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THE INFLUENCE OF ASPERGILLUS ORYZAE FERMENTATION PRODUCT EITHER ALONE OR WITH MALATE SALT SUPPLEMENTATION TO DAIRY BUFFALO COWS ON SOME BLOOD TRAITS AND REPRODUCTIVE PERFORMANCE DURING TRANSITION PERIOD

By

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ABSTRACT

The study was performed to evaluate the influence of dietary supplementation of *Aspergillus oryzae* (AO) extract (Amaferm®) alone or combined with malate salt on blood constituents and reproductive parameters in fifteen multiparous buffalo's cows during the last two months of pregnancy and up to three months postpartum. Animals were used with an average live body weight of 446.53 ± 29.23 kg and 2-5 parities selected randomly and divided into three similar experimental groups according to their body weight and parity(5animalseach).The randomized design in experimental groups was as follows: 1-The control group received the basal ration without any supplementation, 2- The 1st treated group (AO) fed on control ration supplemented with 15g/h/d of Amaferm®. The 2nd treated group (AO+MS) was fed on control ration supplemented with combination 15g/h/d of Amaferm®/h/d plus10g/h/d malate salt. The basal ration was composed of concentrate feed mixture, berseem and rice straw. Results indicated that treated buffalo cows achieved a significantly higher in calf birth weight and calves weaning weight as well as faster average of daily gain in calves and treated dams body weights at 3months post-calving than those of the control group. Moreover, addition of AO+MS and AO improved plasma total protein and albumin during late pregnancy (LP) as compared to control animals while no significant difference was observed during post pregnancy (PP) period. On the other hand blood glucose level, blood urea nitrogen (BUN) and T4 levels were higher (P< 0.05) in treated groups during PP period, with no significant difference during LP period. In addition, higher levels with no significant differences in blood globulin concentrations, A/G ratio, blood creatinine concentration and level of plasma

T3 within two stages. AO and AO+MS treated rations tented to lower (P<0.05) cholesterol level during LP without significant differences in PP period however Estradiol 17 β concentrations in blood were greater significantly (P< 0.05) in animals fed on treated rations through the two stages than that of control. AO+MS buffalo treated dam recorded shorter periods from parturition until the first detected estrus and insemination, as well as days open, conception rates and the lowest number of service per conception followed by AO and the control groups respectively. It can be concluded that combination of AO and MS supplementation for ration of buffalo cows improved calf birth weight and performance as well as dams parameter in some of reproductive performance traits.

Keywords:

Buffalo cows, *Aspergillus oryzae*, malate salt, performance, blood components and reproductive parameters.

INTRODUCTION

The transition period extended from, three weeks before calving, to the lactating state (3 weeks postpartum) is considered as a critical determinant of productivity and profitability management in dairy farms **(Grummer, 1995).** That was accompanied with several biochemical and physiological stress changes to animals making them more susceptible to infectious diseases and various metabolic disorders. Furthermore energy and protein requirements deficit with increase in nutrients requirement for fetal, placental development and postpartum milk synthesis also, reduced feed intake capacity a few weeks before parturition while the lowest level occurred at calving, **(Ingvartsen and Andersen, 2000).** Cattle showed ability to compensate the feed energy deficits by a few adaptations in the animal's body including increased hepatic gluconeogenesis and adipose reserves mobilization with body fat β-oxidation reserves, so that, the dry cow fat depots decline and feed intake becomes higher with peak milk yield (**Shared *et al.*, 2016**). During prepartum animals that fail to transit were successfully into lactation as related to poor of nutrition require management considering some problems in early lactation period associated with suboptimal milk production, diminished reproductive performance, increased morbidity, mortality ,treatment cost, involuntary culling and immune depression. Increased dietary fat and diet's energy density with concentrate one of a the common practice alternative feed strategies which enhances milk production with minimize metabolic disorders on both animal health and the

