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RESEARCH ARTICLE

EFFECT OF MATERNAL CONTACT, HOUSING SYSTEM AND PROBIOTICS SUPPLEMENTATION ON SOME PLASMA METABOLITES AND THYROID HORMONES LEVELS OF CAMEL-CALVES DURING WEANING PERIOD

Abdel-Fattah M.S., Hashem A.L.S., Reda A. Abd-Elazem, Azamel A.A. and Badawy M.T.A
Department of Animal Physiology, Animal and Poultry Production Division, Desert Research Center, Cairo, Egypt.

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Abstract

There are some factors influence on productive performance and physiological responses of camel-calves during weaning period. Mothering is an important factor for maintain the biological functions, immune-competence and calf survival. At weaning, calves were housed either with their dams or separated from their dams. Therefore, this study performed to investigate the effects of calf-dam contact system [completely contact system, (CCS) vs. completely separation system, (CSS)]; housing system [grouping, (G) vs. individually, (I)] and probiotics supplementation [control, (C) vs. probiotic, (P)] on some plasma biochemical parameters and thyroid gland activity of Maghreby camel-calves for 28 days period. Twenty female of camel calves aged 280 days with initial live body weight (LBW) of 236.76 ± 0.224 kg were used. The study was carried out at Maryout Research Station of the Desert Research Center (DRC), Cairo, Egypt. Weekly blood plasma samples was analyzed for plasma total proteins (TP), albumin (A), plasma urea nitrogen (PUN), total cholesterol (CHO), triglycerides (TG), glucose (GLU), alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) concentrations and thyroid gland activity (T_3 and T_4 hormones) were determined. Results indicated that calf-dam contact system as Psychological stress and housing system as social stress, influenced significantly (P<0.05) on plasma TP, G, PUN, CHO, TG and GLU concentrations whereas the presence of dam and grouping housing system has the higher levels of these parameters compared with absence of dam and individually housing system. Calves housed under completely contact with their dams or grouped housing system has higher (P<0.05) concentrations in circulatory T_3 and T_4 hormones compared with calves separated from their dams or individually housed during weaning period, while probiotics supplementation had no statistically significant effect on circulatory T_3 and T_4 levels. Interactions of treatment and sampling time (**P** < 0.01) were detected for plasma T_3 and T_4 levels under all three experimented factors. Liver enzymes (ALT, AST, ALP and LDH) activities were unaffected significantly (P>0.05) by calf-dam contact system, housing system and probiotics supplementation.It be concluded that camel-calves were more sensitive to the effects of calf-dam contact and

Corresponding Author:-Reda A. Abd-Elazem
Address**:-**Department of Animal Physiology, Animal and Poultry Production Division, Desert Research Center, Cairo, Egypt.

housing system during weaning process. The results suggest that the calves remained with their dams and group-housing system can be the better as anti-psychological and social stressors than those separated from their dams or individually housed ones as management system at weaning process.

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Introduction:-

There are different methods used for weaning process to evaluate the effects of these methods on dams and their offspring's. The common method of weaning performed by breeders are either early or late weaning (based on the age or body weights of young calves) and their effects on the reproductive performance of dams. Chibsaet al. (2014) recommended that weaning camel-calves at 8 months of age and supplemented concentrates to 12 months of age yield optimum growth rate and increase survivability of camel-calve. Recently, Orihuela and Galina, (2019) reviewed summarizes knowledge resulted in numerous published scientific studies on the separation of cows and calves and its effect on dams and their calves, where weaning stress is composed of the psychological stress of breaking maternal and social bonding and the physical stress of nutritional change. Weaning stress in animals is recognized as one cause of many health problems in cattle with important economic implications (Lee et al. 2008).

Weaning performed by breeders is sometimes associated with a complete separation of the two partners. Therefore, such practices may cause more profound behavioral and/or physiological disturbances (Orgeuret al, 1999). Also, Pérez et al. (2017) found that separation of the offspring has a stressful effect on both the mothers and the offspring as shown by the display of behaviors associated with stress, like vocalizations and locomotor activity, increase in cortisol concentration, loss of weight, and close proximity to fence-line, among others. Orihuela and Galina, (2019) mentioned that, although separation young calves from their mothers is stressful, keeping the calves in a familiar environment with other pen mates may allow them to adjust to separation from their dams more easily than moving them to an un-familiar pen (individually).

Early and McGee, (2011) mentioned that weaning can be a multifactor stressor, in which, nutritional, social, physical, and psychological stress are combined. Physical and nutritional stressors are often present through the introduction and adaptation to a new diet and new environment, whereas, psychological stress in the form of maternal separation and social disruption.

Lambs were generally found to be disturbed when they were separated from their mothers than when they were reunited (Abdul-Rahmanet al, 2012). Maternal separation has been identified as a potent stressor in infants and young animals that may have long-term psychological and physical effects Faturi et al., (2010). Abrupt weaning or separation of suckling calves from their dams is a common husbandry practice in both the beef and dairy industries. In 5 to 6-month old beef calves, abrupt maternal separation results in both the psychological stress of breaking the maternal bond as well as nutritional changes associated with dietary changes (Haley et al, 2005). In sheep, isolation of mothers from lambs has been shown to be a strong stress, particularly for the lamb (Niezgodaet al., 1993).

Previous study (LeNeindre, 1993) has assessed the effect on animal welfare of management stressors, such as isolation stress on productive performance during post-weaning period. The use of group housing might be beneficial when considering the welfare and socialization of the calf (Gulliksenet al., 2009). Group housing, containing 2 to 6 calves, provides more calf interactions and enriches their environment by adding stimulus (Stull and Reynolds, 2008). Besides housing systems, the type of diet and level of feeding are known to influence the hemato-biochemical parameters of animals (Katungukaet al. 1987). Studies on cattle suggest the physiological effects of separating a calf from its mother may contribute to a performance decrease several days after weaning. Physiological effects may be classified into three general categories; gross clinical signs, blood composition changes and endocrine interposed changes (Buenoet al, 1998).

Möstl and Palme, (2002) reported that there is a need for additional biochemical or endocrine parameters for detection of disturbances. Determination of the serum biochemistry reflects the physiological responsiveness of the animals to its internal and external environment (Esonuet al. 2001). The present study aimed to elucidate the influence of the presence of dams with their calves or the separation between them, housing system during post-weaning period and the supplementation of probiotics on some blood plasma parameter0s and thyroid gland hormones of camel-calves.

Materials and Methods:-

Animals and experimental procedure:-

The study was carried out at Maryout Research Station belonging to the Desert Research Center (DRC), Cairo, Egypt. Twenty females camel calves (Maghraby breed) were weaned using either completely contact system with their dams (CCS, 10 calves) or completely separation system from their dams (CSS, 10 calves) during weaning period under two housing systems [6 calves in grouping (G) and 4 calves in individually (I) housing system], half of calves in each housing system were supplemented with probiotics (P), while the other half was not-supplemented with probiotics (C). Calves were weaned at 280 days of age with initial live body weight (LBW) of 236.76 ± 0.224 kg (pictures from 4 to 9 showing maternal contact system, housing system and probiotics supplementations).

