

## **EFFECT OF FORMALDEHYDE TREATMENT AND FEEDING REGIME OF DIETS FOR CROSSBRED FRIESIAN COWS ON MILK PRODUCTION AND MICROBIOLOGICAL STATUS**

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### **SUMMARY**

*Sixteen lactating crossbred Friesian cows, in early lactation, were divided into 4 groups using 4 x 4 Latin square design throughout 30 days experimental period. Rations used consisted of berseem hay (BH) or whole corn silage (WCS) as a source of roughage and concentrate feed mixture either untreated or formaldehyde-treated. The ratio of roughage to concentrate was 40 to 60 on the basis of dry matter. Before the commencement of the experiment, the experimental rations were evaluated on sheep for their digestibility and nutritive values.*

*Results indicated significant ( $P < 0.05$ ) increase in digestibility coefficients of organic matter (OM) and nitrogen-free extract (NFE) of the whole corn silage (WCS) compared to BH rations. The same effect was found for the nutritive value in terms of digestible energy (DE) and metabolizable energy (ME) of WCS. On the other hand, a significant effect was recorded on digestible crude protein (DCP) values for animals when received BH rations compared to those received WCS. Formaldehyde treatment of concentrate feed mixture (CFM) significantly increased the CP digestibility coefficients and DCP% as well. No significant differences were recorded for other nutrients digestibility coefficients and/or the feeding values of the tested rations.*

*Yield of 4% fat corrected milk (FCM) was improved by 8.3% for cows fed on formaldehyde-treated concentrate feed mixture (F-CFM) than those fed untreated ones. Milk component yields were significantly higher in case of formaldehyde treated group than untreated corresponding. Residual formaldehyde levels in milk from cows fed F-CFM found to be negligible.*

*The microbiological examination included nine pathogenic bacteria namely *E. coli*, *Shigella* spp., *Salmonella* spp., *Proteus* spp., *Yersinia* spp., *Brucella* spp., *Staphylococcus* spp. and *Enterococcus* spp. besides fecal coliforms and total bacterial count. In addition, some mesophilic and thermophilic members of lactic acid bacteria as useful and naturally occurring microinhabitants in milk were also examined in the produced milk. The obtained milk was also used for making yoghurt to examine the effect of the residual formaldehyde on the yoghurt characters such as pH, acidity, fats, total solids and specific gravity.*

*Results showed that *Proteus* spp. exhibited the highest decrease percentage (58.91) followed by *Enterococcus* spp. (52.43). *E. coli* and *Shigella* spp. exhibited decrease equal to (49.93) and (49.91), respectively. *Streptococcus lactis* showed the*

highest decrease percentage being 14.3 followed by *Streptococcus thermophilus* (10.35). No significant differences between yoghurt made from treated or untreated milk when compared with the standards of the tested parameters.

Finally, it could be concluded that WCS seems to be better source of roughage than BH in formulating rations of lactating cows, based on better feeding values. In addition, low concentration of formaldehyde (1%) used for treatment is strongly recommended for the protection of CFM protein to improve milk yield and its components by high yielding cows. In the meantime, formaldehyde inhibits undesirable bacteria such as pathogenic ones with low destruction percent for desirable species such as lactic acid bacteria.

**Keywords:** Lactating cows, diets, formaldehyde treatment, milk quality and quantity, microbiology yoghurt

## INTRODUCTION

In Egypt, most farm animal breeders depend mainly on berseem hay (BH) or whole corn silage (WCS) for feeding during summer season with supplementation of concentrate feed mixture (CFM). Many sources of plant proteins are used as supplements in CFM for lactating and growing animals. Among those, undecorticated cotton seed meal, linseed meal and wheat bran, are characterized by high rumen degraded protein (Mehrez, 1981; NRC, 1989; El-Shabrawy, 1996). Two main sources of roughage such as BH and WCS are also characterized by high rumen degraded protein (Mabjeesh *et al.*, 1997 and El-Fadaly *et al.*, 2003).

Milk yield and its protein content can be affected by the amount of CP flow into the small intestine (Hof *et al.*, 1994). In order to magnify available protein level, undegradable protein must be added to the ration above the amount of microbial protein synthesised in the rumen (NRC, 1989). There are many methods for protein protection to increase the undegradable CP in rumen, e.g. 1% (w/w) formaldehyde treatment (Ferguson *et al.*, 1967), heat treatment (Stern *et al.*, 1985), tannin treatment (Pace *et al.*, 1993) and hence increasing the amount of CP escaping to the small intestine. The formaldehyde treatment method is apparently the more suitable for protection of these ingredients without severely affecting enzymatic digestion of protein (El-Shabrawy, 1996).

