

EFFECT OF ANISE SEEDS (*PIMPINELLA ANISUM L*) AND ACTIVE DRY YEAST (*SACCHAROMYCES CEREVISIAE*) SUPPLEMENTS AS FEED ADDITIVES ON THE PRODUCTIVE PERFORMANCE OF LACTATING EGYPTIAN BUFFALOES

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SUMMARY

This study was conducted to evaluate the effect of supplementing rations with Anise seeds (AS) and Active dry Yeast (Y) in Egyptian buffaloes on milk yield, milk composition and some blood parameters. Twelve multiparous pregnant buffaloes in third and fourth lactation, weighing in average 465 ± 2.5 kg and at 6 weeks before parturition were divided randomly into four equal groups. Control group (T₁) fed concentrate feed mixture, Egyptian clover and wheat straw without supplements (basal diet). The 2nd group (T₂) was given the basal diet plus 50 gram/head/day of Anise seeds. The 3rd group (T₃) was given the basal diet plus 20 gram/head/day of active dry yeast (*Saccharomyces Cerevisiae*). The 4th group (T₄) was given the basal diet plus 10 gram of active dry yeast and 25 g of Anise seeds/head/day. Buffaloes were fed individually and the experimental period started form 6 weeks before calving and extended for 15 weeks after calving periods. The results indicated that the dry matter intake (DMI) was significantly ($P < 0.05$) increased in T₂ and T₄ than control treatments. Also, Anise seeds and Yeast supplements significantly ($P < 0.05$ or $P < 0.01$) increased nutrient digestibility (DM, OM, CP, EE). Total milk yield, daily milk yield and fat, lactose, total solid percentage significantly ($P < 0.05$ or $P < 0.01$) increased in treated buffaloes. Also, calculated efficiency of milk/DMI was improved ($P < 0.05$) in treated buffaloes. However, values of blood plasma for total cholesterol and triglyceride concentrations significantly ($P < 0.01$) decreased in treated buffaloes compared to control treatment. The addition of Anise seeds and Yeast increased ($P < 0.05$) the levels of blood plasma T₃, T₄ hormones in lactating buffaloes.

Keywords: Anise seeds, dry yeast, Egyptian buffaloes, milk yield, triglyceride.

INTRODUCTION

Egypt has approximately 3.8 million heads of buffaloes and their milk contributes to 60% of the total milk production in Egypt (Ministry of Agriculture, Dokki, Egypt, 2003). Buffalo's milk preferred by the Egyptian consumers for its high percent of fat and good tastes. It is whiter in color than local cow's milk. Therefore, buffalo's milk gets almost double the price of cow's milk in the local market (Abdel-Aziz, 2005). Generally; there is an increasing demand for buffalo's milk in Egypt. Many attempts have been made to improve milk production, increase growth rate and profits of buffaloes. Feed additives are important material that can improve feed efficiency, milk yield and composition of lactating buffaloes (Khattab *et al.*, 2010). Dietary supplements of live yeast culture had been reported by El-Ashryet *et al.*, (2001) and Abd El-Hakeem, (2011) to improve feed utilization, milk production and composition in buffaloes. Also, El-Garhy and Mustafa (2007) reported that Anise seeds (*Pimpinella Anisum L*) might be useful as a milk stimulant for lactating animals. Sallam *et al.*, (2012) reported that Anise seeds diet supplementation could be used as natural growth promoters in ewe diets to improve milk yield, milk composition. Generally, feed additives supplementies in the diet led positive (increase) or negative (decrease) or unchanged effect on the concentrations of blood (Abo El-nor *et al*, 2007, Khattab *et al*, 2011) on lactating buffaloes. The objective of this study was to evaluate the effect of Anise seeds (*Pimpinella Anisum L*) and Active dry Yeast

(*Saccharomyces Cerevisiae*) supplements as feed additives on lactating buffalo's performance, feed efficiency, milk yield, milk composition, and some blood parameters.

MATERIALS AND METHODS

This study was carried out at the experimental farm station belonging to the Animal production Department, Faculty of Agriculture, Al-Azhar University, Assuit Branch, during the period from 2014 to 2016 years.

