). Postweaning growth and reproduction characteristics of heifers sired by bulls of seven breeds and raised on different levels of nutrition. J Anim Sci., 75(11): 2841-2851. https://doi.org/10.2527/1997.75112841x.
Frizzo LS, Soto LP, Bertozzi E, Zbrun MV, Signorini ML, Sequeira GR, Rodriguez A, Rosmini MR. (2011). Intestinal populations of Lactobacilli and coliforms after in vivo Salmonella Dublin challenge and their relationship with microbial translocation in calves supplemented with lactic acid bacteria and lactose. Anim. Feed Sci. Technol. 170 (1): 12-20. https://doi.org/10.1016/j.anifeedsci.2011.07.016.
Garton GA, Lough AK, Vioque E. (1961). Glyceride hydrolysis and glycerol fermentation by sheep rumen contents. J Gen Microbiol., 25: 215-225. https://doi.org/10.1099/00221287-25-2-215.
Ghoniem AH, Safaa ES Atia (2020). Effect of addition protected fatty acids in ruminant rations on productive performance of suffolk x ossimi crossbred ewes during different production stages. Egyptian J. Nutrition and Feeds, 23(3): 369-383. https://doi.org/10.21608/EJNF.2020.148119.
Goff JP, Horst RL. (2001). Oral glycerol as an aid in the treatment of ketosis/fatty liver complex. International Animal Agriculture and Food Science Conference, Jul. 25, 2001. (https://www.ars.usda.gov/research/publications/publication/?seqNo115=124812).
Hammon HM, Metges CC, Junghans P, Becker F, Bellmann O, Schneider F, Nürnberg G, Dubreuil P, Lapierre H. (2008).

Metabolic changes and net portal flux in dairy cows fed a ration containing rumen-protected fat as compared to a control diet. J Dairy Sci., 91(1): 208-217. https://doi.org/10.3168/jds.2007-0517.

Henry JB. (2001). Clinical Diagnosis and Management by Laboratory Methods. Twentieth Edition, Page 485. ISBN 0-7216-8864-0.

Herdt TH, Emery RS. (1992). Therapy of diseases of ruminant intermediary metabolism. Vet. Clin. North. Am. Food. Anim. Pract., 8(1): 91-106. https://doi.org/10.1016/s0749-0720(15)30761-1.

Hoffman PC. (1997). Optimum body size of Holstein replacement heifers. J Anim Sci., 75(3): 836-845. https://doi.org/10.2527/1997.753836x.

IBM Corp. Released (2017). IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp. (https://www.ibm. com/support/pages/how-cite-ibm-spss-statistics-or-earlierversions-spss).

Jaeger SMPL and Oliveira RL. (2007). Uso de gordura protegida sobre o desempenho e a digestibilidade para diferentes grupos genéticos de bovinos em confinamento. Magistra, 19: 135-142.

Jenkins TC, McGuire MA. (2006). Major advances in nutrition: impact on milk composition. J Dairy Sci., 89(4): 1302-1310. https://doi.org/10.3168/jds.S0022-0302(06)72198-1.

Jolazadeh AR, Mohammadabadi T, Dehghan-Banadaky M, Chaji M, Garcia M. (2019). Effect of supplementation fat during the last 3 weeks of uterine life and the preweaning period on performance, ruminal fermentation, blood metabolites, passive immunity and health of the newborn calf. Br. J. Nutr., 122(12): 1346-1358. https://doi.org/10.1017/S0007114519002174.

Kalyesubula M, Rosov A, Alon T, Moallem U, Dvir H. (2019). Intravenous Infusions of Glycerol Versus Propylene Glycol for the Regulation of Negative Energy Balance in Sheep: A Randomized Trial. Animals (Basel), 9(10): 731. https://doi.org/10.3390/ani9100731.

Kawakami SI, Yamada T, Nakanishi N, Cai Y. (2010). Feeding of lactic acid bacteria and yeast on growth and diarrhea of Holstein calves. J. Anim. and Vet. Advances. 9(7): 1112-1114. https://doi.org/10.3923/javaa.2010.1112.1114.

Klebaniuk R, Kochman G, Kowalczuk-Vasilev E, Grela ER, Kowalczyk-Pecka D, Bąkowski M. (2017). Dietary supplementation with glucogenic precusors and fatty acids improves performance and health of periparturient dairy cows. Anim. Prod. Sci., 59: 109-121. https://doi.org/10.1071/AN16550.