The main objective of this study was to investigate the effect of feeding CFM either untreated or formaldehyde-treated with different roughage sources such as BH or WCS on nutrients digestibility, nutritive values of the tested rations. Milk production and its components were also studied. The quality of produced milk was evaluated by making yoghurt and monitoring the microbiological status represented by pathogenic and lactic acid bacteria existence.

## MATERIALS AND METHODS

This study was carried out at El-Serw Experimental Station, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture and Land

Reclamation, and Department of Microbiology, Faculty of Agriculture, Mansoura University, Egypt.

#### **Animals and feed formulation**

Sixteen lactating crossbred Friesian cows in early lactation were balanced for body weight, milk yield, days after calving and parity. The animals were divided into 4 groups and each received 4 rations using 4 x 4 Latin square design with 30 days experimental period.

The 4 experimental rations used were the following: 1) Untreated concentrate feed mixture + berseem hay (U-CFM + BH), 2) Formaldehyde treated - CFM + BH, (F-CFM + BH), 3) U-CFM + whole corn silage (U-CFM + WCS) and 4) F-CFM + WCS.

The CFM consisted of 22% yellow maize, 26% wheat bran, 32% undecorticated cotton seed cake, 5% linseed meal, 9% rice bran, 3% molasses, 2% limestone and 1% sodium chloride. The CFM pellets were ground (6 mm particle size) and sprayed with commercial formalin solution at the rate of 1 g HCHO / 100 g CP (w/w) of the tested material according to Ferguson *et al.* (1967). All animals were fed on the tested rations formulated of 60% CFM (untreated or treated) and 40% roughage (BH or WCS) to satisfy their requirements, calculated according to NRC (1989).

The rations were individually weighed for animals and offered twice daily. Animals were fed individually in tie-stalls, kept in their stalls (usually 8 h), then were exercised in a drylot with free access to water.

#### **Chemical analysis**

The chemical composition of ingredients and rations was carried out by the methods of AOAC (1980). The animals were machine milked twice daily. Milk samples were obtained monthly from each cow from two consecutive milking and composited proportionally for determination of fat, protein, lactose, solids-not fat (SNF) and total solids (TS) by milkoscan 133 B (AISN Foss Electric, 69 Slangeupgade DK 3400 Hillerd, Denmark). Fat corrected milk (4% FCM) for each cow was calculated using the formula of Gaines and Overman (1938).

Formalin in the produced milk was assessed using Nash's reagent according to Naiem (1999) to measure the intensity of color developed using 412 nm wavelength. Quantity of formalin was calculated from the standard curve prepared by the same procedure.

#### **Digestibility trials**

Prior to commencement of the experiments, four digestion trials were conducted to evaluate the digestibility and nutritive value of the four experimental rations used to cow's feeding. Three mature Rahmani rams in average weight of 63 kg were involved in these trials. The rams were assigned to receive 85% of their *ad lib.* dry matter intake at 1600 g/h/d (960 g CFM either untreated or treated and 640 g roughage either BH or WCS). One month as a transition period was allowed between the evaluation of the two roughages. Each trial lasted for 28 days, of which the first 21 days were considered as a preliminary period and the last 7 days were the collection period. The feeds were offered twice daily at 8 a.m. and 4 p.m. Drinking water was available all time. Samples of WCS after drying at 60°C for 48 hours in forced air oven, berseem hay and CFM as well as feces samples were dried at 105°C

for 3 hours, ground through a 1-mm screen hummer mill and analyzed for ash, crude protein (CP), ether extract (EE) and crude fiber (CF) according to the AOAC (1980).

#### **Microbiological determination**

##### **Media used**

The following specific media were used, brilliant green agar for *Shigella* spp. at 37°C for 24 h, MacConkey agar for *Proteus* spp. and *E. coli* at 37°C for 24 h (Merck, 1994). Peptone sorbitol bile agar medium was used for *Yersinia* spp. at 35°C for 48 h (Klausner and Donnelly, 1991). *Listeria* spp. was enumerated at 35°C for 48 h using tryptose phosphate agar according to Pini and Gilbert (1988). For *Salmonella* spp., the selenite agar medium was used at 37°C for 24 h (Collins and Lyne, 1985). *Staphylococcus* spp. was counted using staphylococci 110 medium at pH 7.0±0.2 for 2 h at 35°C. For *Brucella* spp., the Brucella agar medium was used. For total bacterial count, TGY medium was used at 35°C for 24 h.