environment, **(Hayirli and Grummer,2004).** However, such strategy can be associated with increased ruminal acidosis risk **(Drackley,1999).** Thus, dietary fungal products can enhance rumen fermentation and ruminal digestive processes, as well as improve animal health and energy status in early lactation **(AlZahal *et al.*,2014)** with reduction of risk of acidosis, bloat or laminitis **(Chiquette, 2009).** Moreover organic acids such as malate salt are a key intermediate of the inverse citric acid and the succinate-propionate cycle **(Martin,1998).** Malate slat enhanced milk fat synthesis and milk yield production in dairy cows and participates in propionate production that increases gluconeogenesis by production of oxaloacetate into intestine as growth substrate for different microbial populations that increase the production of acetate diets due to fiber digestion. Also, improving rumen ecosystem conditions due to increased amount of lactate utilized **(Sniffen *et al.*, 2006).**In addition, such strategy leads to beneficial production performance of dairy cows fed under stressful conditions **(Hutjens,2008)** and increased protein synthesis with reduced gluconeogenesis deficiency in dairy cows associated with increased production of total volatile fatty acids by amilolytic and cellulolytic flora **(Lui *et al.*, 2009)**.Therefore, the objectives of the present study were to evaluate the effects of addition of *Aspergillus oryzae* (AO) fermentation extract as a natural feed additive alone or with malate salt to buffalos dams rations during the transition period on the weight gain, some blood metabolites, some reproductive parameters of lactating buffaloes dam and their offspring's,

MATERIAL AND METHODS

The experimental study was conducted at the experimental buffalo's farm of Mahallet Mousa, Kafer El-Sheikh Governorate belonging to the Animal Production Research Institute, (APRI), Agricultural Research Centre, Egypt**.**

Experimental Design:

The experiment was carried on 15 multiparous of Egyptian buffaloes weighing 446.53 ± 29.23 kg at 2nd to 5th parities during the last two months of pregnancy (expected calving) and continued up to three months of postpartum. Animals were classified randomly into three equal groups **(**Five in each) according to age, parity, live body weight (LBW), previous milk yield, and expected calving dates. According to the feeding regimen, buffaloes of the 1st group (Control diet) were fed on basal diet and received no treatment while 2nd (AO) group received the basal diet plus 15g/h/d of Amaferm (*Aspergillus oryzae* fermentation extract)

that were purchased from Biozyme Enterprises Inc. (St. Joseph, MO65404, USA). Buffaloes of the 3rd (AO+MS) group received the basal diet plus 15g/h/d of Amaferm accompanied with 10g/h/d of malate salt (carboxylic acid salt as commercial product composed of disodium malate - calcium malate (0·16: 0·84, w/w) (Laboratorios Ovejero S.A., Leo ´n, Spain). Rumalate© (Norel and Nature, S.A., Barcelona, Spain). All animals were fed on basal diet composed of concentrate feed mixture (CFM) (Table 1), Berseem hay (BH) and rice straw. Chemical analysis of CFM, BH and RS are presented in (Table 2). Animals fed individually on balanced ration formulated to meet established nutrient requirements of pregnant and lactating dairy animals according to **Kearl (1982)** and the amounts of feeds were recalculated biweekly based on the milk yield and reproductive status. Water and mineral blocks were available to all animals. At the time of feeding, daily amounts of feed additives were directly handly mixed well with the concentrate , animals groups were fed individually in two equal meals daily at 7 a.m. and 3 p.m.

Table (1): The concentrate feed mixture (CFM) consists of,

Ingredients	%
Undecorticated cotton seed cake	**32**
Rice bran,	**12**
Wheat bran,	**24**
Yellow corn,	**22**
Linseed cake,	**5**
Molasses,	**3**
Limestone and	**1**
Common salt	**1**

Table (2): Chemical analysis of different feed stuffs as DM basis (%).

Item	Chemical composition			
Calculated chemical composition (DM%):				
	CFM	**Berseem Hay**	**Rice straw**	**Basal ration**
Dry matter (DM)	**90.87**	**87.85**	**88.86**	**90.54**
Organic matter (OM)	**92.96**	**88.06**	**85.67**	**89.53**
Crude protein (CP)	**16.06**	**11.70**	**2.83**	**13.57**
Crude fibre (CF)	**12.61**	**29.45**	**39.61**	**19.25**
Ether extract (EE)	**3.68**	**2.61**	**1.67**	**3.41**
Nitrogen-free extract (NFE)	**60.61**	**44.30**	**41.56**	**53.3**

Ash	**7.14**	**11.94**	**14.30**	**10.29**

Buffaloes were housed in an open free-stall barn under natural temperature and light until the time of delivery then transferred to the maternity unit. Each group was placed in a shaded pen equipped with free stalls with fresh water access except at the milking time. After delivery, all buffalo cows were allowed to nurse their calves for one week only postpartum (Period of colostrum intake), then dams were transferred to the milking unit and milked twice daily at 7 a.m. and 3 p.m. and they were subjected to . managerial practices of the breeding stock.