All calves were fed commercial concentrate mixture to meet nutrient requirements for a post-weaning period to achieve adequate growth as suggested by Davis and Drackley (1998). Commercial concentrate feed mixture (CFM) was fed once a day in the morning (at 08:00 am). All calves had clover hay (CH) ad libitum as roughage throughout the experiment. The chemical composition of CFM and CH (on DM basis %) are presented in Table (1). Proximate analyses were determined by the standard AOAC (2005), while nitrogen-free extract (NFE) was determined by the calculated difference. Fresh water was offered once daily. All calves, were provided free-choice access to a salt-based trace mineral mix that contained 12.0% Ca, 9.0% B, 9.0% Na, 0.30% Zn, 0.15% Cu, 0.05% Mn, 0:02% I, 0.005% Co, and 0.004% Se; in a blocks form.

Table1:- The chemical composition (on DM basis, %) of concentrate feed mixture (CFM) and clover hay (CH).

Item	DM	OM	CP	CF	EE	Ash	NEF
CFM*	92.83	92.79	16.97	10.21	4.37	7.44	61.01
CH	90.77	84.91	14.84	24.83	1.78	15.03	43.52

***CFM:** concentrate feed mixture contained; 36% yellow corn; 31% un-decorticated cotton seed; 19% wheat bran; 7.5% rice bran ; 2.5% molasses; 3% limestone and 1% common salt.

Anti-suckling mean:-

For calves (10 calves) under completely contact with their dams system (CCS-calves), the appealing to use anti-suckling mean for dams to prevent calves from nursing while allowing the calf and their dam to remain together because it facilitates a relatively low-stress weaning approach. The simple common form of anti-suckling is tie of thick cloth coated the dam udder, so that the calf cannot get the teat into its mouth to nurse. This type of anti-suckling mean is low cost and can be reused (Pictures 1: 3).

Preparation of probiotics:-

A probiotic compound (BIOVET-YC) was used where each kg containing 7.500 x10^6c.f.u.Lactobacillussporogenes; 30.000 x10^6c.f.u. ; 125.000 x10^6c.f.uSaccharomyces cerevisia SC-47 ; 5 g Alpha amylase; 100 g sea wood powder and q.s excipient. 20 g BIOVET-YC was dissolved in 200 ml warm fresh water plus 30 ml molas. This dose of probiotics was given involuntary and orally to each calf in probiotics supplemented group daily at 10:00 am by using drench-revolver at the beginning of weaning and lasted 28 days. Same volume (220 ml) of fresh water plus 30 ml molas was also given to each calf in un-supplemented probiotics calves once daily at 10:00 am by using drench-revolver.

Blood collection and biochemical analysis:-

Weekly blood sample was collected via jugular venipuncture into 10 ml vacutainer tubes containing Lithium heparin as anticoagulant. Plasma samples were analyzed for total proteins (TP, g/dl), albumin (A, g/dl), urea nitrogen (PUN, mg/dl), triglycerides (TG, mg/dl), cholesterol (CHO, mg/dl), and glucose (GLU, mg/dl) concentrations were estimated using kits from Hoffmann-La-Roche. Alanine aminotransferase (ALT,u/l) , aspartate aminotransferase (AST,u/l) were determined according to Retiman andFrankel (1957). Alkaline phosphatase (ALP, u/l) was estimated according to Roy (1970) and lactate dehydrogenase (LDH, u/l) estimated using available kits supplied by bio Me'ricux-france. Concentrations of Triiodothyronine (T_3, ng/dl) and Thyroxine (T_4, µg/dl) hormones were determined with RIA as recently described (Hammon and Blum 1998). Globulin was calculated (as the difference between total proteins and albumin).

Statistical analysis:-

Data analyses were conducted using General Linear Model (GLM) procedures (SAS,2008). Data were analyzed using an analysis of variance for a repeated measures design. Sources of variation included calf-dam contact system, housing system, probiotics supplementation and their interactions. Mean comparisons were evaluated by Duncan's Multiple Range Test (1955), least significant difference with a single degree of freedom. a= ($P<0.05$) level of significance was chosen.

Results and Discussion:-

We found relevant interactions among the three main studied factors (calf-dam contact system as psychological stress, housing system as social stress and probiotics supplementation as biological additives); therefore, the obtained results of blood plasma parameters and thyroid gland activity under each factor will be discussed independently from the other factors as follow:

Maternal contact effect (Psychological stress):

Plasma metabolites, Plasma proteins:

Plasma concentrations of TP, A, G and A/G ratio are shown in Table 2. Regarding the effect of calf-dam contact system solely, results indicated that separation of calves from their dams during weaning period declined significantly ($P<0.01$) plasma TP and G concentrations while A and A/G ratio increased (7.67, 3.11, 4.65 g/dl and 0.67%) compared with calves under completely contact with their dams (8.06, 2.91, 5.15 g/dl and 0.56%). This reduction suggests that maternal separation caused psychological stress by breaking the stable bonds between calves and their dams and subsequently some biological problems may arise. Hickey et al. (2003) mentioned that weaning stress lead to break the social bond between the calf and its dam in addition disrupts their familiar social which is an inherent aspect of weaning. Also, Stehulovaet al. (2003) mentioned that the cow-calf separation has been reported to be stressful to cows and calves.

Statistical analysis showed that there is a significant effect of the interaction between treatment and sampling time ($P<0.05$). Fig.(1) illustrated that Plasma TP and A concentrations decreased rapidly in both CCS-calves and CSS-calves on D_7 post-weaning as a result of immunoglobulin absorption into blood circulatory, this might explain the response to the effect of the a abrupt shifting from liquid to solid feeds. In accordance, Coppo, (2000) and Coppoet al, (2003) reported that plasma TP, A, PUN, CHO and TG as indicators of nutrition state were significantly lower in early weaned calves than control, these modifications could be attributed to nutritional imbalances caused by change of the feeding method. Atasogluet al. (2008) reported that plasma total protein concentration increased (**P**<0.001) significantly by the periods in weaned kids during post-weaning.

As shown in Fig. (1), plasma TP and G concentrations tended to significantly increase ($P<0.05$) for CCS-calves compared with CSS-calves, while plasma A concentration was stable in both CCS-calves and CSS-calves from D_{14} till D_{28}. Therefore, the increase in plasma TP associated with elevated globulin concentrations. This increase in plasma G concentration indicates improving the immune status in CCS-calves than CSS-calves. Accordingly, the interactions between treatment and sampling time resulting changes in plasma proteins concentrations and is considered important factor in the plasma proteins traits of stressed calves. Peak values of plasma TP and G concentrations of both CCS and CSS-calves were observed at D_{21} of experimental period.

Liver and kidney functions:-

Means of PUN, CHO, TG and GLU concentrations for CCS-calves and CSS-calves are presented in Table 2. Concerning the effect of calf-dam contact system, results indicated that PUN, CHO, TG and GLU concentrations increased significantly ($P<0.01$) in CCS-calves (39.08, 51.50, 34.62 and 108.20 mg/dl) compared with CSS-calves (35.01, 48.42, 30.96 and 104.54 mg/dl). In contrast, Ali et al. (2015) reported that, lambs weaned at age of 45 days had more stress when compared with lambs weaned at 90 days based on the observed increase in blood glucose and urea nitrogen when lambs were completely separated from their dams.