##### **Counting of pathogenic bacteria**

One ml of milk samples was dispersed in 9 ml of sterile distilled water and decimal serial dilutions were prepared with vigorous shaking. From the third ( $10^{-3}$ ) dilutions, one-ml sample was taken and plated on different media and temperature and period of incubation varied according to the microorganisms as mentioned above. Therefore, developed colonies were counted.

##### **Counting of lactic acid bacteria**

One ml of the produced milk was used for making serial dilutions with vigorous shaking. From the 5<sup>th</sup> dilution ( $10^{-5}$ ), one ml was taken and plated on the agar medium of Lee *et al.* (1974) at 35°C for the mesophilic lactic acid bacteria or at 55°C for thermophilic ones. Colonies were counted after incubation period of 48 h.

##### **Statistical analysis**

The obtained data for the parameters of metabolism trails, nutritive value, milk yield and its composition were statistically analyzed by the analysis of variance using factorial design (2 x 2) in a Latin Square Design. The data of milk microbiological studies were analyzed using the completely randomized design. Duncan's Multiple Range Test was used for comparison among means at 0.05 level (Duncan, 1955). Computation was performed using SAS computer program package (SAS, 1994).

## **RESULTS AND DISCUSSION**

### **Chemical composition**

Results in Table (1) show that the chemical analysis of the ingredients used in this study were in normal ranges as previously reported (Maklad *et al.*, 2000; El-Deeb, 2001). The chemical composition of the tested rations seemed similar in all nutrients, except for DM, CP and ash content which was higher in BH rations than those of WCS. The NFE was higher in WCS rations than those of BH rations.

### **Digestibility coefficients and nutritive values**

Results of digestibility and feeding values are presented in Table (2). There were no significant differences among nutrients digestibility of all tested rations, except

EE digestibility which was significantly ( $P < 0.01$ ) different. A slight increase was also observed in CP digestibility of the tested rations containing F-CFM. The feeding values expressed as TDN %, DCP%, DE MJ/kg DM and ME MJ/kg DM were not affected by the experimental rations. This might be related to the similar values obtained for digestibility of all used rations.

**Table 1. Chemical composition of tested feed ingredients and their formulated rations (% DM basis)**

Examined Items*	DM (%)	Chemical composition						
		OM	CP	EE	CF	NFE	Ash	GE**
U-CFM	90.40	91.78	16.20	2.65	11.24	61.69	8.22	17.82
F-CFM	87.96	91.75	16.30	2.63	11.12	61.70	8.25	17.80
BH	86.92	91.14	12.17	2.05	28.33	48.59	8.86	16.91
WCS	28.75	91.92	8.42	2.24	25.81	55.45	8.08	17.58
Calculated chemical composition of the formulated experimental rations								
U-CFM + BH	89.01	89.93	14.59	2.41	18.07	54.86	10.07	17.45
F-CFM + BH	87.55	89.91	14.65	2.40	18.00	54.86	10.09	17.46
U-CFM + WCS	65.74	91.84	13.09	2.49	17.06	59.20	8.16	17.72
F-CFM + WCS	64.28	91.82	13.15	2.48	16.99	59.20	8.18	17.72

\* U-CFM: Untreated concentrate feed mixture, F-CFM: Formaldehyde treated concentrate feed mixture, BH: Berseem hay, WCS: Whole corn silage

\*\* GE: Gross energy, calculated according to MAFF (1975) using the following equation: GE (MJ / kg DM) = 0.0226 CP + 0.0407 EE + 0.0192 CF + 0.0177 NFE

**Table 2. Effect of roughage source and formaldehyde treatment on nutrient digestibility coefficients and nutritive values of the experimental rations**

Examined Items	Digestibility (%)						Nutritive values			
	DM	OM	CP	EE	CF	NFE	TDN (%)	DCP (%)	DE* (MJ / kg DM)	ME** (MJ / kg DM)
Experimental rations										
U-CFM+BH	57.23	59.10	63.50	67.39	43.31	62.30	57.34	11.70	10.13	8.31
F-CFM+BH	60.88	63.18	67.09	81.49	46.80	66.26	61.70	12.39	10.83	8.88
U-CFM+WCS	61.19	63.79	62.60	89.04	46.88	67.83	61.41	8.14	11.13	9.13
F-CFM+WCS	63.54	65.94	64.82	73.90	50.11	70.34	62.94	8.47	11.51	9.44
Roughage source										
BH	59.05	61.14 <sup>b</sup>	65.29	74.44	45.05	64.28 <sup>b</sup>	59.52	12.04 <sup>a</sup>	10.48 <sup>b</sup>	8.59 <sup>b</sup>
WCS	62.37	64.86 <sup>a</sup>	63.71	81.47	48.49	69.09 <sup>a</sup>	62.17	8.30 <sup>b</sup>	11.32 <sup>a</sup>	9.28 <sup>a</sup>
Formaldehyde treatment										
Untreated	59.21	61.44	63.05 <sup>b</sup>	78.21	45.09	65.07	59.37	9.92 <sup>b</sup>	10.63	8.72
Treated	62.21	64.56	65.96 <sup>a</sup>	77.69	48.45	68.30	62.32	10.43 <sup>a</sup>	11.17	9.16

