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METABOLIC ATTRIBUTES OF USING OREGANO LEAVES EXTRACT ON BLOOD PARAMETERS AND PRODUCTIVE PERFORMANCE OF DAIRY BUFFALOES

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ABSTRACT

The goal of this study was to evaluate the impact of feeding oregano *(Origanum vulgare)* leaves extract on metabolic , performance and health of lactating buffalos, blood constituents, digestibility, milk production, and milk composition of dairy buffalos. Twenty dairy buffalos were chosen at random to assign treatments: control, 10, 20, and 30 g/head/day of oregano leaves extract as CON., OR1, OR2, and OR3, respectively. Results showed that the addition of oregano leaves extract had a significant effect (P<0.05) on all parameters of digestion coefficients DM, OM, NFE, CP, CF, EE, DCP, and TDN. The addition of Oregano leaves extract (OR2 and OR3) substantially raised the digestibility coefficient of CP and CF (P<0.05), with the inclusion of 20 and 30 g/head/day oregano leaves extract yielding the highest values. OR2 and R3 supplemented with 20 and 30 g/head/day oregano leaves extract had the best total digestible nutrients (TDN) and digestible crude protein (DCP). The groups (OR2 and OR3) showed significantly (P<0.05) the highest milk yield of both actual and 4% fat-corrected milk, followed by group (OR1), while the control group (CON.) had the lowest milk yield. Animals fed 20 and 30 g/head/day Oregano leaves extract recorded significantly (P<0.05) better values of feed conversion ratio (FCR) compared with control. The experimental groups showed more significant (P<0.05) variations in the milk content of protein, fat, TS, SNF, lactose, and ash than the control group when supplemented with oregano leaves extract. Supplementation with 30 g/head/day oregano leaves extract decreased serum total protein, albumin, urea, creatinine, cholesterol, LDL, VLDL, and triglyceride concentrations (P<0.05) compared with other groups, without difference (P > 0.05) among groups in ALT and AST. The average value of serum globulin was significantly higher in oregano leaves extract groups than the control group. The obtained results demonstrated that supplementation groups also tended to increase glucose value as the BHBA and NEFA levels decreased (P > 0.05) that protected against ketosis.

Key Words: Dairy buffalos, oregano leave extracts, digestibility, milk production

INTRODUCTION

Around the world, the livestock production sector is under significant governmental and community pressure to produce safe products with little usage of antibiotics or other compounds derived from synthetic compounds. **(Mousa *et al.,* 2022).** Antibiotics are widely used in dairy farming to boost immunity, reduce stress and sensitivity to infections, enhance the development of rumen & performance of livestock, and lower the risk of calf mortality because of newborn diarrhea **(Poudel *et al.,* 2019)**. To increase animal welfare and maximize livestock production while assuring no dangers to the environment or human health, an appropriate substitute to chemicals is required **(Cheng *et al.,* 2014)**. Animal feed additives are considered an essential component that improves animal performance and increases production **(El- Sherif *et al.,* 2021)**. Due to their wide biological effects, plant extracts have been considered the most effective alternatives to antibiotics **(Diao *et al.,* 2014)**. Bioactive plants and plant compounds, when used as feed components, have a variety of effects on ruminant digestibility and serum biochemicals. They may affect blood parameters in sheep by maintaining plasma glucose levels **(Raghuvansi *et al.,* 2007)**, whereas others may increase plasma glucose concentration in steers or alter serum insulin concentration in young Holstein bulls fed a high concentrate diet **(Devant *et al.,* 2007)**. Transitioning from a non-lactating period or pregnancy to a lactating period or non-pregnancy necessitates sophisticated metabolic adjustments to assure the availability of glucose required to initiate lactation **(McCarthy *et al.,* 2020)**.

Natural phytogenic feed additives have demonstrated excellent outcomes in terms of weight gain, feed efficiency, and decreased mortality rate in animals **(Alatrony *et al.,* 2022)**. According to **(El-Sherif *et al.,* 2021)** phytogenic chemicals are intended to enhance animal function by encouraging the synthesis of digestive enzymes, which facilitates better absorption and digestion.

Oregano (Origanum vulgare) yields essential oils consists of carvacrol (3.1%), carvacrol methyl ether (5.6%), sabinene (6.5%), linalyl acetate (7.2%), p-cymene (8.6%), -terpinene (10.6%), Z-sabinene hydrate (13.4%), and thymol (15.9%) **(Simirgiotis *et al.,* 2020)**. Thymol and carvacrol make up around 80% of the essential oil of oregano, and they are primarily the cause of its efficiency as an antioxidant **(Zhai *et al.,* 2018)**. According to **Mousa *et al.,* (2022)**, the active components of oregano essential oil effectively prevent rumen methane mitigation *in vitro*. Additionally, according to **Shen *et al.*, (2010)**, oregano essential oil is a good source of anti-inflammatory substances.