Experimental procedures:

Body weight and daily gain:

At the start of the experimental period (2 months pre-partum), all experimental dams groups were weighted to get the initial LBW, then before and after calving and biweekly during post-partum. Newly born calves were recorded, sexed, weighed and numbered at the day of birth (Birth weight, BW) and were kept for suckling until weaning (at three months of age) and then weighed at weaning (Weaning weight). The growth rate (g /day) of calves was calculated from birth until weaning.

Chemical analyses:

Weekly feed samples were composited over the entire feeding period. To determine the contents of feedstuffs, the official methods of chemical analysis of **AOAC, (1990)** used as described by the procedures (DM, method number 930.15), crude protein (CP, method number 976.05) and ash (Method number 927.02) while, nitrogen-free extract (NFE) was calculated by the differences also, organic matter (OM) content was calculated as the difference between DM and ash.

Blood Sampling and Hormonal Assay:

Blood samples were taken before morning feeding and drinking from all animals during late pregnancy (LP) and postpartum (PP) period, 10 ml of blood samples were collected biweekly from the jugular vein in dried clean EDTA tubes and plasma was separated after centrifugation at 4000 r.p.m for 20 minutes and then plasma was stored frozen at- 20°C until analysis. Plasma was used to determine total protein according to **(Henry,1974),** albumin **(Doumas *et al.*, 1971),** liver function by measuring the activities of AST (Alanine amino transferase) and ALT (Aspartate amino transferase) as described by **Reitman and Frankel,**

(1957), total cholesterol and urea **(Henry, 1965)** and creatinine **(Bartels, 1971)** using spectrophotometer.

Concentration of globulin was calculated by the difference between total protein and albumin concentrations. Meanwhile Glucose was determined by enzymatic method according to **Trinder (1969)** and direct radioimmunoassay technique was performed for determination of progesterone (P4), estradiol 17β, triiodothyronine (T3) and thyroxin (T4) hormones in plasma samples. Kits of ‘‘Diagnostic Products Corporation,(DCP) Los Angles,USA’’ as ready antibody coated tubes were used according to the procedures outlined by the manufacturer.

Reproductive traits:

In order to assess the uterine involution, the reproductive tract was rectally palpated once every two days untill 21 days postpartum and once every three days after according to **El-Fadaly (1978).** As a method of breeding in the station, all experimental buffalo cows were observed twice daily for estrous activity and buffaloes in heat were natural inseminated 12hrs after estrus detection thereafter the non-return animals were rectally-palpated and examined after 50 to 60 days of insemination for pregnancy diagnosis. Reproductive parameters for each buffalo cow were recorded after parturition immediately,as the interval elapsed for complete fetal membranes drop (Hours). Moreover, the period from calving to first detected estrus (In Days), service period length (Date of first insemination) (Day), number of services per conception (NS/C) (conception rate within 120 day postpartum period), were recorded for each pregnant buffalo, 1st service (PPFSI) or to conception (days open), and pregnancy rate (%), were recorded.

Statistical analysis:

Data obtained were tested by analysis of variance using the General Linear Model (GLM) procedures of the Statistical Analysis Systems **(SAS, 2002)**, with one way design to test the group differences according to the following model:

$Yij = \mu + Ti + eij$; where: Yij= observed values, μ=overall mean, Ti=experimental group and eij=random error.All statements of significance were based on ($P<0.05$) using Duncan multiple range test within the computer program.

RESULTS AND DISCUSSION

Body weight of dams and born calves:

The results of BW (Dams and calves) and calves BW gain are summarized in (Table 3). Dam BW before parturition (2,1 month), at calving and fetal fluid were non-significant in all experimental groups. AO group achieved a significant higher CBW/Dam BW post-partum and calf birth weight than other groups. Meanwhile, at 3 months post-calving dams body weight and calves weaning weight were higher significantly with faster calves average daily gain and total gain in AO+MS and AO treated dams than those of the control one.