Means within the same column for each effect having different superscripts are significantly different ( $P < 0.05$ ).

\* DE (MJ/Kg DM) = Digested organic matter x 19 (MAFF, 1975).

\*\* ME (MJ/Kg DM) = DE x 0.82 (MAFF, 1975).

These results came on line with those obtained by Krastanova *et al.* (1995) and Majeesh *et al.* (1997). They reported that the protection of dietary protein led to non-significant effect on nutrients digestibility with different sources of feedstuffs and protection methods.

There was no significant effect of roughage source on DM, CP, EE and CF digestibility of the tested rations. On the other hand, OM and NFE digestibilities were significantly ( $P < 0.05$ ) higher with WCS than those of BH rations. The higher ( $P < 0.05$ ) nutritive value in terms of DE and ME of WCS than BH rations could be associated by higher OM and NFE digestibilities of the former rations than the latter ones. The reverse was true for DCP%, since it was higher in case of BH containing rations. This could be explained by the increase in the favorable N source for rumen microbes beside the reduced dietary energy escaping ruminal degradation. These results are in harmony with the findings of El-Shabrawy *et al.* (2004).

Regarding the effect of protection method, the higher ( $P < 0.05$ ) CP digestibility values (65.96%) were obtained with the F-CFM ration than the untreated one (63.05%). The protection of protein CFM enhanced DCP content of the ration from 9.92% for the untreated ration to 10.43% for the formaldehyde treated ration. The improvement in CP digestibility may be related to the formaldehyde as a protective agent of protein source. Hence reducing protein solubility and degradability in the rumen could provide more dietary protein for digestion and absorption in the small intestine, which was probably better than that of microbial proteins as reported by Atwal *et al.* (1995), El-Shabrawy (1996) and El-Shabrawy (2000).

#### ***Milk yield***

Table (3) shows that there were no significant differences in daily milk yield and its components among the four used rations.

Although the daily milk yield and its components except fat were increased ( $P < 0.05$ ) in BH compared with WCS rations, the numerical values were close and did not exceed 2 percentage units. This indicates that neither BH nor WCS exhibited deleterious effect.

The effects of protection using formaldehyde on milk and its components and 4% fat-corrected milk yields were significantly different where values were higher for cows fed the formaldehyde treated rations than those fed untreated ones. The increase of rumen undegradable protein (RUP) resulted in improving the yield of milk and its components, probably because of high flow of nitrogen and essential amino acids to the small intestine (Cunningham *et al.*, 1996). These results are in agreement with those of Atwal *et al.* (1995). They found that the increased amount of RUP in diets of dairy cows tended to increase milk yield because of improved protein status and improved intake of metabolizable energy or both of them.

**Table 3. Effect of the experimental rations, source of roughage and formaldehyde treatment on daily milk yield and its components**

Examined Items	Yield of milk and milk components (kg / head / day)						
	Milk	4% FCM	Fat	Protein	Lactose	SNF	TS
<b>Experimental rations</b>							
U-CFM + BH	15.62	14.94	0.58	0.44	0.74	1.28	1.86
F-CFM + BH	16.91	16.14	0.62	0.48	0.80	1.40	2.02
U-CFM+ WCS	15.24	14.66	0.57	0.42	0.72	1.24	1.82
F-CFM + WCS	16.56	15.92	0.62	0.47	0.79	1.37	1.99
± SE	0.11	0.11	0.004	0.003	0.005	0.01	0.01
<b>Roughage source</b>							
BH	16.26 <sup>a</sup>	15.54 <sup>a</sup>	0.60	0.46 <sup>a</sup>	0.77 <sup>a</sup>	1.34 <sup>a</sup>	1.94 <sup>a</sup>
WCS	15.90 <sup>b</sup>	15.29 <sup>b</sup>	0.59	0.44 <sup>b</sup>	0.75 <sup>b</sup>	1.30 <sup>b</sup>	1.90 <sup>b</sup>
± SE	0.08	0.08	0.003	0.002	0.004	0.007	0.01
<b>Formaldehyde treatment</b>							
Untreated	15.43 <sup>b</sup>	14.80 <sup>b</sup>	0.57 <sup>b</sup>	0.43 <sup>b</sup>	0.73 <sup>b</sup>	1.26 <sup>b</sup>	1.83 <sup>b</sup>
Treated	16.73 <sup>a</sup>	16.03 <sup>a</sup>	0.62 <sup>a</sup>	0.48 <sup>a</sup>	0.80 <sup>a</sup>	1.38 <sup>a</sup>	2.01 <sup>a</sup>
± SE	0.08	0.07	0.003	0.002	0.004	0.007	0.01