According to **Oh *et al*,. (2017)** carvacrol is the primary secondary metabolite extracted from oregano plants. This substance has mainly served as antidepressants, antibacterial, and also as antioxidant stimulation and boosting immune systems in humans and monogastric animals **(Gonçalves *et al*., 2015)**. On the other hand, they mostly control ruminal fermentation in ruminants **(Kolling *et al*., 2018)**. This includes carvacrol and thymol. Polyphenols and essential oils have been shown to influence rumen fermentation **(Heleno *et al*., 2015)** and have an impact on methanogenesis by directly or indirectly inhibiting the growth, activity, development, and metabolic processes of the methanogenic *Archaea*. This may reduce emissions of CH4 **(Szumacher-Strabel and Cieślak, 2012)**.

The lactation period is critical for a lactating dairy buffalo's health and milk production. This period of high milk production causes metabolic stress by increasing the apparent incidence of conditions like metritis, mastitis, and ketosis in lactating dairy cows due to increased nutrient requirements, high energy requirements, and production and secretion of milk. This metabolic stress results in a decrease in yield and an increase in concerns about animal welfare, **(Sharma *et al*., 2011; Sordillo and Mavangira, 2014)**. Negative energy balance marked by rather high concentration of ketone bodies (acetoacetate, β-hydroxy butyrate, and acetone) and a parallel reduction of blood glucose levels causes ketosis, a serious metabolic disorder. **(Dann *et al.* 2005)**.

Testing graded amounts of oregano leaves extract was the goal of the current study. The aim of this study was to investigate how oregano extract supplementation levels affected dairy buffalo's performance, digestibility, milk production, milk composition, blood constituents and the metabolic changes associated with various levels of energy deficit would investigated to improve the performance and health of dairy buffalo's

MATERIAL AND METHOD

Location:

The dairy farm at the Agricultural Research Center's El Sero Experiments Station served as the site of this study's experimental activities. Blood samples were examined at the hormone laboratories at the Faculty of Agriculture Research Park (FARP), while milk examinations were carried out at the Cattle Information System/Egypt (CISE). The laboratories of the Regional Center for Food and Feed, Agriculture Research Center, Ministry of Agriculture, Egypt, performed the chemical analyses of feeds and excrement.

Preparation of oregano plant extracts:

After being cut, the oregano plant was let to dry out for two weeks at room temperature. The moisture was subsequently fully removed by dehydrating it for 24 hours at 45 °C in an oven made by Thermo Scientific®. The plant (leaves) was dried and crushed to get the extracts. Alcoholic extraction was used for the extracts.

Using 250 g of ground sample, 2 L of pure ethanol (J.T. Baker) and triple-distilled water (80:20, v/v) were used for the alcohol extraction process. The mixture was then placed in an amber flask and soaked for a month, shaking every third day. It was then filtered through Whatman paper No. 4, and 70% of the solvent was evaporated in a Soxtec System HT type extractor (Fisher Scientific 1043) at 85 °C for 45 minutes, **Pesewua *et al*., 2008)**.

Gas chromatography (GC), using an Agilent Technologies series 6890N, was used to determine the concentration of the active ingredients in oregano extracts. The following set of criteria was followed: Film, ID 0.320 mm, length 30 m.

Animals, Diets and Experimental Design

Twenty lactating buffalos were divided into four groups (five buffalos each). From the second to the third season. CON. was the control group, with no plant extract supplementation; OR1, OR2, and OR3 had oregano extract additions of 10, 20, and 30 g/head/day. Each group received rations at 7 AM and 7 PM, twice a day. According to **NRC (2001)**, the provided feeds were assessed to determine if they matched each dairy buffalo's nutritional needs. All of the diets were supplied with a concentrate-to-roughage ratio of around 63:37% on a DM basis. During the day, there was always access to drinking clean water. Tables (1 and 2) presented the formulation and chemical composition of the constituents in experimental diets and feed. Feed refusal was recorded once per day. Weekly changes were made to feeding allowances based on variations in both milk output and body weight.

Table 1. Concentrate feed mixture supplemented with oregano extract/g on a dry matter basis.

Item	Experimental concentrate feed mixture (kg/100kg)			
	CON.	OR1	OR2	OR3
Yellow corn	30	30	30	30
Sunflower meal	26	26	26	26
Wheat bran	39	39	39	39
Molasses	2	2	2	2
Limestone	2	2	2	2
Sodium chloride (Na Cl)	1	1	1	1
<u>Additive</u>				
Oregano extract/ g/ head/day	0	10	20	30

CFM: concentrate feed mixture
CON.: lactating buffalos fed control diet without any supplementation.
OR1: lactating buffalos fed control diet supplemented with 10 g/head/day Oregano leaves extract.
OR2 lactating buffalos fed control diet supplemented with 20 g/head/day Oregano leaves extract.
OR3: lactating buffalos fed control diet supplemented with 30 g/head/day Oregano leaves extract.

Table 2. The chemical components of the rice straw, clover hay, and concentrate feed mixtures includes the experimental complete feed mixtures (on a DM base of 100%).