Experimental animals and feeding system.

Twelve pregnant buffaloes in the third and fourth lactation, weighing in average 465 ± 2.5 kg and at 6 weeks before parturition were divided randomly into four equal groups. Control group (T₁) fed concentrate feed mixture, Egyptian clover and Wheat Straw without supplements (basal diet). The 2nd group (T₂) was given the basal diet plus 50 g/head/day of Anise seeds. The 3rd group (T₃) was given the basal diet plus 20 g/head/day of Active dry Yeast (*Saccharomyces Cerevisiae*). The 4th group was given the basal diet plus 10 g of Active dry Yeast + 25 g of Anise seeds/head/day. Buffaloes were fed individually and the offered daily feeds were assessed to cover the maintenance and production requirements for each animal according to Shehata (1971). Buffaloes were fed individually and the experimental period started from 6 weeks before calving and extended for 15 weeks after calving period. Rations were offered twice daily at 7.00 a.m and 3.00 p.m. Fresh water and mineral salts were continuously available during the trial period. Daily amounts of feed consumed and the residuals were weight and recorded. The chemical compositions of ingredients are shown in Table (1).

Table (1). Chemical composition of concentrate feed mixture (CFM), Egyptian clover (EC) and wheat straw (WS), anise seeds (AS), active dry yeast (Y), percentage of dry matter.

Item	Diet ingredient				
	CFM	Egyptian clover	Wheat straw	Anise Seed	Yeast
Dry matter (DM) %	89	17.19	90.63	90.24	92.23
Organic matter (OM) %	88.5	87.29	88.2	89.47	94.77
Crude protein (CP) %	14.32	13.49	3.00	17.47	33.67
Crude fiber (CF) %	15.1	23.37	39.33	19.2	6.24
Ether extract (EE) %	4.22	1.68	1.73	26.35	10.42
Nitrogen free extract (NFE) %	54.86	48.75	44.14	26.45	44.44
Ash %	11.5	12.71	11.8	10.53	5.23

Chemical analysis:

Fecal samples were collected at the last week of the experimental period twice daily at 7.00 a.m and 4.00 p.m directly from the rectum of each buffalo then it was stored at -5° C until analysis. Sub samples of fecal and experimental ration were dried at 105° C in a forced air oven for 3 hours or till reached a constant weight to determine dry matter (DM), while other subsamples were dried on 60° C in a forced air oven till constant weight for feed and fecal nutrients determination. Proximate chemical analysis was applied according to A.O.A.C (1995). Organic matter (OM) and Nitrogen free extract (NFE) were calculated. Digestibility coefficients of DM, OM, CP, CF, EE and NFE, were determined using acid insoluble ash (AIA %) as natural marked according to Van keulen and Young (1977). The nutritive values as total digestible nutrients (TDN) and digestible crude protein (DCP) of the experimental rations were calculated.

Blood plasma constituents.

Heparinized blood samples were collected from the jugular vein from each of the experimental buffaloes at the last week of feeding trial. Blood samples were centrifuged at 4000 r.p.m for 20 minutes for separation of plasma that were stored at -20° C till analysis. Part of blood plasma samples was used to measure, by spectrophotometer, the concentration of total cholesterol according to Ellefson and Caraway (1976), triglycerides according to Stein (1987). The second part of blood plasma samples was used to determine the concentration of triiodothyronine (T₃) (Chopra *et al*, 1971), thyroxine (T₄) (Irvine and Standeven 1968) and prolactin according to (Downing *et al.*, 1995) using radioimmunoassay technique.

Milk yield and Composition:

Buffaloes were hand milked twice daily at 6.00 a.m and 4.00 p.m during the period from the fifth days of calving till the end of the 15th week thereafter and milk yields were recorded individually at each milking. Milk samples were collected from experimental animals every two weeks. The samples were kept under freezing at - 20 ° C until the chemical analysis. All samples were mixed for each animal in one sample for analysis. Milk sample were analyzed for fat, total solids, total protein and ash (Ling, 1963), solids not fat (SNF) was calculated by difference. Lactose percentage was calculated as following: Lactose % = 100 – (total protein% + fat% + ash% + moisture %), according to Economides (1986).