(Fig.2) stated that there was a significant effect of the interaction between treatment and sampling time ($P<0.05$) where plasma PUN, CHO and TG concentrations had increased with the advancement of sampling time in both groups, but CCS-calves had the higher in rate of change (14.67, 10.75 and 25.71%) compared with CSS-calves (2.84, 4.37 and 12.70%) for PUN, CHO and TG, respectively. Atasogluet al. (2008) reported that plasma glucose urea and cholesterol concentrations were significantly (**P** <0.003) affected by the periods in weaned kids during post-weaning. In agreement, Yanchevet al. (2008) reported that glucose levels increased significantly ($P<0.01$) by 4 and 9 days following weaning in Black-and-White calves, thus the increased plasma GLU level after weaning could reflect a new level of homeostatic balance aimed at reducing cortisol induced gluconeogenesis, by maintaining low plasma cortisol level. In contrast, they also reported that plasma urea and cholesterol levels following weaning declined significantly in Black-and White calves. Coppo, (2001) reported that the early weaning of calves caused significantly ($P<0.05$) increase in plasma GLU concentration and suggested that this increase could probably be attributed to medulla-adrenal sympathetic alarms caused by handling, blood sampling, feeding system change and separation of the calves from their dams.

Enzymes activity response:-

Concerning the effect of maternal contact system, results in Table 2 indicated that there were non-significant differences in plasma ALT, AST, ALP and LDH levels between CCS-calves (10.54, 29.98, 81.72 and 473.75 u/l) and CSS-calves (10.62, 30.30, 80.96 and 472.96 u/l), respectively. As shown in Figure (3) plasma ALT, AST and ALP enzymes decreased significantly ($P<0.01$) in both CCS-calves and CSS-calves on D7 followed by significantly increase ($P<0.01$) from D_{14} till the end of study (D_{28}). The observed decrease in plasma ALT, AST and ALP levels on D7 indicated to the acute response of weaning shock in both groups; while, the similarity increase in these parameters reflect the healthy indicator of liver function for all calves. No main or interactions with sampling time for CSS group were detected on all plasma enzymes activity during pot-weaning. Oler and Glowinska, (2013) mentioned that ALT and AST are enzymes associated with liver parenchymal cells, when body tissue or an organ, such as the liver, is diseased or damaged, AST is released into the blood; thus, the amount of that enzyme in the blood is related to the extent of damage.

Table 2:- Effect of Calf-dam contact system (CCS vs. CSS), housing system (G vs. I) and probioticspplementation (C vs. P) on plasma metabolites of camel-calves during weaning period.

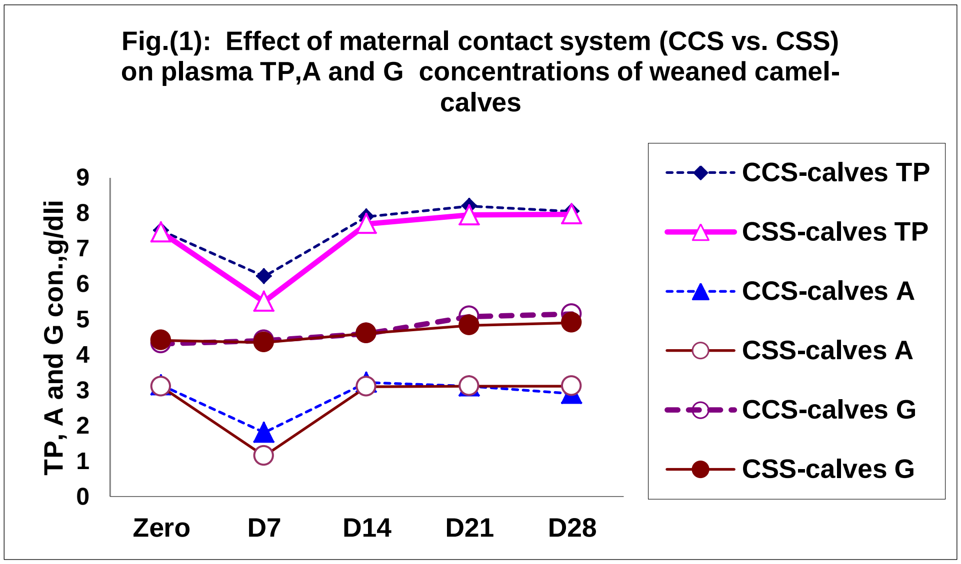
Traits	**Maternal contact System**			**Housing system**			**Probiotics supplementation**		
	CCS	**CSS**	**±SE**	**G**	**I**	**±SE**	**C**	**P**	**±SE**
TP, g/dl	8.06^a	7.67^b	**0.02**	8.30^a	7.95^b	**0.02**	7.71^b	8.32^a	**0.02**
A, g/dl	2.91^b	3.11^a	**0.01**	3.29^a	2.93^b	**0.01**	3.02^a	2.99^b	**0.01**
G, g/dl	5.15^a	4.65^b	**0.02**	4.99^a	5.02^a	**0.03**	4.68^b	5.33^a	**0.02**
A/G ratio	0.56^b	0.67^a	**0.01**	0.63^a	0.60^b	**0.01**	0.65^a	0.60^b	**0.21**
PUN, mg/dl	39.08^a	35.01^b	**0.25**	35.42^a	34.75^b	**0.26**	34.54^b	35.62^a	**0.35**
CHO, mg/dl	51.50^a	48.42^b	**0.35**	51.92^a	48.00^b	**0.34**	47.70^b	52.52^a	**0.33**
TG, mg/dl	34.62^a	30.96^b	**0.50**	33.83^a	31.75^b	**0.29**	32.96^b	35.62^a	**0.29**
GLU, mg/dl	108.20^a	104.54^b	**0.50**	109.58^a	108.98^b	**0.50**	106.92^a	105.7^b	**0.50**
ALT (u/l)	10.54^a	10.62^a	**0.08**	10.55^b	10.37^a	**0.09**	10.56^a	10.60^a	**0.08**
AST (u/l)	29.98^a	30.30^a	**0.25**	29.97^a	29.90^b	**0.28**	29.90^a	30.30^a	**0.25**
ALP (u/l)	81.72^a	80.96^a	**0.60**	81.67^a	80.71^a	**0.60**	81.30^a	81.10^a	**0.60**
LDH (u/l)	473.75^a	472.96^a	**0.19**	474.42^a	471.75^a	**1.1**	474.1^a	473.9^a	**0.19**
T_3 (ng/dl)	2.66^a	2.51^b	**0.28**	2.69^a	2.44^b	**0.28**	2.57^a	2.56^a	**0.27**
T_4 (µg/dl)	168.21^a	144.20^b	**0.55**	164.12^a	162.37^b	**0.60**	165.1^a	164.9^a	**0.60**