Item	Chemical composition on DM basis%						
	DM	OM	Ash	CP	EE	CF	NFE
CFM	87.89	92.7	7.3	15.89	3.13	11.35	62.33
CH	89.2	90.02	9.98	12.01	2.41	28.56	47.04
RS	90.5	84.8	15.2	2.32	1.48	33.63	47.37

CFM : Concentrate feed mixture, CH: Clover hay, RS: Rice straw, DM: Dry Matter, OM: Organic Matter, CP: Crude protein, EE: Ether extract, CF: Crude fibre and NFE: Nitrogen free extract.

Milk and blood samples:

At six in the morning and six in the evening, dairy buffalos were mechanically milked. The amount of milk produced was recorded every day. **Gaine's (1928)** formula was used to calculate the fat corrected milk (4%): FCM% = actual milk yield (kg) x 0.4 + 15 x fat yield (kg). Milk samples were taken from five cows in all groups and analyzed using a Milk-Scan Model (133 B).

Blood samples were collected from three buffalos in each dietary supplement just before meals, through the jugular vein in non-heparinized vacationer tubes. Serum was obtained by centrifuging blood samples for 30 minutes at 4,000 pm, and it was stored at -20 °C until further analysis. With the aid of readily available kits and an ultraviolet spectrophotometer UV4802 (Unico Co., Dayton, OH, USA), serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, and creatinine were determined (Biosystem S.A.,* Costa Brava,-30 Barcelona,. Spain). Glucose was measured spectrophotometrically using commercial kits from Biodiagnostic Company. Both colorimetric and electrophoretic methods were used to quantify the serum proteins. Serum protein electrophoresis was carried out using the electrophoresis apparatus and the cellulose acetate electrophoresis kit (Biotec-Fischer GmbH, Germany). Using spectrophotometry, measurements of serum total proteins and albumin were made by UV 3220 (Unico Co., Dayton, OH, USA) and commercial kits from Spinreact. The albumin was subtracted from the total protein to determine the globulin values. Bio-Merieux kits (Mary o1, Eliot Charbnniere-Les, Beins, France) used for determining triglycerides, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and total cholesterol in plasma. An analytical kit from photometric systems was used to evaluate non-esterified fatty acids (NEFA) (DIA Lab, Austria). Using an analytical kit, the levels of beta-hydroxybutyric acid (BHBA) were determined (POINTE Scientific Inc., USA).

Digestibility trails:

Following the feeding trial, a digestibility trial was conducted to ascertain the feeding nutritional value and digestibility coefficients of the diets under study. This was done using the acid-insoluble Ash technique, a natural internal biomarker originally described by **Van Keulen and Young (1977)**. Fecal sample collections were made twice a day, at 8 a.m. and 8 p.m., for a period of five days. Faeces samples were kept in freezers at -18°C to facilitate additional chemical analysis. As stated by **AOAC. (1995)**, chemical analyses of feeds were performed for digestion and feeding experiments as well as feces. According to **Schnider and Flatt's (1975)** formulae, the DM digestibility and digestibility coefficient have been calculated and as follows:

$$\text{Dry matter digestibility (\%)} = 100 - [100 * \frac{\text{AIA\% in feed}}{\text{AIA\% in feces}}]$$

$$\text{Digestion coefficient of nutrient} = 100 - (100 \times \frac{\text{AIA \% in feeds} \times \text{Nutrient in feces}}{\text{AIA \% in feces} \times \text{Nutrient \% in feeds.}})$$

Van Keulen and Young (1977) suggest using acid insoluble ash (AIA) in this manner. An estimate of digestibility can be obtained by dividing the concentration of AIA in the feed by the concentration of AIA in the feces **(Osuji *et al.*, 1993).**

Chemical composition:

According to **AOAC (2000)**, ground dried samples of feed and faeces were analyzed for (moisture, CP, Ash, EE and CF %), and calculated as dry matter.

Statistical Analysis

ANOVA (one-way) was used for analyses of variance of the data. All data were statistically analyzed using the **SAS (2013)** statistical analysis system's general linear models technique. When the main effect was significant, Duncan's multiple range test **(Duncan, 1955)** was employed to separate the means. The significance of differences was set at ($p<0.05$).

RESULTS

The concentration of active ingredients of oregano leaves extract (mg/mL):

Table 3 findings demonstrated the high thymol content of the oregano leaves extract (25.13 mg/ml), followed by terpinene (14.08 mg/ml), linalool (9.64 mg/ml), and finally carvacrol (4.25 mg/ml). Therefore, oregano leaves extract is a good source of bioactive compounds.

Table 3. The concentration of active ingredients of oregano leaves extract.