Statistical Analysis:

The statistical analysis system (SAS, 2008) was used for least square of variance for separated measures of milk yield, milk composition, feed efficiency parameter and data of blood plasma analysis. The following model was applied: $Y_{ij} = \mu + A_i + E_{ij}$. Where: Y_{ij} = the studied trait, μ = the overall mean. A_i = the effect of treatments (i=1, 2, 3, 4). E_{ij} = the experimental error. (0, σ^2). Significant differences among means for each trait were detected using (Duncan's multiple range tests, 1955).

RESULTS AND DISCUSSION

Feed intake and nutrients digestibility:

Dry matter intake (DMI) for buffaloes fed T₄ ration was significantly (P<0.05) higher than those recorded for T₁, T₂ and T₃ ration as shown in Table (2). These results agree with those reported by Abo El-nor *et al.*, (2007); Sallam and Mahgoub (2015), they found that DM intake was increased when lactating animals fed different level of medical plant seeds. Results in Table (2) showed that the digestibility coefficients of DM, OM, CP, EE and feeding values (TDN and DCP) were significantly (P<0.05 or P<0.01) increased in supplemented groups than control. These results agree with finding of Sadek *et al.*, (2013); Sallam and Mahgoub (2015) with Egyptian buffaloes, they reported that medicinal plant seeds significantly improved the digestibility coefficient of DM, OM, CP and EE and TDN, DCP compared with the control. Also, the present results agree with finding of Salem *et al.*, (2002); Abd El-Hakeem, (2011) they found that digestibility coefficient of DM, OM, CP and EE and TDN, DCP in lactating buffaloes increased by Yeast culture added compared with the control.

The improvement in nutrient digestibility and feeding value of supplemented groups than control Table (2) could be due to the stimulation of rumen micro flora activity through one of the following: (1- decreasing number and activity of antagonistic organisms), (2- saving some micro factors to rumen micro flora, micro elements, vitamins, hormone, enzymes or unknown factors) which are required to the efficient digestion, absorption and metabolism and available as effective groups or components in medical plants), (3- decreasing hazard some harmful heavy metals) and (4- minimizing effectively hazards of mycotoxins by inhibition of fungi growth and aflatoxins production. (Mohamed *et al.*, 2003).

Table (2). Effect of treatment on total dry matter intake (DMI) and digestibility coefficients of lactating buffaloes, fed the experimental ration.

Item	Total dry matter intake (TDMI)				± SE	Sign	p.value
	T ₁	T ₂	T ₃	T ₄			
CFM Intake _(kg)	6.87 ^c		7.8 ^b	6.76 ^c	8.54 ^a	0.49	* 0.021
WS Intake _(kg)	4		4	4	4	-	- -
EC/Intake _(kg)	2		2	2	2	-	- -
DMI/h/d _(kg)	12.87 ^c		13.80 ^b	12.76 ^c	14.54 ^a	0.49	* 0.021
Anise seeds, (gm)	-		50	-	25	-	- -
Active dry yeast, (gm)	-		-	20	10	-	- -
Digestibility coefficients %							
DM		62.46 ^c	66.51 ^b	65.79 ^b	71.63 ^a	1.74	* 0.034
OM		67.38 ^c	70.62 ^b	71.95 ^b	75.25 ^a	1.11	** 0.0068
CP		64.25 ^c	70.47 ^b	71.44 ^b	74.53 ^a	0.85	** 0.0002
EE		66.22 ^b	76.25 ^a	77.23 ^a	75.95 ^a	1.52	** 0.0028
CF		53.95	55.74	60.67	59.60	2.13	NS 0.163
NFE		69.47	70.92	70.26	71.50	1.32	NS 0.729
Feeding value %							
TDN		61.74 ^c	64.66 ^b	65.27 ^a	66.11 ^a	1.28	* 0.0168
DCP		9.20 ^c	10.09 ^b	10.23 ^b	10.67 ^a	0.12	** 0.0002

^{a,b,c} Means within the same row having different superscripts are significantly different (P<0.05)

NS= Not significant (P>0.05), *=(P<0.05), **=(P<0.01)

T₁= control group (basal ration); T₂= basal ration plus 50g Anise seeds/ head/day; T₃= basal ration plus 20g active dry yeast / head/ day; T₄= basal ration plus 25g Anise seeds+10g Active dry yeast /head/day.