Means within the same column for each effect having different superscripts are significantly different ( $P < 0.05$ )

#### **Milk components**

Results presented in Table (4) indicated that the mean effect of the experimental rations on percentages of milk components was not significant. These results contradict those of Zerbini *et al.* (1988), who found that increased dietary RUP reduced milk fat percentage and milk fat yield. On the other hand, obtained results are in agreement with those obtained by Khorasani *et al.* (1996). Such discrepancy might be due to milk productivity level of cows in the different studies.

Concerning the effect of roughage source, the milk fat, lactose and SNF percentages significantly increased in WCS compared with BH rations. The increase of milk fat, lactose and SNF percentages of cows fed WCS rations may be due to better ruminal fermentation, which was observed by El-Fadaly *et al.* (2003). In contrast, significant ( $P < 0.05$ ) increases of milk protein and TS were obtained in milk of cows fed BH rations. The results are in agreement with those reported by Maklad *et al.* (2000).

In respect to the effect of protection using formaldehyde; milk protein, lactose, SNF and TS percentages significantly increased for cows fed F-CFM than those fed untreated rations (CFM). There was no significant effect of formaldehyde protection on milk fat percentage. Higher percentages of most milk components were obtained with cows fed the F-CFM ration than those of untreated one. The increase in TS in milk by protection treatment was mainly due to the form of protein and lactose in obtained milk.

**Table 4. Mean effect of the experimental rations, source of roughage and formaldehyde treatment on percentage of milk components**

Examined Items	Milk components, %				
	Fat	Protein	Lactose	SNF	TS
<b>Experimental rations</b>					
U-CFM + BH	3.71	2.81	4.71	8.20	11.91
F-CFM + BH	3.69	2.85	4.74	8.27	11.96
U-CFM+ WCS	3.75	2.76	4.73	8.17	11.92
F-CFM + WCS	3.74	2.82	4.76	8.26	12.00
± SE	0.007	0.004	0.01	0.01	0.001
<b>Roughage source</b>					
BH	3.70 <sup>b</sup>	2.83 <sup>a</sup>	4.72 <sup>b</sup>	8.23 <sup>a</sup>	11.93 <sup>a</sup>
WCS	3.74 <sup>a</sup>	2.79 <sup>b</sup>	4.75 <sup>a</sup>	8.22 <sup>a</sup>	11.96 <sup>b</sup>
± SE	0.005	0.003	0.006	0.009	0.008
<b>Formaldehyde treatment</b>					
Untreated	3.73	2.78 <sup>b</sup>	4.72 <sup>b</sup>	8.18 <sup>b</sup>	11.91 <sup>b</sup>
Treated	3.72	2.83 <sup>a</sup>	4.75 <sup>a</sup>	8.26 <sup>a</sup>	11.98 <sup>a</sup>
± SE	0.005	0.003	0.006	0.009	0.008

Means within the same column for each effect having different superscripts are significantly different ( $P < 0.05$ ).

The effect of protection using formaldehyde of plant protein sources on milk components in the literature was inconsistent (Bruckental *et al.*, 1996; Cunningham *et al.*, 1996). In the present study, residual formaldehyde concentration in milk from cows fed F-CFM have been found to be negligible (2.05 ppm).

Syrjala-Qvist and Setala (1982), Ismail and El-Shabrawy (2002) and El-Shabrawy *et al.* (2004) found that when cows were fed grass silage, CFM treated with formaldehyde, or alfalfa silage the residual formaldehyde content in milk was related to intake, being 4.00, 3.11 or 1.6 ppm, respectively. In cows fed whey treated with formaldehyde at levels of 0, 13.8, 27.7 and 41.6 g / day, the contents of formaldehyde in their milk were 0.017, 0.034, 0.095 and 0.223 mg/kg as found by Buckley and Fisher (1984).