Extract	Terpinene (mg/mL)	Linalool (mg/mL)	Thymol (mg/mL)	Carvacrol (mg/mL)
Oregano leaves extracts	14.08	9.64	25.13	4.25

Effects of Oregano leave extracts on digestibility coefficients and nutritive values of dairy buffalos:

Table (4) shows the impact of oregano leaves extract on the nutritional values and digestibility coefficients of dairy buffalos. The inclusion of oregano leaves extract significantly (P<0.05) affected all parameters of the digestive coefficients, including DM, OM, NFE, CP, CF, and EE, according to the results. In comparison to the control group (CON.), which appeared the lowest values of the digestibility coefficients of DM, OM, NFE, CP, CF, and EE, the addition of oregano leaves extract (OR2 and OR3) significantly increased the digestibility coefficient of CP and CF (P<0.05). The inclusion of 20 and 30 g/head/day oregano leaves extract yielded the highest values, followed by R2 added with 10 g/head/day oregano leaves extract. when compared to the control group, which displayed the lowest levels of both total digestible nutrients (TDN) and digestible crude protein (DCP). The addition of oregano leaves extract significantly (P<0.05) improved both TDN and DCP. OR2 and OR3 supplemented with 20 and 30 g/head/day oregano leaf extract had the best values of TDN and DCP, followed by OR1. These improvements in the nutritive values and digestibility coefficients among the groups given diets supplemented with extract from oregano leaves could be the result of active components on their extracts which have several advantageous impacts on animal nutrition, such as enhancing the release of digestive enzymes, immune response activation, hunger stimulation, antioxidant, antiviral, and antibacterial properties.

Table 4. Effect of oregano extracts on coefficients of nutrient digestibility and nutritive values of lactating buffalos

Item	Experimental Groups					
	CON.	OR1	OR2	OR3	±SE	*p-value*
Digestion coefficients %						
DM	60.22^{b}	72.22^{a}	73.70^{a}	73.45^{a}	1.08	< 0.001
OM	69.16^{b}	76.87^{a}	78.06^{a}	78.68^{a}	0.79	< 0.001
CP	89.35^{b}	90.96^{b}	93.38^{a}	93.12^{a}	0.52	< 0.001
CF	33.11^{c}	53.55^{b}	58.16^{a}	62.05^{a}	1.45	< 0.001
EE	94.12^{b}	95.90^{a}	95.93^{a}	96.64^{a}	0.30	< 0.001
NFE	81.36^{b}	85.62^{a}	84.44^{a}	84.21^{a}	0.77	< 0.001
Nutritive value %						
DCP	14.02^{c}	14.28^{b}	14.97^{a}	14.62^{a}	0.08	< 0.001
TDN	73.02^{b}	79.65^{a}	81.09^{a}	81.22^{a}	0.68	< 0.001

a,b,c, mean within some rows with differing superscript are significantly differ (P<0.05).

CON.: lactating buffalos fed control diet without any supplementation.

OR1: lactating buffalos fed control diet supplemented with 10 g/head/day oregano leaves extract.

OR2 lactating buffalos fed control diet supplemented with 20 g/head/day oregano leaves extract.

OR3: lactating buffalos fed control diet supplemented with 30 g/head/day oregano leaves extract.

Efficiency of Feed and Milk Yield

The experimental buffalos' milk yield and feed efficiency results are shown in Tables 5 and 6 indicated that the groups that extracted leaves of oregano had significantly (P<0.05) higher milk yields. The groups with the highest milk yield (OR2 and OR3) were followed by G2 in a significant way (P<0.05), while the group with the lowest milk yield (CON.) was the control group.

According to Table 6. Over the course of the trial, the groups fed diets supplemented with Oregano leaves extract recorded considerably (P<0.05) higher yields of both real and 4% fat-corrected milk than the control group, as evidenced by the average daily milk yield and fat-corrected milk (4%) (FCM) results. Feed conversion ratio (FCR) results are expressed as the amount of DM required to produce one kilogram actual, or 4%.

Table 5. Dairy buffalos at different degrees of milk production during different experimental groups.

Item	Milk production (kg/day)					
Week 1	**CON.**	**OR1**	**OR2**	**OR3**	**±SE**	***p-value***
Week 2	13.47	14.05	14.19	13.95	0.29	< 0.001
Week 3	13.04^{b}	14.47a	14.90a	14.80a	0.22	< 0.001
Week 4	12.57^{b}	14.66^{a}	14.71^{a}	14.62^{a}	0.18	< 0.001
Week 5	11.62^{b}	13.38^{a}	13.62^{a}	13.76^{a}	0.25	< 0.001
Week 6	11.28^{c}	12.85^{b}	13.00^{b}	14.23^{a}	0.29	< 0.001
Week 7	9.83^{b}	12.76^{a}	13.05^{a}	12.61^{a}	0.42	< 0.001
Week 8	8.69^{c}	11.54^{b}	13.66^{a}	12.31^{b}	0.31	< 0.001
Week 9	8.23^{b}	11.52^{a}	11.33^{a}	11.33^{a}	0.28	< 0.001
Week 10	7.71^{b}	12.33^{a}	12.21^{a}	12.35^{a}	0.27	< 0.001
Week 11	7.24^{b}	11.57^{a}	11.76^{a}	11.71^{a}	0.36	< 0.001
Week 12	6.47^{b}	11.95^{a}	11.95^{a}	12.04^{a}	0.16	< 0.001
Week 13	6.28^{c}	12.00^{b}	12.04^{b}	12.57^{a}	0.13	< 0.001
Week 14	6.57^{c}	11.42^{b}	12.09^{b}	12.95^{a}	0.21	< 0.001
Week 15	6.60^{c}	11.38^{b}	12.43^{a}	12.47^{a}	0.26	< 0.001
Week 16	6.28^{c}	11.52^{b}	12.62^{a}	13.00^{a}	0.17	< 0.001
Week 17	6.71^{d}	11.18^{c}	12.42^{b}	13.42^{a}	0.25	< 0.001
Week 18	6.92^{c}	11.37^{b}	11.96^{b}	12.85^{a}	0.23	< 0.001