The improvement of calves daily gains were 5.43 and 3.99 % for AO+MS, and AO groups than the control respectively. Malate feed additive increased milk production and dairy cows persistence and improved calves performance as well as dams milk production **(Kung *et al.*, 1982; Devant and Bach, 2004; Sniffen *et al.*, 2006; Wang *et al.*, 2009 and Hayat *et al.*, (2009)** Also, **Mallinckerodt *et al.* (1993**) noticed that calf birth weight and weaning weight increased when their dams fed malate during prepartum, led to improved rumen function and increased protein digestion,TDN,colostrum immunoglobulin concentrations, milk protein and milk lactose without deficiency in reproductive capacity**.** Also, **AlZahal *et al.*, (2014)** stated that, the improvement in calves performance as reflected to body reserves mobilization by negative energy balance development and progeny as well as growth dam body weight losses during late pregnancy.

Table (3): Body weights of buffalo dams' and their born calves as affected with AO alone or with MS supplemented fed dams ration during late pregnancy and suckling periods.

Items	Treatment			SEM
	Control	AO	AO+MS	
Average of buffalo dam LBW (kg):				
Dam LBW 2 months pre-partum.	**446.00**	**451.00**	**442.584**	**2.02**
Dam LBW One month pre-partum.	**465.00**	**468.75**	**461.25**	**2.11**
Dam LBW At calving .	**497.25**	**512.50**	**501.00**	**2.58**
Dam LBW Post-calving .	**434.00**	**440.00**	**438.43**	**1.69**
Dam LBW 3 months Post-calving.	**439.71^{c}**	**443.85^{b}**	**446.18^{a}**	**1.90**
Fetal fluid, (kg)	**10.65**	**10.73**	**10.95**	**0.45**
Calf birth weight (CBW) kg .	**28.80 ^{c}**	**30.05^{a}**	**30.55 ^{b}**	**0.70**
CBW / Dam Post-calving (%).	**6.636 ^{c}**	**6.829 ^{a}**	**6.968 ^{b}**	**0.48**
Calving loss%*.	**7.93**	**7.81**	**8.28**	**0.35**
Calf weaning weight (kg).	**85.12 ^{c}**	**88.67 ^{b}**	**89.95 ^{a}**	**1.40**
Total gain of calve (kg) .	**56.32 ^{c}**	**58.62 ^{b}**	**59.40 ^{a}**	**0.79**
Daily gain of calve (g/day).	**0.626 ^{c}**	**0.651 ^{b}**	**0.660 ^{a}**	**0.07**

Means bearing different superscripts in the same raw are significantly different (P < 0.05).

***Calving loss%= (CBW + fetal fluid) / dam BW before parturition.**

Bertics *et al.*, (1992) detected that LBW in the first weeks of the dry period decreased by 2% and 1.4% in the 7-10 days before calving with 30% decreased in DMI that appear to occur very rapidly in the transition period. **Wang *et al*. (2009)** reported less loss of cows BW during the 63-DMI period of malate supplemented as related to the rapid growth of fetus taking up abdominal space and displacing rumen volume with no milk production thus sending negative signals to the satiety center of hypothalamus and getting into negative energy balance (NEB) by low voluntary feed intake which may occur already before calving. Moreover, **Grummer (1995)** noticed reduction in LBW at the last trimester of pregnancy (Dry period) due to decrease in dry matter intake (DMI) with the lowest level occurring at calving and decreased plasma estradiol concentration with the parturition approaches.

Bell (**1995)** found that reduce in cows feed intake and negative energy balance through the reduction in using glucose and increasing in non-esterified fatty acids (NEFA) mobilization from peripheral tissue and adipose. On the other hand, **Kellems *et al.*** (**1990**) observed that adding Amaferm to Holstein cow's diets had no effects on body condition scores or BW changes during early lactation. **Bisinotto *et al.* (2012**) found that increase prepartum body weight, BCS and pre adipose by increased lipid mobilization in early lactation as related to fed cow ad libitum nutrient during the entire dry period tended to.