Blood parameters.

Cholesterol and triglycerides levels were decreased significantly (P<0.01) in treated buffaloes (T₂, T₃, T₄) compared with control group (Table 3 and Fig 1). This result might be attributed in somehow to the higher nutritive values of medicinal herbs ration. However, it was of interest to point out to the fact that not all medicinal herbs to have similar positive influences on reducing cholesterol biosynthesis in animal blood. These results are in agreement with those reported that by Othman (2005) found that medicinal plant seeds additives significantly decreased Cholesterol and triglycerides levels in lactating animals. Similarly, Zeid (2004) showed that total lipids and cholesterol concentrations were lower (P<0.05) with both of *Nigella sativa* and *Chamomile* when they compared with the control ration. Also, the present results are in agreement with those reported by Temim *et al.*, (2009) found that supplementation of dairy cows with *Saccharomyces cerevisiae* significantly lowered the cholesterol and triglycerides concentration levels.

Table (3). Effect of treatments on some blood parameters.

Item	Treatments , LSM±S.E				Sign	P.Value
	T ₁ (control)	T ₂	T ₃	T ₄		
Cholesterol (mg/dl)	186.9±7.95 ^a	145.9±7.95 ^b	148.6±7.95 ^b	138.2±7.95 ^b	**	0.001
Triglyceride (mg/dl)	45.52±5.16 ^a	34.26±5.16 ^b	38.20±5.16 ^b	35.66±5.16 ^b	**	0.008
T3 (ng/dl)	1.16±0.01 ^c	1.33±0.76 ^b	1.37±0.59 ^b	1.79±0.05 ^a	*	0.052
T4 (ng/dl)	35.73±1.98 ^c	47.24±7.38 ^b	48.1±7.45 ^b	52.5±1.00 ^a	*	0.017

^{a,b,c} Means within the same row having different superscripts are significantly different (P<0.05)

*=(P<0.05); **=(P<0.01).

The highest blood plasma Triiodothyronine (T₃) and thyroxine (T₄) hormones levels were found with buffaloes fed T₄ ration followed by T₃ treatment then T₂, while the lowest blood plasma levels of T₃ and T₄

hormones was found with the control group buffaloes. The differences were significant ($P < 0.05$) among treatments (Table 2 and Fig 2). The significant increase in the secretion of thyroid hormones (T_3 and T_4 ng/dl) in T_4 , T_3 and T_2 treatments might be due to (1) the increase of carbohydrate, fat and protein metabolism, which it was reflected in a positive effect on digestibility coefficient of carbohydrate (as NFE), fat (as EE) and protein (as CP) it was reported in literature by Kassab (2007). These results are in agreement with those reported that by Zanonny *et al.*, (2013) they found that added medicinal plant seeds significantly increase ($P < 0.05$) the level of T_3 and T_4 hormone concentrations in Ossimi lambs. Also, the present results are in agreement with the finding of Yousef *et al.*, (1996) found that, feeding on diet supplemented with 15 g YC/h/d increased T_3 hormone level of lactating buffaloes during summer season.