Lammoglia MA, Bellows RA, Grings EE, Bergman JW, Bellows SE, Short RE, Hallford DM, Randel RD. (2000). Effects of dietary fat and sire breed on puberty, weight, and reproductive traits of F1 beef heifers. J. Anim. Sci., 78(9): 2244-2252. https://doi.org/10.2527/2000.7892244x.

Lesmeister JL, Burfening PJ, Blackwell RL. (1973). Date of first calving in beef cows and subsequent calf production. J. Anim. Sci., 36: 1-6. https://doi.org/10.2527/jas1973.3611.

Linke P. (2005). Ruminal and Plasma Responses in Dairy Cows to Drenching or Feeding Glycerol. The Journal of Undergraduate Research, 3: Article 8. http://openprairie.sdstate.edu/jur/vol3/iss1/8.

Loor JJ, Ferlay A, Ollier A, Doreau M, Chilliard Y. (2005). Relationship among trans and conjugated fatty acids and bovine milk fat yield due to dietary concentrate and linseed oil. J Dairy Sci., 88(2): 726-740. https://doi.org/10.3168/jds.S0022-0302(05)72736-3.

Lucy MC, Staples CR, Michel FM, Thatcher WW, Bolt DJ. (1991). Effect of feeding calcium soaps to early postpartum dairy cows on plasma prostaglandin F2 alpha, luteinizing hormone, and follicular growth. J. Dairy Sci., 74(2): 483-489. https://doi.org/10.3168/jds.S0022-0302(91)78195-2.

Mach N, Bach A, Devant M. (2009). Effects of crude glycerin supplementation on performance and meat quality of Holstein bulls fed high-concentrate diets. J. Anim Sci., 87(2): 632-638. https://doi.org/10.2527/jas.2008-0987.

Mohd Azmi AF, Ahmad H, Mohd Nor N, Meng GY, Saad MZ, Abu Bakar MZ, Abdullah P, Jayanegara A, Abu Hassim H. (2021). Effects of Concentrate and Bypass Fat Supplementations on Growth Performance, Blood Profile, and Rearing Cost of Feedlot Buffaloes. Animals, 11: 2105. https://doi.org/10.3390/ani11072105.

Marei WFA, Khalil WA, Pushpakumara APG, El-Harairy MA, Abo El-Atta AMA, Wathes DC, Fouladi-Nashta A. (2018). Polyunsaturated fatty acids influence offspring sex ratio in cows. Int. J. Vet. Sci. Med., 15-6(Suppl): S36-S40. https://doi.org/10.1016/j.ijvsm.2018.01.006.

Mattos R, Staples CR, Thatcher WW. (2000). Effects of dietary fatty acids on reproduction in ruminants. Rev Reprod., 5(1): 38-45. https://doi.org/10.1530/ror.0.0050038.

Mattos R, Guzeloglu A, Badinga L, Staples CR, Thatcher WW. (2003). Polyunsaturated fatty acids and bovine interferon-tau modify phorbol ester-induced secretion of prostaglandin F2 alpha and expression of prostaglandin endoperoxide synthase-2 and phospholipase-A2 in bovine endometrial cells. Biol. Reprod., 69(3): 780-787. https://doi.org/10.1095/biolreprod.102.015057.

Mattos R, Staples CR, Arteche A, Wiltbank MC, Diaz FJ, Jenkins TC, Thatcher WW. (2004). The effects of feeding fish oil on uterine secretion of PGF2alpha, milk composition, and metabolic status of periparturient Holstein cows. J. Dairy Sci., 87(4): 921-932. https://doi.org/10.3168/jds.S0022-0302(04)73236-1.

McGowan MW, Artiss JD, Strandbergh DR, Zak B. (1983). A peroxidase-coupled method for the colorimetric determination of serum triglycerides. Clin. Chem., 29(3): 538-842. PMID: 6825269.

McNamara S, Butler T, Ryan DP, Mee JF, Dillon P, O'Mara FP, Butler ST, Anglesey D, Rath M, Murphy JJ. (2003). Effect of offering rumen-protected fat supplements on fertility and performance in spring-calving Holstein-Friesian cows. Anim Reprod Sci., 20: 79(1-2): 45-56. https://doi.org/10.1016/s0378-4320(03)00111-8.