Blood biochemical parameters:

Data in (Table 4) illustrate that blood plasma total protein and albumin in supplemented groups were higher significantly (P<0.05) than those of the control during LP. Higher values were achieved with AO+MS and AO supplemented groups, but their no significant difference was observed during PP period. However, blood globulin concentration or A/G ratio was not affected in supplemented ration with no damage effects on the liver functions (AST and ALT) in the two stages. Treated groups increased serum albumin and total protein during gestation period as related to the improvement in protein digestion, protein synthesis, protein turnover and total protein in blood **(Chiou *et al.*, 2000).Wiedmeier *et al.* (1987)** found that, fed AO as diet supplementation enhencing rumen protein digestibility and increased in blood urea concentrations. **Ashour *et al.* (2004)** reported a positive correlation between plasma TP, ALB levels as reflection of the liver function and higher animal ability

to synthesize, absorb and store more protein. **Al-Saied *et al*. (1999)** in Friesian cows and **Abdel-Ghani *et al*. (2003)** in Egyptian buffaloes noticed that decreases in the blood globulin concentration in late pregnancy**. Abdel-Hafez (2002)** reported an increase in pre-partum fetus weight and increased protein breakdown required for gluconeogenesis that could be attributed to decreases in blood protein fractions. On the other hand, **Kowalik *et al.* (2012) and Malekkhahi *et al.* (2015)** found that plasma total protein were not affected by fed lambs' on 4 g/day malate in high-concentrate diets. Data in (Table 4**)** revealed that blood glucose levels was significantly higher (P< 0.05) in AO+MS treated group during post-partum period, than AO and control while, there was no significant difference during late pregnancy period which could be due to the high demand for energy especially glucose. However, there was an increase in milk lactose synthesis and consequently milk production in post calving.

Manston and Allen, (1981) Waterman *et al*., (2006) and Wang *et al*., (2009) reported that glucose was heavy demand during late pregnancy period and 1-2 days after parturition as indicating to blood sugar level reduction. similarly, **El-Malky (2007)** found that, decreases in blood glucose during late pregnancy followed increasing at 3 months postpartum in Egyptian buffalos.

Table (4): Blood plasma biochemical components of buffalo dams fed on supplemented rations with AO alone or with MS during late pregnancy and postpartum periods.

Blood Parameter	late pregnancy (LP)				Postpartum (PP)			
	Control	AO	AO+MS	SEM	Control	AO	AO+MS	SEM
T. protein (g/dl)	6.43 ^{c}	6.68 ^{b}	7.16 ^{a}	0.11	7.09	6.91	7.33	0.12
Albumin (A) (g/dl)	3.03^{b}	3.18^{ab}	3.28^{a}	0.02	3.67	3.38	3.63	0.04
Globulin (G) (g/dl)	3.4	3.5	3.88	0.03	3.42	3.53	3.70	0.05
A / G ratio	0.89	0.91	0.81	0.02	1.07	0.95	0.98	0.01
AST activity (U/L)	39.28	42.11	43.01	0.32	45.02	45.93	46.11	0.56
ALT activity (U/L)	22.64	23.01	23.35	0.89	26.14	26.66	25.04	0.14
Glucose(mg/dl)	53.85	56.14	56.72	0.26	61.24 ^{b}	62.56 ^{b}	68.02 ^{a}	0.24
BUN (mg/dl)	35.51	38.44	40.96	0.77	36.06 ^{c}	42.10 ^{b}	46.21 ^{a}	0.85
Creatinine (mg/dl)	1.30	1.25	1.26	0.06	1.27	1.28	1.31	0.08
Cholesterol (mg/dl)	83.51 ^{a}	79.45 ^{b}	75.11 ^{b}	0.51	87.22	83.54	80.88	0.61
T3 (ng/dl)	98.56	110.45	115.55	2.42	103.25	121.36	130.59	5.22
T4 (ug/dl)	2.13	2.52	2.98	0.22	2.74 ^{c}	3.58 ^{b}	4.17 ^{a}	0.23
P4 (ng/dl)	3.48	3.13	3.31	0.16	1.38 ^{b}	1.71 ^{a}	1.77 ^{a}	0.11
EST 17β (pg/ml)	78.49 ^{c}	95.25 ^{b}	116.58 ^{a}	5.32	35.73 ^{c}	41.78 ^{b}	46.34 ^{a}	1.38

a, b and c: Means in the same rows with different superscripts are significantly different (P < 0.05).