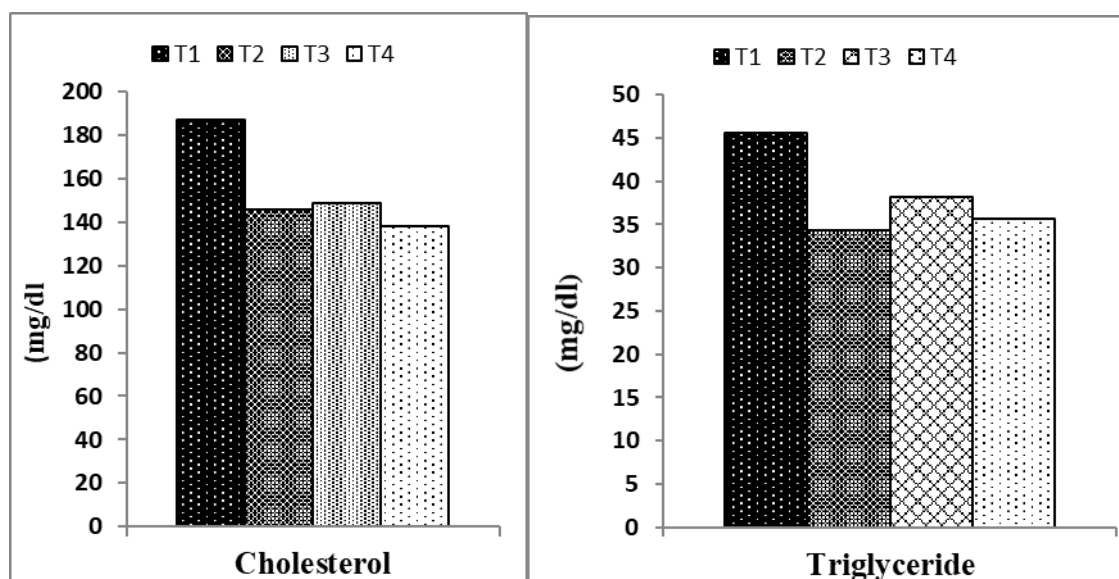


Figure (1): Cholesterol and Triglyceride levels.

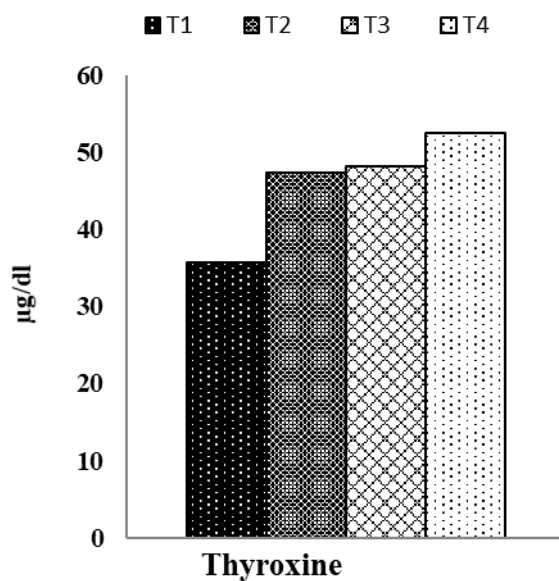


Figure (2): Triiodthyronine (T_3) and Thyroxine (T_4) levels.

Milk Yield and Composition.

The total milk yield for T₁, T₂, T₃ and T₄ treatments were 540.01, 621.20, 641.88 and 670.86 kg, respectively. Also, the overall daily milk means for T₁, T₂, T₃ and T₄ treatments were 5.14, 5.92, 6.11 and 6.39 kg/h/d, respectively (Table 4). Results in Table (4) and Fig (3) revealed that total and daily milk yield in the treated animals were significantly (P<0.05) higher compared to the control.

The increase in milk yield by addition of Anise seeds may be due to the galactogoetic effect of active components that Anise may contain. El-komey, (1996) found that the term galactopoietic arises from medicinal seeds Anise and Caraway which contain active substances which stimulate milk secretion. Sallam *et al.*, (2012) reported that Anise seeds diet supplementation improves milk yield in Ossimi ewes. The improvement in milk yield could be due to biological properties of Anise oil that are inhibiting for bacterial (Sagdic and Ozcan, 2003) and Fungi growth (Soliman and Badeaa, 2002), which stimulating the secretion of digestive enzymes and appetizing. In addition, the estrogenic agents of Anise can also contribute to better milk secretion (Albert-Buleo, 1980). Concerning the effect of supplementation Yeast culture in diets on milk yield, many investigator reported that milk yield had increased significantly (P<0.05 or P<0.01) by dietary Yeast culture addition (Khattab *et al.*, (2010); Mousa *et al.*, (2012).