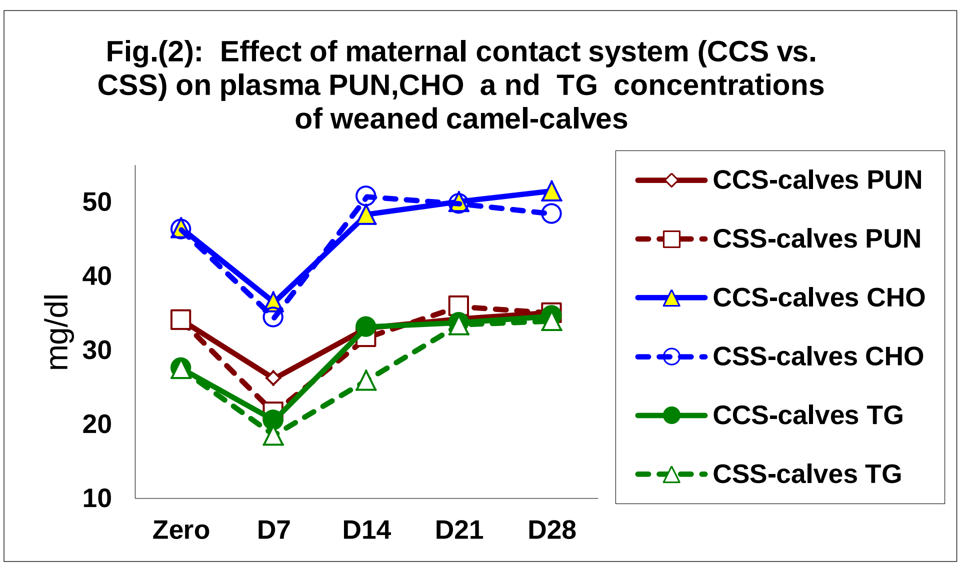
CCS= completely contact system, CSS= completely separation system, G= grouping housing, I= individually housing, C= control group, P=probiotics group, TP= total protein; A= albumin, G=globulin; A/G= albumin/globulin ratio; PUN= plasma urea nitrogen; CHO= cholesterol; TG=triglycerides; GLU=glucose; ALT=alanine amino transferase; AST= aspatate amino transaminase; ALP= alkaline phosphatase; LDH= lactate dehydrogenase; T_3= triiodothyronine hormone, T_4= thyroxine hormone;^{a,b}= different letters indicate significant differences within the same raw ($P<0.05$)

Thyroid hormones response:-

Regarding the effect of calf-dam contact system; results indicated that both groups (CCS vs. CSS) influenced to weaning shock where CSS-calves recorded the lower levels of plasma T_3 and T_4 (2.04 $ng^{-1}ml$ and 157.24$\mu g^{-1}dl$) during the first week (D_7) compared with CCS-calves (2.19 $ng^{-1}ml$ and 159.77 $\mu g^{-1}dl$). This difference could be attributed to the effect of the higher level of imposed stresses (the shift from liquid to solid feeding and completely maternal deprivation) for CSS-calves than CCS-calves. Therefore, this type of weaning (completely separation between calves and their dams) may be cause a lot of stresses to both mother and young that are express for both calves and their dams in their physiological responses. At the second week (D_{14}) the levels of T_3 and T_4 recorded

2.31 $ng^{-1}ml$ and 161.23$\mu g^{-1}dl$ for CSS-calves versus 2.39 $ng^{-1}ml$ and 163.54 $\mu g^{-1}dl$ for CCS-calves. This finding stated that plasma T_3 was the lower while plasma T_4 increased for CCS-calves. In agreement, Fazio et al., (2015) reported that weaned lambs showed significantly decrease in serum T_3 and elevated T_4 concentrations at two weeks after weaning where weaning was done by abrupt visual and acoustical separation of the ewe and lamb. Therefore, calves were allowed continuous contact with their dams not marked stress response for thyroid gland activity. Thereafter plasma T_3 and T_4 concentrations for CSS-calves increased ($P<0.01$) significantly from D_{14} till the end of study (D_{28}) where their values of T_3 and T_4 on D_{28} were nearly to the values of T_3 and T_4 for CCS- calves. (2.53 $ng^{-1}ml$ and 167.92 $\mu g^{-1}dl$ vs. 2.62 $ng^{-1}ml$ and 168.61 $\mu g^{-1}dl$). Therefore, calves were allowed continuous contact with their dams had lower stress on thyroid activity. Dvorak and Neumannova (1986) reported increased serum concentration of T_4 among piglets in response of weaning induced stimulated adrenocortical activity. They suggested that both specific stressor effects and circulating corticosteroids are responsible for the changes of T_3 and T_4 concentrations in blood sera of weaned animals. The significant changes of total iodothyronines in weaned camel-calves confirm data previously observed in foals after weaning, hence the thyroid responses may, therefore, be the physiological consequence of post-weaning adaptations and may be both the cause and/or the consequence of growth programming, confirming previous results obtained in growing foals (Fazio et al. 2007).





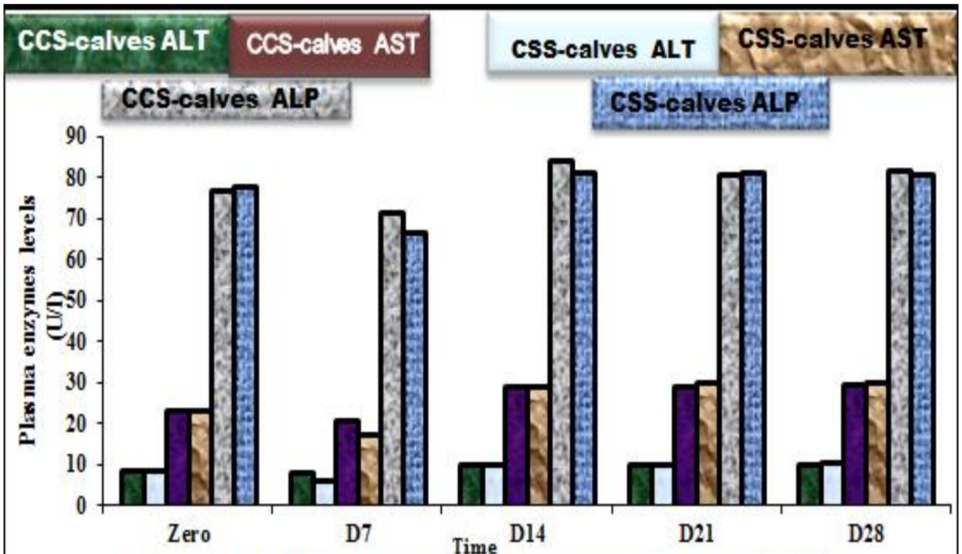


Fig. (3): Effect of maternal contact system (CCS vs. CSS) on plasma ALT, AST and ALP levels of weaned camel-calves

Housing system effect:-

Plasma proteins:-

With respect the effect of housing system [grouping (G) vs. individually (I)], the obtained results in Table 2 indicated that plasma TP, A, G and A/G ratio increased significantly (P<0.05) in G-calves (8.30, 3.29, 4.99 and 0.63) compared with I-calves (7.95, 2.93,5.02 g/dl and 0.60%). In accordance, Babuet al. (2009) reported that plasma total protein increased in group housed calves compared with individually housed calves. In disagreement, Maccariet al. (2014) found that serum albumin was higher at an age of 21 days under individually housing calves in relation to grouped housing calves. Abd-Allah et al., (2015) reported that plasma total protein and albumin levels increased significantly (P≤0.01) in calves housed individually than calves housed in groups, in spite of plasma globulin concentration remained lower in buffalo calves treated by two housing systems (group vs. individually). There was interaction (P<0.05) between housing system and sampling time on plasma concentrations of TP, G and A/G ratio for both grouped and individually housed calves from D_{14} till the end of experimental period except on D_7 (Fig.4), this could suggest that calves have more difficulty adapting to the housing system during the first week after weaning.