Abdel-Khalek *et al*. (2000) found that increase in blood glucose level by a rapid higher rate of hydrolysis and absorption dietary carbohydrates by alimentary tract. **Abdel-Hafez (2002)** found decrease in blood glucose for suffolk x Ossimi ewes at the last week of pregnancy. Meanwhile, **Chan and Freedland (1972)** and **Rutter *et al.* (1983)** recorded that malate fed supplementation increased blood glucose during PP due to the sudden increase of ruminal propionate production, which begins after the metabolic system adaptation. Otherwise, the addition of malate increased the cellulolytic bacteria activity and increase the glycogenic precursor propionate in rumen or decrease plasma insulin and insulin-glucose ratio leading to an increase in gluconeogenesis **(Dawson,1993).Williams (1989)** detected that AO supplementation increased amylase activity and carbohydrates hydrolysis. Results in (Table 4) denote that blood urea nitrogen (BUN) of buffalo dams that increased insignificantly during LP as response with AO and AO+MS fed supplementation. However, treated groups showed higher significant values (P<0.05) in postpartum periods as compared with the control group. Meanwhile, blood creatinine concentration was non-significantly affected in both period stages for AO and AO+MS treatments. Afinding that may indicate a tendency for improved N utilization of feed, and increased propionate in treated animals that agrees with a slight increase in protein catabolism. The excess amount of rumen ammonia by increased bacterial ammonia removal capability may provide high soluble protein fractions and high blood urea nitrogen concentration during the milking period **(Chiou *et al.*, 2000**). **Wiedmeier *et al.* (1987)** declared that, the AO inclusion in the diet increased blood urea concentrations and protein turnover by increase protein deamination and rumen digestibility. Concerning T3 and T4 levels in blood, there was a non- significant increase in plasma T3 during LP and post-partum while T4 showed a significant increase during post-partum. These results are consistent with those of **Todini *et al*. (2007)** who recorded that, the higher feed energy in diet induced to higher plasma T3 and T4. Data in (Table 4) show that, the concentration of blood cholesterol was lower (P<0.05) in AO and AO+MS than the control group during LP and without a significant differences in PP period.There is a reverse relationship between diet fed energy and blood cholesterol concentration that occurred during treatment, but the effect on cholesterol synthesis was lower than that on cholesterol excretion; the net result was a

decrease in plasma cholesterol concentration.**(Salem *et al.* 2002)** reported that ,the blood cholesterol concentration in lactating buffaloes was increased till the 2nd month postpartum. However, serum cholesterol varies with a variety of factors as the nature of the diet, hepatic function and other factors **(Kaneko, 1980).**

On the other hand **El-Ashry *et al*. (2004)** found that there was a decrease in cholesterol concentration when buffalo heifers were fed on fungi treated diets as anti-cholesterolemic effect due to stimulation of bacterial lipids synthesis **(Williams, 1989).** The present data show that treatment of lactating buffalo cows did not affect the plasma progesterone level (P4) profile in prepartum periods. However, analysis of variance revealed that, the average of P4 concentration tended to be slightly higher in AO+MS and AO treated groups than the control during postpartum period as related to the ovaries activity. **Gordon (1996)** observed a sharp decrease of P4 just before parturition. A findings that is similar to those mentioned by several authors by **Bushmich *et al.*, (1980)** who found an increase in mean luteal progesterone level per heifer and per corpus luteum in heifers with an increase in molar proportions of ruminal propionate**.Wang *et al.*, (2009)** reported that there was an increase t in progesterone production with increase in the ruminal propionate production in malate treated group . A result that may be attributed to increase of serum insulin level**.** The presence of insulin receptors have been reported on bovine corpus as evidenced by the direct effect of insulin on corpus luteum or due to regression of corpus luteum in all cows at parturition **(El-Moghazy, 2003).**The decline level of P4 is accompanied by a gradual increase in PGF2α until 24 h before calving in buffaloes **(Gordon, 1996). Smith *et al.* (1973)** indicated that the decline of P4 level stimulates the uterus to be under dominance of estrogen at a time when coordinated uterine contractions begin in cattle. In contrary, **Nanda *et al.* (1981)** noticed that P4 levels in normal pregnant buffaloes remained almost constant from day 60 before calving to the last week of pregnancy. The drop of P4 level before onset of calving is important to prevent P4 inhibitory effect upon myometrial contraction as well as the release of oxytocin **(Batra *et al.*, 1982).** As shown in (Table 4), the level of estradiol 17β concentrations in blood of treated groups were significantly greater ($P< 0.05$) than that of control group within the two stages. concentrations of EST17 β increased linearly toward the time of parturition in all groups and decreased sharply after delivery, in comparison with its level during late pregnancy. However, in the first months

postpartum the ovaries were still inactive so that P4 concentrations tended to change slightly. These results were in agreement with those of **Gordon (1996) and Prakash and Madan (1986)**. Moreover, normal calving requires softening and dilation of the cervix particularly during late pregnancy due to the influence of relaxin and estrogen when P4 dominance decline and uterine prostaglandin production increases **(Taverne, 1992).**

The increase in blood constituents studied may be due to the role of feed additive in improving all nutrient digestibility values and the increase in the absorption rate from the digestive tract especially CP of buffalos fed AO or AO+MS. In general, blood parameters estimated in the present study were within the normal range for blood constituents of buffaloes.