Official Journal of the European Union. (2010). Directive 2010/63/ EU of 22 September 2010 on the protection of animals used for scientific purposes. L276/33-792010. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF.

Osman MA, Allen PS, Mehyar NA, Bobe G, Coetzee JF, Koehler KJ, Beitz DC. (2008). Acute metabolic responses of postpartal dairy cows to subcutaneous glucagon injections, oral glycerol, or both. J. Dairy Sci., 91(9): 3311-3322. https://doi.org/10.3168/jds.2008-0997.

Otaru SM, Adamu AM, Ehoche OW, Makun HJ. (2011). Effects of varying the level of palm oil on feed intake, milk yield and composition and postpartum weight changes of Red Sokoto goats. Small Ruminant Research, 96: 25-35. https://doi.org/10.1016/j.smallrumres.2010.11.004.

Palmquist DL, Jenkins TC. (2017). A 100-Year Review: Fat

feeding of dairy cows. J. Dairy Sci., 100: 10061-10077. https://doi.org/10.3168/jds.2017-12924.

Perry GA. (2016). Factors affecting puberty in replacement beef heifers. Theriogenology., 86(1): 373-378. https://doi.org/10.1016/j.theriogenology.2016.04.051.

Piantoni P, Allen MS. (2015). Evaluation of propylene glycol and glycerol infusions as treatments for ketosis in dairy cows. J. Dairy Sci., 98(8): 5429-5439. https://doi.org/10.3168/jds.2015-9476.

Porcu C, Manca C, Cabiddu A, Dattena M, Gallus M, Pasciu V. (2018). Effects of short-term administration of a glucogenic mixture at mating on feed intake, metabolism, milk yield and reproductive performance of lactating dairy ewes. Anim. Feed Sci. Technol., 243: 10-21. https://doi.org/10.1016/j.anifeedsci.2018.06.012.

Pryce, J. E. and Royal, M. D, Garnsworthy PC, Mao IL. (2004). Fertility in the high-producing dairy cow. Livest. Prod. Sci., 86:125–35. https://doi.org/10.1016/S0301-6226(03)00145-3.

Putrino SM, Leme PR, Silva SL, Alleoni GF, Lanna DPD, Grossklaus C. (2006). Exigências líquidas de proteína e energia para ganho de peso de novilhos Nelore alimentados com dietas contendo grão de milho úmido e gordura protegida. Revista Brasileira de Zootecnia, 35: 301-308. https://doi.org/10.1590/S1516-35982006000100038.

Reitman S, Frankel S. (1957). A colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. American J. Clin. Pathol., 28: 56-63. https://doi.org/10.1093/ajcp/28.1.56.

Richmond W. (1973). Preparation and properties of a cholesterol oxidase from Nocardia sp. and its application to the enzymatic assay of total cholesterol in serum. Clin Chem., 19(12): 1350-1356. PMID: 4757363.

Rizos D, Kenny DA, Griffin W, Quinn KM, Duffy P, Mulligan FJ, Roche JF, Boland MP, Lonergan P. (2008). The effect of feeding propylene glycol to dairy cows during the early postpartum period on follicular dynamics and on metabolic parameters related to fertility. Theriogenology., 69(6): 688-699. https://doi.org/10.1016/j.theriogenology.2007.12.001.

Rodney RM, Celi P, Scott W, Breinhild K, Lean IJ. (2015). Effects of dietary fat on fertility of dairy cattle: A meta-analysis and meta-regression. J. Dairy Sci., 98(8): 5601-5620. https://doi.org/10.3168/jds.2015-9528.

Rodney RM, Celi P, Scott W, Breinhild K, Santos JEP, Lean IJ. (2018). Effects of nutrition on the fertility of lactating dairy cattle. J. Dairy Sci., 101: 5115-5133. https://doi.org/10.3168/jds.

Saleem AM, Zanouny AI, Singar AM. (2018). Effect of glycerol supplementation during early lactation on milk yield, milk composition, nutrient digestibility and blood metabolites of dairy buffaloes. Animal., 12(4): 757-763. https://doi.org/10.1017/S175173111700180X.

Sallam SMA, Mahmoud AAA, Hashem NM, Attia MFA, Mesbah MM, Zeitoun MM. (2021). Alterations of Productive and Reproductive Efficiencies in Dairy Cows by the Inclusion of Protected Fats and Full-Fat Soy in the Diet. Animal Science, MedDocs Publishers. Vol. 1, Chapter 1, pp. 1-13. http://meddocsonline.org/.