The improvement in milk yield, in the present study may also due to the higher DM, OM, CP, EE, and NFE digestibility's and the feeding value expressed as TDN and DCP (Table 2). Moreover, the increase of milk yield from treated buffaloes in the present study may be due to the positive effect of Anise seeds and Active dry Yeast supplementation on secretion of thyroid hormones and the level of prolactin hormone concentration. The efficiencies in milk yield calculated as DMI/kg milk yield and milk yield /DMI were improved (P<0.05) in treated groups (T₂, T₃ and T₄) as compared with control group (Table 4). These results are in agreement with those reported by Abo El-Nor *et al.*, (2007); Sallam and Mahgoub (2015), they found that DMI/kg milk yield and milk yield /DMI in Egyptian buffaloes were improved (P<0.05) in treated groups as compared with the control by supplementation medicinal plant seeds. Milk fat, lactose, total solids, solid not fat and ash contents were higher (P<0.05 or P<0.01) in T₂, T₃ and T₄ than control. While, milk protein content did not differ significantly (P>0.05) between treatments (Table 4 and Fig 4).

Table (4). Milk yield and composition of lactating buffaloes as affected by dietary treatments.

Item	Treatment				S.E	Sig	P.Value
	T ₁ (control)	T ₂	T ₃	T ₄			
Lactation length(d)	105	105	105	105			
Total milk yield (kg)	540.01 ^b	621.20 ^a	641.88 ^a	670.86 ^a	49.93	*	0.037
Daily milk yield (kg)	5.14 ^b	5.92 ^a	6.11 ^a	6.39 ^a	0.48	*	0.036
	<i>Feed efficiency</i>						
DMI(kg/h/d)	12.87 ^c	13.80 ^b	12.76 ^c	14.54 ^a	0.49	*	0.021
DM I/ milk	2.50 ^a	2.33 ^b	2.09 ^c	2.28 ^b	0.18	*	0.031
Milk/DMI	0.40 ^b	0.44 ^a	0.48 ^a	0.44 ^a	0.04	*	0.042
	<i>Milk composition (%)</i>						
Fat	5.90 ^c	6.78 ^a	6.31 ^b	6.62 ^{ab}	0.13	**	0.0003
Protein	3.52	3.48	3.34	3.61	0.9	NS	0.221
Lactose	4.34 ^c	4.79 ^{ab}	4.59 ^{bc}	4.92 ^a	0.11	**	0.0032
Total solid	14.34 ^b	15.80 ^a	14.94 ^b	15.74 ^a	0.21	**	0.0001
Solid not fat	8.44 ^c	9.02 ^{ab}	8.63 ^{bc}	9.12 ^a	0.20	*	0.013
Ash	0.69 ^b	0.73 ^b	0.82 ^a	0.70 ^b	0.02	**	0.0003

^{a,b,c} Means within the same row having different superscripts are significantly different (P<0.05)

NS= Not significant (P>0.05), *= (P<0.05); **=(P<0.01).

In some reports, it was demonstrated that many plants increased milk production in animals through the induction of lactogenic hormone (prolactin) Lompo-Ouedraogo *et al.* (2004) and Patel and Kanitkar, (1969). Some plants contain estrogenic substances such as anethole that increase the secretion of milk. Structurally, anethole is similar to dopamine and shows a competitive antagonism at the dopamine receptor site. Thus, it can increase the liberation of prolactin and induce the production of milk (Lis-Balchin., 2006). *P. anisum* seeds have many components such as Tanethole (Chandler and Hawkes., 1984), and the lactogenic activity of this plant may be related to this constituent. In addition, the growth of pups and gain weight in treated

groups may be attributed to induction of milk production and milk constituents. These results are in agreement with those reported by many investigators Salem *et al.* (2002); Khattab *et al.* (2010) and Sallam and Mahgoub (2015).