The concentration of formaldehyde in milk in the present study was probably safe for human nutrition. This is confirmed by Ismail and El-Shabrawy (2002), who found that Domiati cheese (8% salt) made from milk produced by cows fed F-CFM contained very small concentrations of formalin, which was 0.650 ppm at zero time. This concentration was decreased gradually during ripening period, which became 0.085ppm after 90 days, since it had no effect on obtained Domiati cheese properties.

Humans and animals exposed to endogenous formaldehyde as an essential intermediate in cellular metabolism that is required for the biosynthesis of purines, thymidine and certain amino acids (Heck *et al.*, 1990). It is involved in methylation reactions through the tetrafolate mechanism, normal blood levels of formaldehyde in humans and animals are approximately 2.5 ppm (2.5 mg/L) and it is rapidly metabolized in the blood with a half-life of approximately 1.5 minutes (Clary and Sullivan, 1992).

It could be concluded that WCS is a better source of roughage than BH in formulating rations of lactating cows, based on better fermentation parameters in the



rumen and improving feeding values as well. In addition, formaldehyde treatment is recommended for the protection of CFM protein at 1% to improve milk yield and its components by high yielding cows.

#### Microbiological status of obtained milk

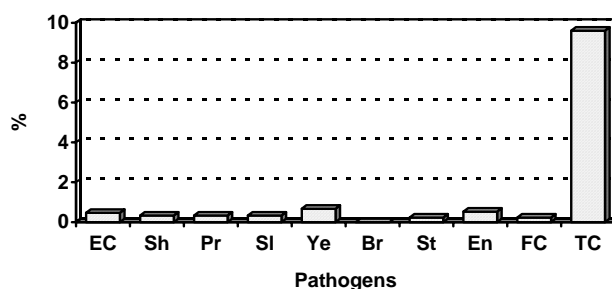
Regarding the pathogenic bacteria, milk from cows either fed both untreated or formaldehyde-treated rations were examined directly after the milking process. Results recorded in Table (5) proved the presence of pathogens in milk containing HCHO and untreated one, but in different levels. Reduction in count as affected by HCHO treatment was calculated for each bacterial group and results are graphically illustrated in Fig. 1.

**Table 5. Microbiological status of produced milk**

Examined pathogenic bacteria	x 10 <sup>3</sup> cfu ml <sup>-1</sup>		
	Untreated	HCHO-Treated	± SE
<i>Escherichia coli</i> (EC)	7.33 <sup>a</sup>	3.00 <sup>b</sup>	0.47
<i>Shigella</i> spp. (Sh)	5.33 <sup>a</sup>	2.67 <sup>b</sup>	0.33
<i>Proteus</i> spp. (Pr)	5.67 <sup>a</sup>	2.33 <sup>b</sup>	0.33
<i>Salmonella</i> spp. (Sl)	5.67 <sup>a</sup>	3.33 <sup>b</sup>	0.33
<i>Yersinia</i> spp. (Ye)	4.33	2.67	0.67
<i>Brucella</i> spp. (Br)	0.00	0.00	0.00
<i>Staphylococcus</i> spp. (St)	4.00 <sup>a</sup>	2.67 <sup>b</sup>	0.23
<i>Enterococcus</i> spp. (En)	6.67	4.67	0.53
<i>Fecal coliform</i> (FC)	7.00 <sup>a</sup>	3.33 <sup>b</sup>	0.23
Total bacterial count (TC)	184.3	173.3	9.61

\* HCHO = Formaldehyde

Means within the same row having different superscripts are significantly different (P<0.05).



**Fig. 1. Reduction percentages in numbers of some pathogenic bacteria as a result of formaldehyde treatment**

*Escherichia coli* exhibited extraordinary reduction being 59.1% followed by *Proteus* spp. (58.9%), fecal coliforms (52.4%), then *Shigella* spp. and *Salmonella* spp. (49.9 and 41.3%, respectively). In addition, 38.3, 33.3, 29.9 and 5.9% decreasing

values were found by *Yersinia* spp., *Staphylococcus* spp., *Enterococcus* spp. and total bacteria, respectively.

As to lactic acid bacteria as beneficial candidates naturally occurring in milk, both mesophilic and thermophilic lactic acid bacteria were tested in obtained milk containing 2.05 ppm residual formaldehyde. Dense populations of up to  $10^7$  cfu ml<sup>-1</sup> for *Lactobacillus plantarum* and *Streptococcus lactis* grown at 35°C as well as *L. thermophilus* and *S. thermophilus* grown at 55°C were recorded (Table 6).