Liver and kidney functions:-

Regarding the effect of housing system [grouping (G) vs. individually (I)], the obtained results in Table 3 indicated that, plasma PUN, CHO, TG and GLU concentrations were significantly higher (P<0.05) in G-calves (35.42, 51.92, 33.83 and 109.58 mg/dl) compared with I-calves (34.75, 48.00, 31.75 and 108.98 mg/dl). In accordance, Terréet al. (2006) and Babuet al. (2009) reported that serum urea concentrations were greater (P<0.01) in grouped calves than in individually housed calves during post-weaning. Abd-Allah et al., (2015) reported that plasma cholesterol concentration remained lower in buffalo calves treated by two housing systems (group vs. individually).

Lynch et al, (2010) reported that weaning and subsequent housing caused transient changes in blood metabolites. In contrast, Maccariet al. (2014) found that serum glucose and urea were higher at age of 21 days under individually housing calves compared to grouped housing calves. On the other hand, Marco et al. (1997)reported that serum CHO level declined significantly during capture stress in adult sheep. Both Pierzchalaet al., (1985) and Niezgodaet al. (1987) found that plasma glucose concentration increased during the initial 2 hour after exposing sheep to isolation stress; however, glucose concentrations quickly returned to levels associated with non-stressed sheep. Gibert, 1991reported that capture stress response caused an increase in serum urea concentration in wild ungulates. Montaneet al (2003) reported that increase in serum urea concentration (P<0.05) over time was observed as response to capture stress in roe deer. Apple et al. (2005) reported that plasma concentrations of glucose was higher (P<0.05) in calves under restraint and isolation stress than in unstressed calves after 80 and 100 minutes of stressor application. Likewise, Apple et al. (1993) reported that glucose concentration elevated in restraint and isolated lambs at the final stressor bout, although serum glucose concentration was unaffected till 18 hours after termination of stressor application.

Concerning the effect of sampling time results showed that in almost sampling time's plasma PUN, CHO and TG were higher for G-calves than I-calves (Fig.5). The rate of change for PUN, CHO, TG and GLU recorded 4.95, 11.20, 21.50 and 1.30%vs**.** 1.05, 4.10,16.22 and 1.30%for G-calves and I-calves, respectively. Statistical analysis showed that there was interaction between housing system and sampling time ($P<0.01$). Results indicated that plasma TG was not significant differed between G and I housed calves. Investigations along experimental period revealed that an opposite trend (reducing) for the levels of PUN, CHO and TG parameters at the first sampling time (D_7) which may be attributed to the effect of weaning shock. Therefore, the superior of G-calves in these parameters is a good indicator of immune activity in G-calves than I-calves and may be this difference are symbolizing to an act of the beneficial effect of socialization which was obviously absent in individually or isolated housed calves.

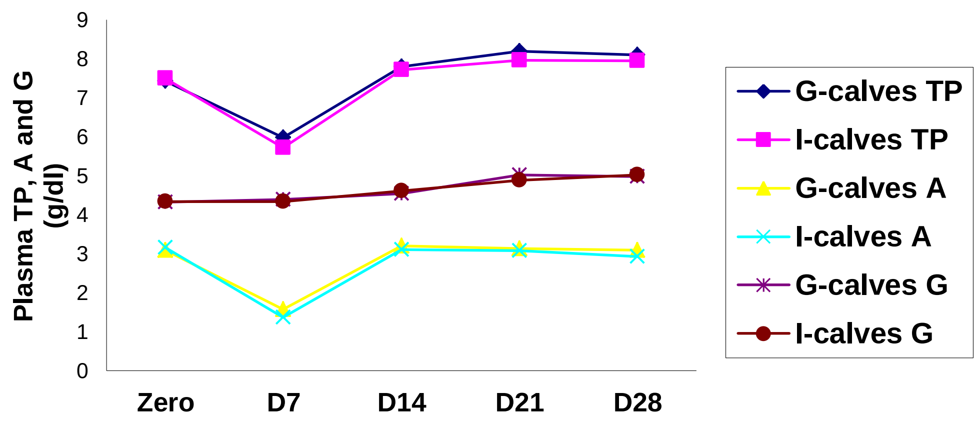
Enzymes activity response:-

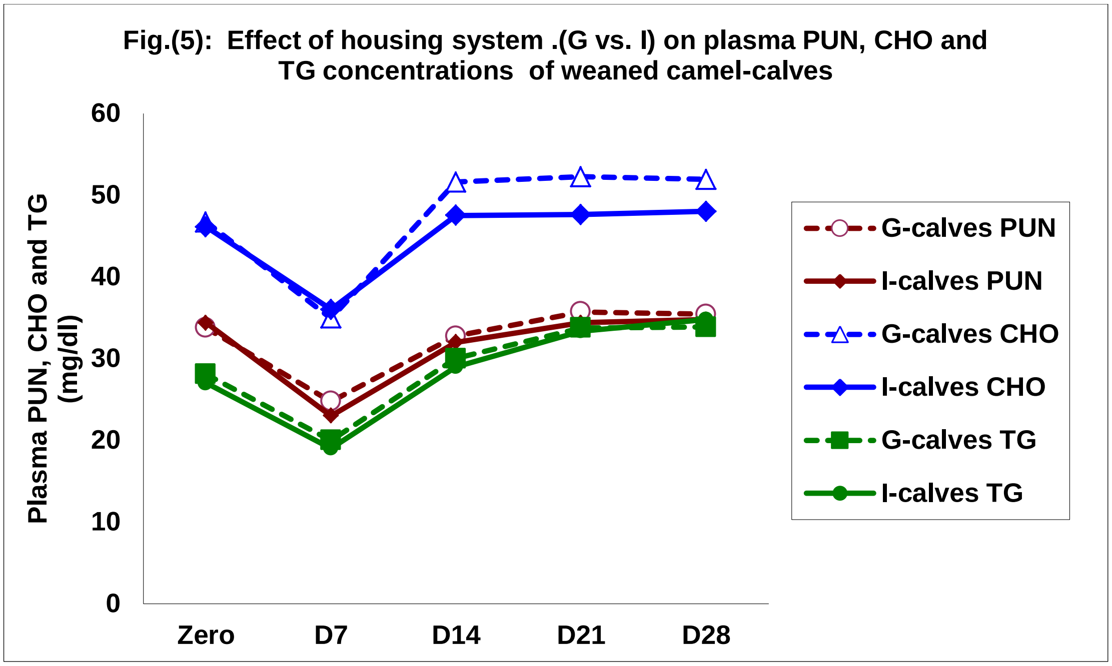
Concerning the effect of housing system, results in Table 2 indicated that ALT, AST, ALP and LDH activities were not significantly differed between G-calves (10.55, 29.97, 81.67 and 474.42u/l , respectively) and I-calves (10.37, 29.90, 80.71 and 471.75u/l , respectively). However, Abd-Allah et al., (2015) reported that plasma ALT was significantly ($P\leq0.05$) affected by the housing system; the level of this enzyme was higher in calves housed individually than calves housed in groups. In spite of plasma AST level remained lower in calvesraised in groups than calves raised as individual but this reduction was not statistically significant. Apple et al. (1993) reported that serum alkaline phosphatase had lower ($P <0.05$) concentration of lambs under restraint and isolation stress (RIS) for 6 h on three consecutive days than did unstressed lambs, while serum glutamic oxaloacetic transaminase (GOT) was increased ($P < 0.05$) 20- to 30-fold in RIS lambs compared with unstressed lambs.