Means followed with the same letter (a, b, c) are not significantly different at 5% level of significant.
CON.: lactating buffalos fed control diet without any supplementation.
OR1: lactating buffalos fed control diet supplemented with 10 g/head/day oregano leaves extract.
OR2 lactating buffalos fed control diet supplemented with 20 g/head/day oregano leaves extract.
OR3: lactating buffalos fed control diet supplemented with 30 g/head/day oregano leaves extract.

Table 6. The feed conversion ratio, milk production, and average daily feed intake

Item	Experimental rations					
	CON.	OR1	OR2	OR3	±S E	*p-value*
Average daily feed intake as feed (kg/h/d)						
Concentrate feed mixture	**7.67^{b}**	**8.67^{a}**	**8.33^{a}**	**8.33^{a}**	**0.36**	**< 0.001**
Clover hay	**3.83**	**3.83**	**4.17**	**4.17**	**0.18**	**< 0.001**
Rice straw	**3.83**	**3.83**	**4.17**	**4.17**	**0.18**	**< 0.001**
Average daily feed intake (on DM basis) kg/h/d						
Total DMI	**13.64**	**14.52**	**14.83**	**14.83**	**0.64**	**< 0.001**
Milk production (kg/ h/ d)						**< 0.001**
Actual daily milk yield	**8.76^{c}**	**12.33^{b}**	**12.81^{a}**	**13.00^{a}**	**0.11**	**< 0.001**
4% fat corrected milk	**12.27^{c}**	**17.53^{b}**	**18.29^{a}**	**18.61^{a}**	**0.17**	**< 0.001**
Feed conversion ratio						
DM kg / kg 4% FCM	**0.90^{b}**	**1.21^{a}**	**1.23^{a}**	**1.26^{a}**	**0.57**	**< 0.001**

Means followed with the same letter (a, b, c) are not significantly different at 5% level of significant.
CON.: lactating buffalos fed control diet without any supplementation.
OR1: lactating buffalos fed control diet supplemented with 10 g/head/day oregano leaves extract.
OR2 lactating buffalos fed control diet supplemented with 20 g/head/day oregano leaves extract.
OR3: lactating buffalos fed control diet supplemented with 30 g/head/day oregano leaves extract..

Effect of oregano leave extracts on milk composition of lactating buffalo:

As presented in Table 7, the contents of protein, fat, TS, SNF, lactose, and ash were shown to differ more significantly (P<0.05) in the experimental groups' milk samples compared to the control group when supplemented with oregano leaves extract. Furthermore, the study's findings disagreed with reports from **Hristov *et al.*, (2013) and Olijhoek *et al.*, (2019)** indicating feeding oregano had no impact on the composition of milk or feed conversion efficiency.

Table 7. Milk chemical composition of the experimental groups

Item	Experimental rations					
	CON.	**OR1**	**OR2**	OR3	**±SE**	***p-value***
Protein %	3.57^{b}	3.63^{ab}	3.65^{ab}	3.72^{a}	0.04	< 0.001
Fat %	6.66^{c}	6.77^{b}	6.82^{ab}	6.93^{a}	0.36	< 0.001
TS %	15.89^{c}	16.00^{b}	16.11^{ab}	16.19^{a}	0.04	< 0.001
SNF %	9.24^{b}	9.25^{b}	9.26^{ab}	9.28^{a}	0.01	< 0.001
Lactose %	4.90^{b}	4.92^{ab}	4.97^{ab}	5.02^{a}	0.03	< 0.001
Ash %	0.96	0.94	0.98	0.96	0.08	< 0.001
Moisture %	84.10^{a}	83.99^{ab}	83.88^{bc}	83.80^{c}	0.04	< 0.001

a,b,c,d Means followed with the same letter (a, b, c) are not significantly different at 5% level of significant.
CON.: lactating buffalos fed control diet without any supplementation.
OR1: lactating buffalos fed control diet supplemented with 10 g/head/day oregano leaves extract.
OR2 lactating buffalos fed control diet supplemented with 20 g/head/day oregano leaves extract.
OR3: lactating buffalos fed control diet supplemented with 30 g/head/day oregano leaves extract.

Serum proteins measured by spectrophotometer and electrophoresis on dairy buffaloes:

Table 8, shows that, the serum total protein constituents. Inclusion of oregano leaves extract in the diets of lactating buffalo at rate 30 g/head/day Oregano leaves extract decreased serum total protein and albumin, The groups who received the extract of oregano leaves had considerably higher average serum globulin values than the control group. The electrophoretic patterns of the supplemented groups were statistically significantly decreased in serum albumin. While total globulin, α-globulin and β-globulin significantly increased as serum γ-globulin levels were highly significantly increased, the majority of globulins were in the form of γ -globulins, followed by α -globulins and then β -globulins.