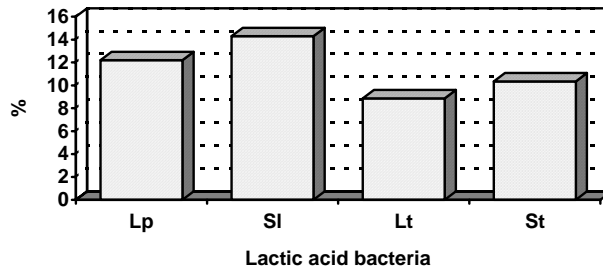
**Table 6. Lactic acid bacterial load of milk samples**

Examined lactic acid bacteria	cfu x 10 <sup>5</sup> /ml of the tested milk		
	Untreated	HCHO-Treated	± SE
<i>Lactobacillus plantarum</i> (Lp)	13.67 <sup>a</sup>	12.33 <sup>b</sup>	0.33
<i>Streptococcus lactis</i> (Sl)	15.00	13.67	0.85
<i>Lactobacillus thermophilus</i> (Lt)	18.67 <sup>a</sup>	16.00 <sup>b</sup>	0.47
<i>Streptococcus thermophilus</i> (St)	19.33 <sup>a</sup>	17.33 <sup>b</sup>	0.33

\* HCHO = Formaldehyde

Means within the same row having different superscripts are significantly different (P<0.05).

Fig. (2) shows the reduction percentages in counts of lactic acid bacteria as affected by HCHO treatment. *Lactobacillus plantarum* (Lp) decreased by 12.2%, while *Streptococcus lactis* (Sl) recorded reduction of 14.3%. In case of thermophilic lactic acid bacteria, *Lactobacillus thermophilus* (Lt) decreased by 8.87%, while 10.35% was found for *Streptococcus thermophilus* (St). At 0.005% formalin, inhibitory effect for *Salmonella* spp., *Staph. aureus*, *E. coli*, *Bacillus* spp., *Streptococcus lactis*, *Streptococcus thermophilus* and *Streptococcus faecalis* was observed (Mahmoud *et al.*, 1985).



**Fig. 2. Reduction percentages in lactic acid bacterial numbers due to formaldehyde treatment**

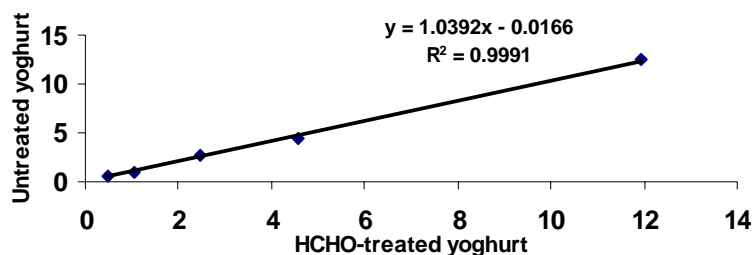
Yoghurt made from obtained milk either treated with HCHO or untreated was evaluated and results are recorded in Table (7). The values of yoghurt pH, acidity (%), fat (%), total solids (%) and specific gravity were almost similar for both yoghurt types. Comparing these parameters with standard parameters, significant correlation with  $r^2 = 0.999$  was obtained as seen in Fig. (3).

**Table 7. Properties of yoghurt obtained from produced milk**

Parameters	Milk used			± SE
	Untreated	HCHO-Treated	Standard	
pH	4.57	4.51	4.57	0.019
Acidity (%)	0.49 <sup>b</sup>	0.51 <sup>a</sup>	0.30 <sup>c</sup>	0.003
Fat (%)	2.47 <sup>b</sup>	2.73 <sup>b</sup>	3.30 <sup>a</sup>	0.098
Total solids (%)	11.93 <sup>b</sup>	12.43 <sup>a</sup>	11.67 <sup>b</sup>	0.110
Specific gravity	1.04	1.04	1.04	0.00

\* HCHO = Formaldehyde

Means within the same row having different superscripts are significantly different (P<0.05).



**Fig. 3. Correlation between yoghurt properties obtained from formaldehyde treated and untreated milk**

It is concluded that the low used concentration of formaldehyde is applicable to protect proteins from degradation in the rumen of animals and to inhibit undesirable bacteria such as pathogenic ones (*Escherichia coli*, *Shigella* spp., *Proteus* spp., *Salmonella* spp., *Yersinia* spp., *Brucella* spp., *Staphylococcus* spp., *Enterococcus* spp. and *Fecal coliforms*) with low destruction percent for desirable bacterial species such as lactic acid producing ones (*Lactobacillus plantarum*, *Streptococcus lactis*, *Lactobacillus thermophilus* and *Streptococcus thermophilus*).