Thyroid hormones response:-

Regarding the effect of housing system, results in Table (2) indicated that grouped housed calves showed significantly ($P<0.05$) higher levels for both plasma T_3 and T_4 being 2.69 $ng^{-1}ml$ and 164.12 $\mu g^{-1}dl$ compared with individually housed calves (2.44 $ng^{-1}ml$ and 162.37 $\mu g^{-1}dl$ for T_3 and T_4, respectively). On the contrary, Wronskaet al. (1990) found that isolation stress acting on fed and fasting sheep groups where increased significantly T_3, T_4 hormones and glucose levels. Friend et al. (1985) reported higher thyroid hormones levels in individually housed calves than in group-housed calves. Mal et al. (1991) found that concentrations of plasma T_3 were higher in isolated mares than in control (allowing social contact) and P (pasture) mares. On the other hand, Casamassimaet al. (2001) reported that neither free-T_3 nor free-T_4 blood levels were changed by housing system of ewes.

Fig.(4): Effect of housing system .(G vs. I) on plasma TP, A and G concentrations of weaned camel-calves





Probiotics effect:-
Plasma metabolites:-
Plasma proteins response:-
Results in Table (2) indicated that there were significant increase (P<0.01) in plasma TP and G concentrations for P-calves (8.32 and 5.33 g/dl) compared with C-calves (7.71 and 4.68 g/dl) while plasma A concentration and A/G ratio decreased significantly where the average values recorded 2.99 and 0.60 vs. 3.02 g/dl and 0.65% for P-calves and C-calves, respectively. There was interaction between treatment and sampling time (P<0.05), the initial values of TP, A, G and A/G ratio were identical in both groups and responded similarly whereas TP and A sharply declined while G was stable at D_7 post-weaning, followed by increasing TP and G levels from D_{14} till the end of study (D_{28}). P-calves was had the higher values than C-calves (Fig.6). The rate of change for TP, A, G and A/G ratio recorded (3.22, -2.6, 7.83 and -9.7% vs. 11.7, -4.46, 23.09 and -16.7%) for C-calves and P-calves, respectively. The higher TP level in P-calves than C-valves may be attributed to the increase in digestibility of crud protein (Yousef and Zaki, 2001). This confirmed by Putnam and Schwab (1994) who reported that YC stimulates rumen microbes that altered microbial protein synthesis and increased protein passage as well as protein yield. Also Komonna,(2007) and Mousaet al. (2012) who found that there was a better utilization of dietary protein through digestive tract after treatment with Saccharomyces cerevisiae.

Our results were paralleled with that recorded by Bakret al. (2009) who reported a significant increase in the levels of serum total proteins of buffalo calves supplemented probiotics. Recently, Izuddinet al. (2019); Ghoneem and Mahmoud. (2014) reported that plasma TP and PUN concentrations increased significantly (P<0.05) with lambs supplemented with postbiotics and inactivated yeast as feed additives, respectively. These results may be due to the improvement in metabolic process as a result of yeast supplementation (Kholif and Khorshed, 2006).

Abdel-Salam et al (2014) found that blood concentrations of total protein, albumin, and globulin were greater when lambs received probiotics treatments compared to the control diets.. Also, Mohanna (2000)with growing Frisian calves, Salem et al. (2000) with growing crossbred sheep, and El-Ashryet al.(2003) with Barki lambs, reported that YC supplementation significantly increased plasma globulin values. Hillalet al. (2011) reported that plasma globulin concentration increased significantly (P<0.05) in growing lambs supplemented probiotics.Soltanet al. (2013) reported that chromium supplementation significantly increased (p<0.05) blood serum globulin concentration in dairy calves by about 79% when compared with the control.
On contrast, Mousaet al., (2019) reported that supplementation of probiotics in drinking water did not display differences in plasma TP and A levels between treated and control groups of Barki lambs. Hossein-Ali Arab (2014)

reported that the supplementation of Bioplus resulted insignificant (P>0.05) changes in the levels of most of the blood metabolites, total proteins, albumin, glucose, triglyceride while cholesterol level decreased in lambs received 0.5 or 1g Bioplus/kg of feed. Also, El-Katchaet al., (2016) and Saleemet al(2016 and 2017) reported that probiotic supplementation in post-weaning lambs diet did not change (p>0.05) blood constituents of total protein, albumin, globulin and glucose, however blood urea and cholesterol concentrations were significantly decreased with probiotics supplementation (p<0.003), (p<0.04), respectively. The decrease in plasma cholesterol level, probably due to microbial feed additives reduced the absorption of lipid from the intestines by de-conjugation (Lubbadehet al., 1999).

Liver and kidney functions:-

Urea in the blood is an indicator of renal function (Oltner and Wiktorson, 1983). Concerning probiotics supplementation, results in Table (2) indicated that the average values showed that there were significant differences (P<0.01) in plasma PUN, CHO, TG and GLU concentrations between the two groups where P-calves tended to be has the higher values (35.62, 52.52, 35.62 and 105.7 mg/dl) compared with C-calves (34.54, 47.70, 32.96 and 106.92 mg/dl) for PUN, CHO, TG and GLU, respectively. Similarly, Raghebet al. (2003) reported that dietary YS and LS supplementation increased plasma urea concentrations of weaned Frisian calves compared with the control group. Abo El-Nor and Kholif (1998) reported higher blood urea nitrogen values in response to probiotics supplementation. This higher in blood urea nitrogen concentration may be due to incapacity of ruminalmicroflora to detain the ammonia optimally (Butler, 1998).

Juchemet al. (2004) suggested that biological additives, such as monensin or live yeast, were reported to influence blood constituents through remodel of ruminal microbial populations, which was reflected by changes in fermentation end products, i.e. the increase of propionic acid was associated with increased plasma glucose concentration. Abo El-Nor and Kholif (1998) reported higher blood glucose concentration in cows fed diets containing probiotics. Hillalet al. (2011) reported that plasma urea and globulin concentrations increased significantly (P<0.05) in growing lambs supplemented probiotics. Our results were paralleled with that recorded by Bakret al. (2009). On contract, Moussaet al., (2019) reported that supplementation of probiotics in drinking water did not display differences in plasma urea nitrogen levels between treated and control groups of Barki lambs.