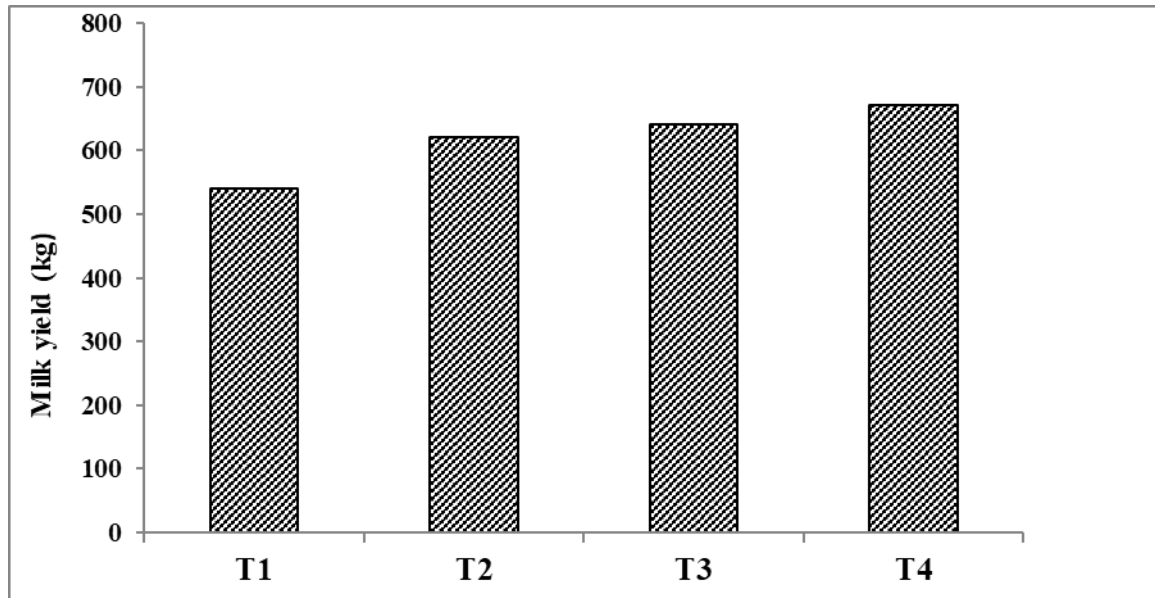


Figure (3): Total milk yield (kg)

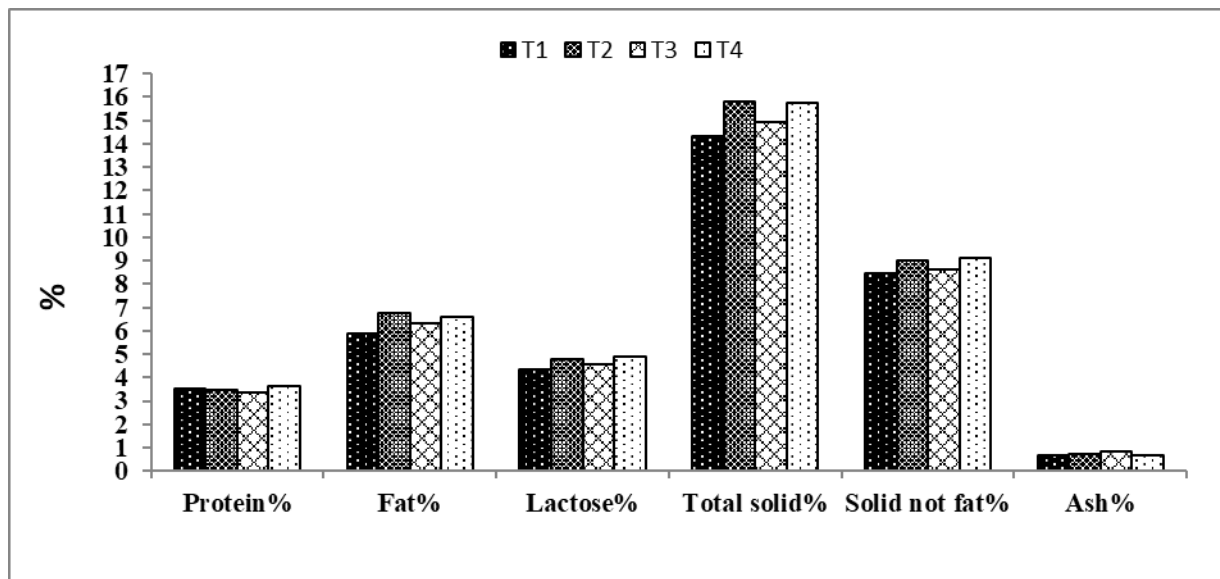


Figure (4): Milk composition.