Table 8. Serum proteins measured by spectrophotometer and electrophoresis on dairy buffaloes.

Item	Experimental rations					
	CON.	OR1	OR2	OR3	±SE.	*p-value*
Spectrophotometer						
Total Protein (g/dl)	7.052c	7.023c	7.416a	7.262b	0.020	<0.001
Albumin (g/dl)	4.021[a]	3.952[b]	3.794[c]	3.382[d]	0.018	<0.001
Globulin (g/dl)	3.031[c]	3.071[c]	3.622[b]	3.880[a]	0.016	<0.001
Protein Electrophoresis						
Albumin (g/dl)	4.020[a]	3.952[a]	3.790[b]	3.380[c]	0.034	<0.001
Total Globulin (g/dl)	3.052[c]	3.100[c]	3.681[b]	3.960[a]	0.021	<0.001
β -Globulin (g/dl)	0.342[d]	0.420[c]	0.550[b]	0.644[a]	0.015	<0.001
α -Globulin (g/dl)	1.01[c]	1.03[c]	1.22[b]	1.324[a]	0.0168	<0.001
γ- Globulin (g/dl)	1.700[b]	1.650[b]	1.911[a]	1.992[a]	0.077	<0.001

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

CON.: lactating buffalos fed control diet without any supplementation.

OR1: lactating buffalos fed control diet supplemented with 10 g/head/day Oregano leaves extract.

OR2 lactating buffalos fed control diet supplemented with 20 g/head/day Oregano leaves extract.

OR3: lactating buffalos fed control diet supplemented with 30 g/head/day Oregano leaves extract.

Table 9 results demonstrated that supplementing 30 g/head/day of oregano leaves extract reduced (P<0.05) the concentrations of urea, creatinine, cholesterol, LDL, VLDL, and triglycerides when compared to the other groups. However, there were no significant deference (P > 0.05) in ALT and AST levels between the groups, Additionally. All groups had decreased levels of serum urea concentration, cholesterol, and triglycerides (P<0.05).

Table 9. Reference values for serum constituents in dairy buffaloes.

Item	CON.	OR1	OR2	OR3	±SE.	*p-value*
Urea (mg/dl)	41.66^{a}	40.06^{b}	39.66^{b}	35.66^{c}	0.121	<0.001
Creatinine (g/dl)	1.25^{a}	1.11^{b}	1.20^{a}	0.80^{c}	0.020	<0.001
Ast(mg/dl)	29.33b	30.33^{a}	28.66^{c}	28.00^{d}	0.072	<0.001
ALt(mg/dl)	30.00^{c}	32.00^{b}	33.00^{a}	29.66^{c}	0.226	<0.001
Cholesterol(mg/dl)	59.87^{a}	59.23^{b}	53.12^{c}	51.11^{d}	0.087	<0.001
Triglyceride(mg/dl)	43.21^{a}	43.66^{a}	38.45^{b}	34.84^{c}	0.578	<0.001
HDL (mg/dl)	14.93^{a}	15.13^{a}	15.12^{a}	15.12^{a}	0.201	<0.001
LDL (mg/dl)	36.31^{a}	35.37^{b}	30.31^{c}	29.60^{d}	0.086	<0.001
VLDL (mg/dl)	8.64^{a}	8.73^{a}	7.96^{b}	7.11^{c}	0.059	<0.001

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

CON.: lactating buffalos fed control diet without any supplementation.

OR1: lactating buffalos fed control diet supplemented with 10 g/head/day oregano leaves extract.

OR2 lactating buffalos fed control diet supplemented with 20 g/head/day oregano leaves extract.

OR3: lactating buffalos fed control diet supplemented with 30 g/head/day oregano leaves extract.

Table 10 indicates that the addition of oregano leaves extract to the diet of lactating buffalo groups had a significant impact on blood glucose levels when compared to the control group. Additionally, the concentration of BHBA and NEFA levels was significantly reduced while glucose levels were increased (P<0.05) by the oregano extract doses. These findings are within the typical and normal range reported by **Fouad *et al.*, (1975)** for healthy buffalos.

Table 10. Ketosis indicators of dairy buffaloes

Item	Experimental rations					
	CON.	OR1	OR2	OR3	±SE.	*p-value*
Glucose(mg/dL)	53.342^{d}	57.140^{c}	63.960^{b}	74.270^{a}	0.310	<0.001
BHBA (mmol/L)	0.830^{a}	0.790^{b}	0.714^{c}	0.632^{d}	0.009	<0.001
NEFAs (mmol/L)	0.610^{a}	0.590^{a}	0.550^{b}	0.520^{c}	0.008	<0.001

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

CON.: lactating buffalos fed control diet without any supplementation.

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DISCUSSION

Analyzed a variety of *Origanum vulgare* obtained from various places, discovering that the amount of active compounds varies depending on the plant's origin **(Acevedo *et al.*, 2013)**. Previous research has found that the main components of oregano extracts include carvacrol, thymol, and terpinene

(Béjaoui *et al.*, 2013). Acevedo *et al.* (2013) found thymol to be the highest component in the essential oil of *Origanum vulgare*, followed by terpinene, according to an investigation of the chemical composition of the oil.