Figure (7) illustrated that plasma concentrations of PUN, CHO and TG followed a pattern which was similar to plasma TP and GL traits whereas P-calves appeared to have higher levels of plasma PUN, CHO and TG than the C-calves from D_{14} till D_{28} post treatment with probiotics while pronounced decrease in both groups occurred at the first week (D_7) post-weaning. Izuddinet al. (2019) reported that no difference was observed in blood triglycerides and cholesterol levels due to the inclusion of postbiotics from L. plantarum RG14 in newly-weaned lambs. In contrast, Saleemet al (2017) and Bunting et al. (1994) reported that probiotics or chromium picolinate supplementation reduced plasma CHO in weaned lambs and growing Holstein calves, respectively.

Ding et al. (2008) reported that plasma urea-N concentration was decreased (p<0.05) of male weaned lambs by Monensin and live yeast supplementations. Sayed (2003) reported no significant effect of probiotics supplementation on PUN in goat's kids. Ghoneem and Mahmoud. (2014) reported that no significant differences in plasma triglycerides concentration (P>0.05) with Barki lambs fed in-activated and dried yeast. Beaucheminet al. (2003) did not observe any change in GLU concentration with yeast supplementation in cattle. On the contrary, Antunovicet al., (2005 and 2006) and Dimovaet al. (2013) reported that lambs supplemented probiotics had lower levels of urea in the blood, indicating a better utilization of nitrogen from diet. On the other hand, the lower level of blood urea, probably due to better utilization of nitrogen from diet in the rumen with probiotic supplementation (Saleemet al 2017).

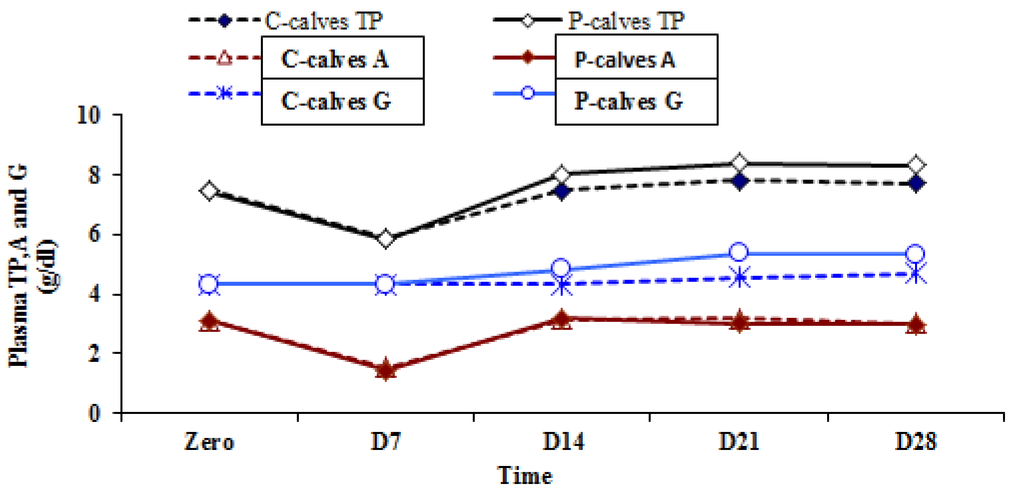


Fig.(6): Effect of probiotis supplementation (C vs. P) on Plasma TP,A and G concntrations of weaned camel-calves

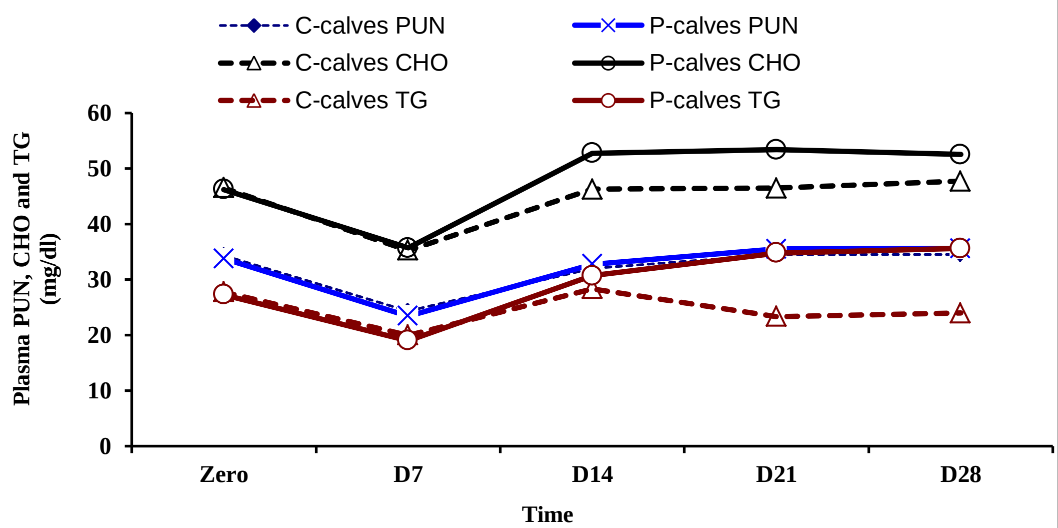


Fig.(7): Effect of probiotics supplementation on PUN,CHO and TG concentrations of weaned camel-calves

Statistical analysis stated that there is a significant increase (P<0.01) in plasma glucose concentration in both groups but P-calves was had the higher levels, this result may be related to rapid rate of hydrolysis and absorption of the dietary carbohydrates in alimentary tract that may be resulted from the effect of probiotics on activity of amylase which lead to increase carbohydrates hydrolysis in the small intestine (Abdel-Khaleket al. 2000). Otherwise, this may be attributed to increase the activity of cellulolytic bacteria that act on cellulose fibers degradation and thus produced more glucose and increased the glucogenic precursor propionate in rumen or decreased plasma insulin and insulin glucose ratio leading to an increase in gluconeogenesis (Dawson, 1993).

Enzymes activity response:-

Concerning the effect of probiotics on plasma enzymes levels, results presented in Table (2) indicated that the overall of plasma enzymes levels were similarly for both control and supplemented with probiotics groups (10.56,29.6, 81.30 and 474.1 vs. 10.60, 30.30, 81.10 and 473.9 µ/l). Thus, no significant effect registered for probiotics supplementation on any enzyme measured, Similarly, Mousaet al., (2019) reported that supplementation of probiotics in drinking water did not display differences in plasma ALT and AST levels between treated and control groups of Barki lambs. Soltanet al. (2013) reported that there were no significant differences in ALT and AST levels between control and supplemented lambs with probiotics. Abdallaet al. (2013) reported that plasma AST and LDH activities are insignificantly changed in buffalo calves post treatment with Saccharomyces cerevisiae. Doležalet al. (2011) said that AST and LDH concentrations are apparently not connected with the yeast culture supplementation but rather with the diet and with the individuality of cows. Also Abdel-Khaleket al. (2000) reported that value of serum AST was not significantly affected by using yeast treatments with suckling Frisian cows. Ghoneem and Mahmoud. (2014) reported that no significant differences in plasma ALT and AST concentrations (P>0.05) with Barki lambs fed inactivated and dried yeast. Hillalet al. (2011) reported that no significant differences were observed in ALT and AST levels between controls and supplemented growing lambs with probiotics. On the contrary, Kassabet al.,(2017) reported that supplementation of probiotics in the diet of beef bulls increased significantly levels of ALT, AST, thyroxin and tri-iodothyronine hormones for treated groups compared with control group.