CONCLUSION

Based on the results of this study, it may be concluded that Anise seeds and Active dry Yeast can successfully be used as feed additives in the ration of lactating buffaloes for increase milk yield by about 19

% more than the unsupplemented groups. Also, the quality of milk yield was improved. Levels of cholesterol and triglyceride were decreased in the blood of treated buffaloes than that of control group.

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تأثير إضافة بذور الينسون والخميرة الجافة النشطة كإضافات غذائية على الأداء الإنتاجي للجاموس المصري الحلاب

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تم دراسة تأثير اضافة بذور الينسون والخميرة الجافة النشطة لعلائق الجاموس المصري على انتاج اللبن وتركيبه وبعض مقاييس الدم. استخدمت في هذه الدراسة 12 جاموسة في الموسم الثالث والرابع للحليب وتزن في المتوسط 465 ± 2.5 كجم، وعند 6 اسابيع قبل الولادة المتوقعة قسمت الحيوانات عشوائيا الى 4 مجاميع متساوية:

مجموعة المقارنة (الكنترول T1) غذيت بمخلوط العلف المركز، البرسيم المصري وتبن القمح (عليقة اساسية). المجموعة الثانية (T2) غذيت بالعليقة الاساسية بالاضافة 50 جم للرأس في اليوم من بذور الينسون. المجموعة الثالثة (T3) غذيت بالعليقة الاساسية بالاضافة 20 جم للرأس في اليوم من الخميرة الجافة النشطة. المجموعة الرابعة (T4) غذيت بالعليقة الاساسية بالاضافة 10 جم من الخميرة الجافة النشطة، 25 جم من بذور الينسون للرأس في اليوم. التغذية كانت فردية وبدات التجربة قبل 6 اسابيع من الولادة المتوقعة لكل جاموسة واستمرت لمدة 15 اسبوع بعد الولادة. اشارت النتائج الى ان الكميات المأكولة من العلف ارتفعت عند مستوى (5% معنوية) في كل من المعاملة الثانية والرابعة بالمقارنة بمجموعة الكنترول. وان اضافة بذور الينسون وكذلك الخميرة الجافة النشطة ادت الى زيادة معنوية (مستوى 1,5%) في معاملات هضم مكونات الغذاء (DM, OM, CP, EE). كمية اللبن الناتج، متوسط انتاج اللبن اليومي للرأس وكذلك النسبة المئوية لكل من الدهن، سكر اللاكتوز، الجوامد الكلية في اللبن الناتج زادت زيادة معنوية (مستوى 1,5%) في الجاموس المعامل. وكانت كفاءة تحويل العليقة المأكولة الى لبن مرتفعة معنويًا (مستوى 5%) في الجاموس المعامل. وقد اظهر النتائج ان مستوى كل من الكوليستيرول والدهون الثلاثية إنخفضت معنويًا (مستوى 1%) في دماء الجاموس المعامل (T₂, T₃, T₄) بالمقارنة بالجاموسة الغير معامل (مجموعة الكنترول). واطهرت النتائج ايضا ان اضافة بذور الينسون والخميرة الجافة الى علائق الجاموس الحلاب ادت الى زيادة معنوية (مستوى 5%) في مستوى هرمونات الغدة الدرقية T₃, T₄.

استناداً إلى نتائج هذه الدراسة، يمكن الاستنتاج أنه يمكن استخدام بذور الينسون والخميرة الجافة النشطة كإضافات غذائية في علائق الجاموس الحلاب لزيادة إنتاج الحليب بنسبة تزيد بنحو 19% أكثر من المجموعات الغير معاملة. أيضا تم تحسين جودة الحليب كما انخفضت مستويات الكوليستيرول والدهون الثلاثية في دم الحيوانات المعاملة مقارنة بمستوى المجموعة الضابطة (الكنترول).