Saunders WB. (1995). Tietz N.W., editor. Clinical guide to laboratory tests. 3rd ed. Philadelphia., 612. https://doi.org/10.1111/j.1537-2995.1995.tb03571.x.

Shahin, FH. (1993). *In vitro* and *in vivo* nutritional evaluation of fat supplements in diets of sheep. MSc. Thesis, Fac. Agric. Cairo Univ. Egypt.

Staples CR, Burke JM, Thatcher WW. (1998). Influence of supplemental fats on reproductive tissues and performance of lactating cows. J. Dairy Sci., 81(3): 856-871. https://doi.org/10.3168/jds.S0022-0302(98)75644-9.

Storry JE. (1981). "1 – The effect of dietary fat on milk composition." Recent advances in animal nutrition. pp: 3-33.

Südekum KH. (2007). Co-products from biodiesel production. The 41st Annual University of Nottingham Feed Conference Proceedings ('Recent Advances in Animal Nutrition - 2007', edited by Phil Garnsworthy and Julian Wiseman) published by Nottingham University Press in the autumn of 2007 (www.nup.com), pp. 201-219.

Tietz NW. (1990) Clinical Guide to Laboratory Tests. 2nd Edition, W.B. Saunders, Philadelphia, 566.

Tietz NW. (1994). Fundamentals of Clinical Chemistry. 2nd Edition, N.W. Tietz, editor, 692.

Trinder P. (1969). Enzymatic determination of glucose in blood serum. Annals of Clinical Biochemistry, 6: 24.

van Knegsel AT, van den Brand H, Dijkstra J, Tamminga S, Kemp B. (2005). Effect of dietary energy source on energy balance, production, metabolic disorders and reproduction in lactating dairy cattle. Reprod. Nutr. Dev., 45(6): 665-688. https://doi.org/10.1051/rnd:2005059.

Wade GN, Schneider JE, Li HY. (1996). Control of fertility by metabolic cues. Am. J. Physiol., 270(1 Pt1): E1-19. https://doi.org/10.1152/ajpendo..270.1.E1.

Wang C, Liu Q, Huo WJ, Yang WZ, Dong KH, Huang YX, Guo G. (2009a). Effects of glycerol on rumen fermentation, urinary excretion of purine derivatives and feed digestibility in steers. Livestock Science, 121(1): 15-20. https://doi.org/10.1016/j.livsci.2008.05.010.

Wang C, Liu Q, Yang WZ, Huo WJ, Dong KH, Huang YX, Yang XM, He DC. (2009b). Effects of glycerol on lactation performance, energy balance and metabolites in early lactation Holstein dairy cows. Animal Feed Science and Technology, 151(1-2): 12-20. https://doi.org/10.1016/j.anifeedsci.2008.10.009.

Waters SM, Coyne GS, Kenny DA, MacHugh DE, Morris DG. (2012). Dietary n-3 polyunsaturated fatty acid supplementation alters the expression of genes involved in the control of fertility in the bovine uterine endometrium. Physiol. Genomics., 44: 878-888. https://doi.org/10.1152/physiolgenomics.00065.2011.

Werner Omazic A, Kronqvist C, Zhongyan L, Martens H, Holtenius K. (2015). The fate of glycerol entering the rumen of dairy cows and sheep. J. Anim. Physiol. Anim. Nutr. (Berl)., 99(2): 258-264. https://doi.org/10.1111/jpn.12245.

Wright DE. (1969). Fermentation of glycerol by rumen micro–organisms. New Zealand J. Agric. Res., 12(2): 281-286. https://doi.org/10.1080/00288233.1969.10421180.

Zeedan KHII. (2003). Effect of dietary fat on performance, rumen fermentation and some blood parameters on buffalo. Ph.D. Thesis. Anim. Prod. Dept., Menoufia University, Egypt.

Zeedan KhII, Komonna OF, Etman KEI, Mousa KhMM. (2010). Effect of using different levels and sources of fats and roughages on productive performance, rumen fermentation and some blood parameters in ruminant. 1- Effect of dry fat supplementation on productive and reproductive performance of Damascus goats. Egyptian J. Nut. and Feeds, 13(1): 21-40.