Figure (8) illustrated that ALT, AST and ALP levels decreased significantly ($P<0.05$) on D_7 followed by rapidly increase from D_{14} till the end of study (D_{28}) with identical values in both groups, this suggests that the shifting from liquid to solid diets stressor (weaning) short lived (may be less than 14 days period) and this behavior reflect reestablishment of the cellular homeostasis in liver and the adaptability of calves to post-weaning conditions. Aida et al. (2008) found that the levels of serum AST, ALP levels were significantly increased ($P < 0.05$) in treated groups with antibiotics compared to controls in growing buffaloes' calves. LDH enzyme behaved the opposite trend on D_7 and behaved the same trend of the other enzymes with similar values between both groups. These findings indicating all calves had become adapted to the solid feeds without any adverse effect on animal health.

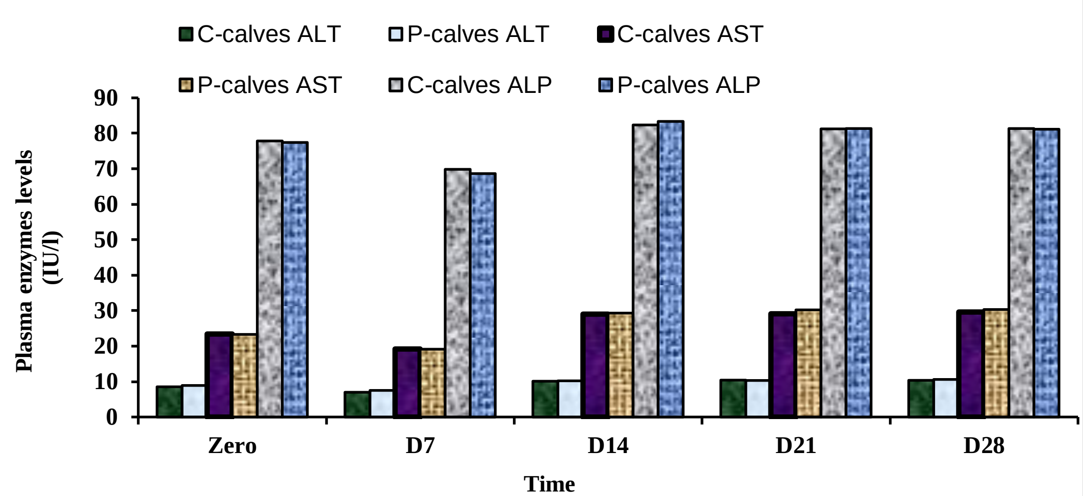


Fig.(8): Effect of propbiotics supplementation on plasma ALT,AST and ALP levels of weaned camel-calves

Thyroid hormones response:-

With respect the effect of probiotics on circulatory T_3 and T_4 levels, results in Table (2) indicated that there was no significant treatment effect on values of plasma T_3 and T_4 concentrations for C-calves (2.57 and 165.1) compared with P-calves (2.56 ng^{-1}dl and 164.9 μg^{-1}dl). In accordance, Estellet al. (1993) found that no beneficial effect ($P>0.10$) of supplementing Se yeast on thyroid hormone concentrations of the calf during post-weaning.

Kassabet al., (2017) reported that supplementation of probiotics in the diet of beef bulls increased significantly levels thyroxin and tri-iodothyronine hormones for treated groups compared with control group, the increase in the secretion of the thyroid hormones may be due to: 1- The increase of carbohydrate, fat and protein metabolism 2- The increase of TDN intake as an indicator for energy metabolism. Also, the increase in the thyroid hormones secretion may be due to there was a positive relationship between energy intake and the concentration of the thyroid hormones as it was reported in literature by Ahmed,2003; Kassab, 2007; Toshihiro 2010; Zanouny ,2011 and Kassab and Hamdon, 2014.

Conclusion:-

Concerning management at weaning process the most important factors which might affect the physiological responses is either allows the attachment between calves and their dams for two weeks at least during post-weaning period. Adaptation to separation calves from their dams was pronounced at the 2^{nd} week post-weaning on all biochemical parameters where calves and their dams gradually becoming more independent of one another. With few exceptions of plasma T_3 and T_4 levels, which were relatively lower levels of CSS-calves and I-calves groups compared with CCS-calves and G-calves. These findings were reflected distress associated with psychological stress during the first two weeks following weaning. In addition, supplementation of probiotics can have small or of minor importance on T_3 and T_4 levels with sampling time progress. The results of the present study suggest that plasma parameters across the experimental period describe changes in the nutritional conditions as a result of the transition from liquid to solid feed in association with weaning process.

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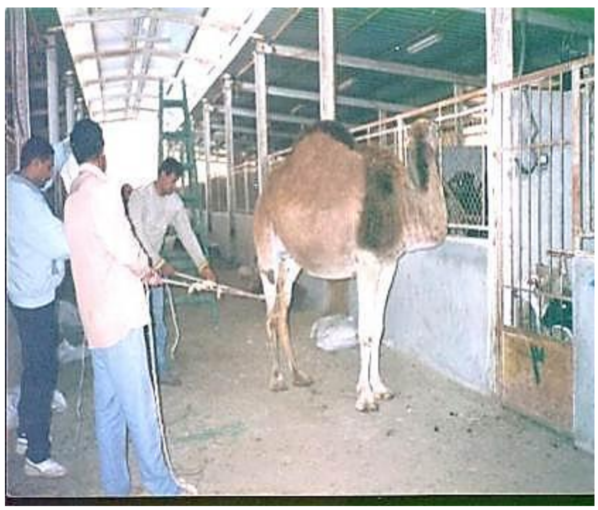

Picture 1:-The first step of anti-suckling preparation.

Picture 2:- The second step of anti-suckling preparation.

Picture 3:- The final step of anti-suckling preparation

Picture 4:- Completely contact system (she-camel with their calf) housed individually and supplemented with probiotics, 20g/h/d.

Picture 5:- Completely separation system (camel-calve housed individually and supplemented with probiotics, 20g/h/d).

Picture 6:- Completely separation system (camel-calve housed individually and un-supplemented with probiotics).

Picture 7:- Completely separation system (camel-calves housed in group and supplemented with probiotics, 20g/h/d).

Picture 8:- Completely contact system (camel-calves housed in group and un-supplemented with probiotics).

Picture 9:- Completely separation system (camel-calve housed individually and un-supplemented with probiotics.

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