The improvement in rumen fermentation may be responsible for the improved nutrient digestibility, specifically if oregano supplementation is used. Additionally, according to **Kholif *et al.* (2017) ; Farghaly and Abdullah (2021)**, herbs may have the capacity to stimulate salivary secretion, pancreatic lipases, and amylases, as well as increase the activity of digesting enzymes in the gastrointestinal mucosa. Furthermore, the presence of bioactive components in the herb plants may have boosted the availability and efficiency of the rumen microorganisms in addition to other ruminal activities **(Kholif *et al.*, 2017)**. In addition, **Williams and Losa (2001)** showed that plant extraction may increase gastrointestinal endogenous digestive enzymes and salivary secretions, which is beneficial in preventing dietary protein from microbial extinction. These results and findings of the present work agree with those of **Hanafy *et al.*, (2009)**.They found that, in comparison to the control, the inclusion of medicinal herbs in diets raised the values of OM, DM, NFE, CF, and EE digestibility. According to **Zhou *et al.*, (2019)** research, feeding sheep 7 g/day of oregano essential oil increased microbial populations, encouraged rumen fungal growth, and may have improved fiber digestion. The groups that received medicinal herbs had considerably ($P<0.05$) higher nutritional values in terms of total digestible nutrients (TDN) and digestible crude protein (DCP) in their diets. This improvement could be attributed to these diets' enhanced nutritional digestibility. Similar findings were reported by **Alatrony *et al.*, (2022)**, who observed a substantial ($P <0.05$) improvement in DCP and TDN in the dietary group that included herbal plant extract as opposed to the control group. Furthermore, **Sabbah-Allam and El-Elaime (2020)** found that, in terms of DCP and TDN, the feeding herbs' nutritional value increased ($P<0.05$) in comparison to the control diet.

Because oregano leaves extracts are rich in essential oils, including thymol and carvacrol, which act to reduce methane production in the rumen and save energy for milk production, this may be the reason why milk production improved as a result of the extract's supplementation. Also, plant bioactive substances, especially essential oil (EO), have also been studied as potential modifiers of rumen bio-hydrogenation of lipids from diets with the objective of improving the health characteristics through the production of milk and meat rich in unsaturated fatty acids **(Lourenco *et al.*, 2008)**. According to **Kolling *et al.*, (2018),** higher digestible dry matter intake and lower methane emissions in these groups could be responsible for the increase in milk production shown in Holstein cows given oregano and green tea extracts, as well as the higher milk yield noticed in Holstein-Gyr crossbred cows.

The primary components of oregano leaves extracts are essential oils (EO), such as thymol and carvacrol; the use of oregano extract supplements in dairy cows has been evaluated, showing positive effects on feeding behavior **(Benchaar, 2020 and Vizzotto *et al.*, 2021)**, decreasing emissions of methane

(Kolling *et al*., 2018), and enhancing the oxidative status of calves **(De Paris *et al.,* 2020)** and dairy cows **(Vizzotto *et al*., 2021)**. In another experiment, lactating dairy cows were given a single dose of *Origanum vulgare* L., which resulted in a significant decrease in rumen production of methane without getting any negative effects on rumen fermentation, digestibility of dietary nutrients, or cow productive performance **(Hristov *et al*., 2013)**. On the contrary, several studies indicated that oregano extract or its essential oils had no effect on milk composition or production **(Vizzotto *et al*., 2021; Benchaar, 2020).**

One measurement utilized to find out about an animal's metabolic and health condition is blood biochemistry analysis. Furthermore, biochemistry parameters are important in assessing organ and tissue damage, **(Klinkon and Jezek 2012)**. The current study's blood results were between predetermined reference levels by **Boyd (1984)**. Protein catabolism and renal function are indicated by serum albumin, total protein, globulin, and urea-N **(Hosten, 1990)**. Steroid flavonoid terpenes, which promote regular cortisol release, may be the cause of the increase in globulin observed when medicinal herbs were supplemented **(Sabbah-Allam and El-Elaime, 2020)**. There were no variances noted in the levels of serum albumin when measured using electrophoresis compared to colorimetric methods. Additionally, the colorimetric method for calculating globulins demonstrated no discrepancy when compared to globulins measured through electrophoresis.

The biological process by it is clear how many medicinal plants can be supplemented to lower cholesterol, **(Kholif *et al.,* 2017)** explained that the hypo-cholesterolemic effect caused by oregano, on the other hand, might be related to its active substances that inhibit hepatic 3-hydroxyl-3-methylglutary co-enzyme A.

According to **Khamisabadi *et al*., (2016)**, When compared to the control diet, the lambs' urea and lipid levels were reduced by adding either peppermint extract or thyme to their diets. However, a number of recent studies founded that include medicinal herbs in sheep diets had no effect on blood biochemical indicators, **(Mohamadi *et al*., 2017 ; Sabbah-Allam and El-Elaime, 2020).**

Due to negative energy balance, almost all dairy cows are at risk for ketosis during early lactation. During this period, ketone levels increase and glucose levels decrease.