Reproductive performance:

Results depicted in (Table 5) indicate the effect of treated ration on reproductive parameters buffalo cow's during the post-partum period. It was found that number of days to first ovulation, first detected estrus (Days to first insemination) and number of days to conception (Days open) and number of services per conception decreased significantly ($P<0.05$) in all treated buffalo groups than in the control one. Also, AO+MS treated group was the highest reproductive parameters followed by than that in the control one. A finding that was . regarding by high rate of conception in treated group associated with increased circulating level of progesterone, also embryo implantation and maintenance of pregnancy during luteal phase, **Fonseca *et al.* (1983) and Bulman and Lamming (1978). Lucy *et al.*, (1992**) reported that conception rate to first insemination enhanced as related to the early resumption of ovulatory cycles post-partum. **Santos *et al.*, (2004**) found that, the improvement of secretion of PGF2α may induce luteolysis of the corpus luteum and termination of pregnancy**. Waterman *et al.* (2006)** reported malate supplementation during post-partum stimulated estrus resumption with shorter interval to first estrus and first ovulation by increase glycogenic potential**.**

Canfield and Butler (1991) found that early development pre-ovulatory follicular in post-partum dairy cow's is a key in ovarian cyclicity return which depends on normal episodic LH release resumption. Malate supplementation increasing the ruminal propionate production that affects LH release as mediated through various metabolic signals **(Rutter *et al.*, 1983). Bushmich *et al.* (1980)** mentioned that the increase in the ruminal propionate might be mediating the enhancement of ovarian sensitivity to gonadotropins in heifers. Also,

increase cells' energy metabolic function by increasing glucose availability enhanced pituitary capacity to response to GnRH **(Rutter *et al.*, 1983).** Hence, serum insulin level associated with malate supplementation **(Wang *et al.*, 2009)** may play a role in the regulation synthesis and release of LH by mediated elevation of insulin receptors in the arcuate nucleus and medial basal hypothalamus (Containing GnRH) in rats **(Rutter *et al.*, 1983). Gong, *et al.* (2002)** reported that enhancement of reproductive performance in post-partum dairy cows by increased circulating estradiol and progesterone level as related to increased insulin ovarian responsiveness to LH (Ovarian insulin receptors). **Bisinotto *et al.* (2012)** reported that, the nutritional status, dietary protein and metabolic health in prepartum associated with successful reproduction by reduce the risk of metabolic disturbances to enhance fertility.On the other hand, reproductive performance was not significantly affected by addation of Amaferm on early lactation in Holstein cows **(Kellems *et al.*, 1990). Santos *et al.*, (2001)** found that altering the protein content of the prepartum diet has little impact on performance of postpartum multiparous cows.

Table (5): Some reproductive performance parameters as affected by fed buffalo dams AO alone or with MS treated ration.

Item	Treatment groups			SEM
	control	AO	AO+MS	
Days to first estrus	63.60 [a]	51.20 [b]	45.30 [c]	2.37
Days to first insemination	97.80 [a]	77.50 [b]	62.10 [c]	4.09
Days to conception (Days open)	139.60 [a]	102.70 [b]	81. 30 [c]	6.43
Number of services/ conception	2.30 [a]	1.90 [b]	1.70 [c]	0.16
Conception rate %	60.00 [b]	80.00 [ab]	100.00 [a]	10.69

a, b, c: Values in the same row with different superscripts differ significantly (P<0.05).

CONCLUSION

It could be concluded that, supplementation of multiparous Egyptian buffaloes diet with 15 g/ head /day Amaferm or Amaferm combined with 10 g/ head /day malate salt during transition period had a beneficial energy balance effect and improved blood biochemical parameters (Glucose level, progesterone level, T4 activity). Also, more effects were noticed on total protein and albumin reducing the risk of metabolic diseases which related to offspring weight

gain as well as hold potential as a natural feed alternative to enhance hormones during post-partum reproductive performance traits.