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## تأثير معاملة العليقة بالفورمالدهيد ونظام التغذية لأبقار الفريزيان الخليط على إنتاجية اللبن ومحتواه الميكروبي

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تم استخدام 16 بقرة فريزيان خليط في مرحلة الإنتاج العالى في تجربة بها 4 معاملات إستمرت 4 شهور بنظام المربع اللاتينى 4 × 4. وقد تكونت العلائق المستخدمة من دريس البرسيم المصرى أو سيلاج الأذرة الكامل بالكيزان ومخلوط العلف المركز سواء غير المعامل أو المعامل بالفورمالدهيد والتي قيمت باستخدام الكباش الرحمانى. وقد شمل الفحص الميكروبيولوجى على اللبن الناتج تسعة من البكتريا الممرضة مثل: *E. coli*, *Shigella* spp., *Salmonella* spp., *Proteus* spp., *Yersinia* spp., *Brucella* spp., *Enterococcus* spp., *fecal coliforms* and *Staphylococcus* spp. هذا بجانب التعداد البكتيرى الكلى. والكشف عن محبات الحرارة المتوسطة وكذلك محبات الحرارة العالية من بكتيريا حمض اللاكتيك كمثال للبكتريا النافعة التى تتواجد طبيعياً في اللبن. كذلك تم استخدام اللبن الناتج لعمل الزبادى وذلك لمعرفة تأثير الميثيق من الفورمالدهيد على عملية تصنيع الزبادى وخصائصه مقارنة بالكنترول. وقد خلصت النتائج إلى الآتى:-

- 1- زيادة معاملات الهضم للمادة العضوية ومستخلص خالى الآزوت وكذلك القيمة الغذائية فى صورة الطاقة المهضومة والطاقة القابلة للتمثيل للعلائق المحتوية على سيلاج الأذرة وذلك بالمقارنة بالعلائق المحتوية على دريس البرسيم المصرى وعلى العكس من ذلك زيادة البروتين الخام المهضوم للعلائق المحتوية على دريس البرسيم المصرى.
- 2- أدت معاملة مخلوط العلف المركز بالفورمالدهيد إلى زيادة معدلات هضم البروتين وكذلك البروتين الخام المهضوم.
- 3- أدت المعاملة بالفورمالدهيد إلى زيادة إنتاج اللبن واللبن معدل الدهن (4%) وكذلك مكونات اللبن الناتج.
- 4- بينت النتائج أن نسبة الفورمالدهيد القليلة المتبقية (2.05 جزء فى المليون) كان لها تأثير إيجابى على انخفاض تعداد البكتريا الممرضة حيث قللت ميكروبات *Proteus* بأعلى نسبة انخفاض تعادل 58.91% ثم إنخفضت ميكروبات *Enterococcus* بنسبة 52.43%، بينما نقصت كل من *E. coli* ، *Shigella* spp. بنسبة 49.93% ، 49.91% على الترتيب.
- 5- سببت النسبة المتبقية من الفورمالدهيد إنخفاض بكتريا حمض اللاكتيك بنسبة 14.3% كما فى حالة البكتريا الكروية السبحية المحبة للحرارة المتوسطة ، بينما نقصت مثيلتها المحبة للحرارة العالية بنسبة 10.35%.
- 6- لم يكن هناك فروق معنوية بين صفات الزبادى المصنع من اللبن المعامل وغير المعامل أخذين فى الاعتبار نسبة الحموضة ، الدهن ، الجوامد الكلية والكثافة النوعية.

أوضحت الدراسة أن سيلاج الأذرة الكامل بالكيزان كمصدر علف خشن أفضل من دريس البرسيم المصرى فى تكوين علائق حيوانات اللبن ، ويرجع ذلك إلى التحسن فى القيمة الغذائية للعلائق. بالإضافة إلى أن المعاملة بالفورمالدهيد بتركيز منخفض (1%) يمكن أن يوصى بها لحماية بروتين العلف المركز لتحسين ناتج اللبن ومكوناته فى الأبقار عالية الإدراة. وفى نفس الوقت أظهرت المعاملة بالفورمالدهيد وجود تأثير مثبط على بكتريا اللبن غير المرغوبة مثل الأنواع الممرضة دون التأثير أو تثبيط الأنواع المرغوبة مثل بكتريا حمض اللاكتيك.