Oregano extract increases propionate levels **(Ozkaya 2020 and Ozkaya *et al.,* 2020),** moreover, research has indicated that propionate possesses anti-ketogenic properties, **(Bush and Milligan 1971)**.

Glucose provides a substrate for the tricarboxylic acid cycle, allowing for the complete metabolism of BHBA **(White, 2015)**. **Bani Ismail *et al.* (2008)** observed a significant negative relationship between glucose concentration and BHBA only when BHBA levels were high. The present results showed that the concentrations of BHBA and NEFA in the serum of buffaloes were decreased significantly ($p<0.05$) in comparison to the control group.

Matthew (2016) found that increasing and/or decreasing the need for oregano leaves extract supplementation can quickly lower circulating BHBA and NEFA levels as long as glucose concentrations are raised. This is because glucose has the ability to release insulin, which increases lipogenesis and decreases lipolysis. The current findings demonstrated the potential of glucose to control BHBA and NEFA production, protect against lipolysis, and enhance lipogenesis.

The anti-ketogenic effects of the oregano and its extracts may be responsible for the increased blood sugar levels and decreased blood BHBA and NEFA concentrations in the supplement group in parallel **(Ozkaya *et al*., 2022)**.

CONCLUSION

It could be stated that inclusion of oregano leaves extract in the diets of lactating buffalo, especially, adding 30 g/head/day oregano leaves extract can be used as an influential source for improving digestion coefficients and nutritive values, milk production, and blood constituents without any negative effect. Similarly, the decrease in blood BHBA and NEFA values, as well as the increase in glucose value, caused the decision that it would be advantageous to prevent ketosis during the early phases of lactation.

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تأثير استخدام مستخلص أوراق نبات الزعتر البرى على خصائص الدم الميتابولزمية والأداء الإنتاجي للجاموس الحلاب

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[4] قسم الأنتاج الحيوانى – كلية الزراعة بمشتهر – جامعة بنها

تهدف هذه الدراسة الى تقيم استخدام مستخلص أوراق نبات الزعتر البرى (*Origanum vulgare*) كأضافة علفية على خصائص الدم الميتابولزمية و تحسين الأداء الإنتاجى للجاموس الحلاب. حيث تم اختيار عشرين جاموسة حلاب و تم توزيعها بشكل عشوائي و قسمت الى أربع مجموعات وغذيت كالاتى المجموعة الأولى(كنترول) ، 10، 20، و 30 جم / رأس / يوم من مستخلص أوراق نبات الزعتر على التوالي. أظهرت النتائج أن إضافة مستخلص

أوراق نبات الزعتر كان له تأثير كبير (P <0.05) على جميع معلمات معاملات الهضم DM و OM و NFE و CP و CF و EE و DCP و TDN. أدى إضافة مستخلص أوراق نبات الزعتر فى المجموعة الثالثة و الرابعة إلى رفع معامل هضم CP و CF بشكل كبير (P <0.05)، مع إضافة مستخلص أوراق نبات الزعتر البرى 20 و 30 جم / رأس / يوم مما أدى إلى أعلى القيم. كان لدى المجموعة الثالثة والرابعة والمضاف إليهما مستخلص أوراق نبات الزعترالبرى 20 و 30 جم / رأس / يوم TDNو DCP .أظهرت المجموعات الثالثة و الرابعة بشكل ملحوظ (P <0.05) أعلى إنتاج للحليب لكل من الحليب الفعلي والحليب المصحح للدهون بنسبة 4٪، تليها المجموعة الثانية، بينما كان لدى مجموعة الكنترول أقل إنتاج للحليب. سجلت الحيوانات التي تغذت على مستخلص أوراق نبات الزعتر البرى 20 و 30 جم / رأس / يوم قيمًا أفضل بشكل ملحوظ (P <0.05) لنسبة تحويل العلف (FCR) مقارنةً بالمجموعة الكنترول. أظهرت المجموعات التجريبية اختلافات معنوية (P<0.05) في التحليل الكيماوى للبن مقارنة بالمجموعة الكنترول . أدى تناول مستخلص أوراق نبات الزعتر البرى بجرعة 30 جرامًا / رأس / يوم إلى انخفاض تركيزات البروتين الكلي والألبومين واليوريا والكرياتينين والكوليسترول والدهون الثلاثية و الدهون منخفضة الكثافة و الدهون مرتفعة الكثافة (P<0.05) مقارنة بالمجموعات الأخرى، مع عدم وجود فرق (P> 0.05) بين المجموعات في إنزيمات الكبد و كانت القيمة المتوسطة للجلوبيولين في الدم أعلى بشكل ملحوظ في مجموعات مستخلص أوراق نبات الزعتر البرى مقارنة بالمجموعة الكنترول. وأظهرت النتائج زيادة قيمة الجلوكوز مع انخفاض مستويات BHBA وNEFA (P> 0.05) التي تحمي الحيوان من الإصابة بالكيتوزية.