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" اثر اضافة مستخلص فطر الاسبرجلس بمفرده او مع ملح المالات لاناث الجاموس الحلابة علي مقاييس الدم والاداء التناسلى خلال المرحلة الانتقالية "

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الملخص العربى

استخدمت هذه الدراسة لتقيم اثر اضافة مستخلص فطر الاسبرجلس وملح المالات على على الكفاءة التناسلية ومقاييس الدم للجاموس المصرى خلال المرحلة الانتقالية الكفاءة وقد اجريت هذه التجربة باستخدام عدد 15 جاموسة عشر بمتوسط وزن 446,53 + 29,23(مابين الموسم الثانى والخامس) وقبل الولادة بشهرين واستمرت التجربة الى مابعد الولادة بثلاثة اشهروتم تقسيم الحيونات عشوائيا لثلاثة مجاميع تجريبية (5 حيونات بكل مجموعة) تبعا لوزن الجسم والموسم وشملت التغذية (كعليقة اساسية) على المركزات ودريس البرسيم وقش الارزوذلك لتغذية المجموعة القياسية بدون اضافات بينما غذيت المجموعة الثانية(الاسبرجلس) على العليقة الأساسية بالاضافة ل15جم /راس/يوم من مستخلص فطر الاسبرجلس (التجارى) اما المجموعة الثالثة المجموعة الثالثة غذيت على العليقة الأساسية بالاضافة لمخلوط مكون من 15جم من مستخلص الاسبرجلس + 10جم من المالات/راس/يوم.

أوضحت النتائج ان الحيونات التجريبية المعاملة باضافة الاسبرجلس او بالاسبرجلس مع المالات قد اظهر تحسن معنوى لوزن الميلاد للعجول ووزن الفطام ومعدل الزيادة الكلية للعجول كذلك ارتفاع فى وزن السوائل بعد الولادة (المشيمة) ووزن الامهات خلال الثلاث اشهر بعد الولادة وذلك بالمقارنة بالمجموعة القياسية. كما ان مجموعات الاضافة قد زاد من مستويات البروتين الكلى والالبيومين بالدم خلال مرحلة ما قبل الولادة ولم يكن للاضافات اثر معنوى لمرحلة ما بعد الولادة، فى المقابل وجد ارتفاع فى مستويات الجلوكوز بالدم وتركيز اليوريا بالدم وتركيز ال T4 ارتفع فى مجموعات الاضافة خلال مرحلة ما بعد الولادة وان لم يكن له تاثير معنوى قبل الولادة بالمقارنة بالجموعة القياسية. اما تركيز جلوبيولين الدم او النسبة بين الالبيومين والجلوبيولين او مستوى الكرياتنين او تركيز الT3 فلم يظهر اختلافات معنويا خلال الفترتين (قبل الولادة وبعدها). اما تركيز الكولستيرول بالدم فتناقص معنويا خلال مرحلة ما قبل الولادة لمجاميع الاضافة ولم يكن هناك اختلافات معنوية بعد الولادة الا ان مستويات هرمون الاسترادايول قد ارتفعت خلال الفترتين نتيجة المعاملة بالمقارنة بالمجموعة القياسية.

المجموعة الثالثة (الخليطة) كانت اقصر المجاميع التجريبية فى الوقت من الولادة وحتى اول شياع والوقت من الولادة وحتى اول تلقيحة مخصبة واقل مدة مابين الولادة والحمل (الفترة المفتوحة) كذلك اقل عدد مرات تلقيح مخصب واكبر معدل حمل يليها مجموعة الاسبرجلس ثم المالات بينما المجموعة القياسية كانت اقل قياسات التناسل.

مما سبق يتضح ان عملية الخلط بين مستخلص الاسبرجلس وملح المالات واستخدامهم كاضافات للجاموس المصرى خلال المرحلة الانتقالية وقبل الولادة بشهرين كان له اثر كبير فى تحسين القدرة التناسلية والاداء التناسلى والتى ظهرت من خلال التحسن فى مقاييس التناسل خلال الفترة مابعد الولادة على الامهات كما حسنت من كفاءة نمو العجول المولودة وحتى الفطام.