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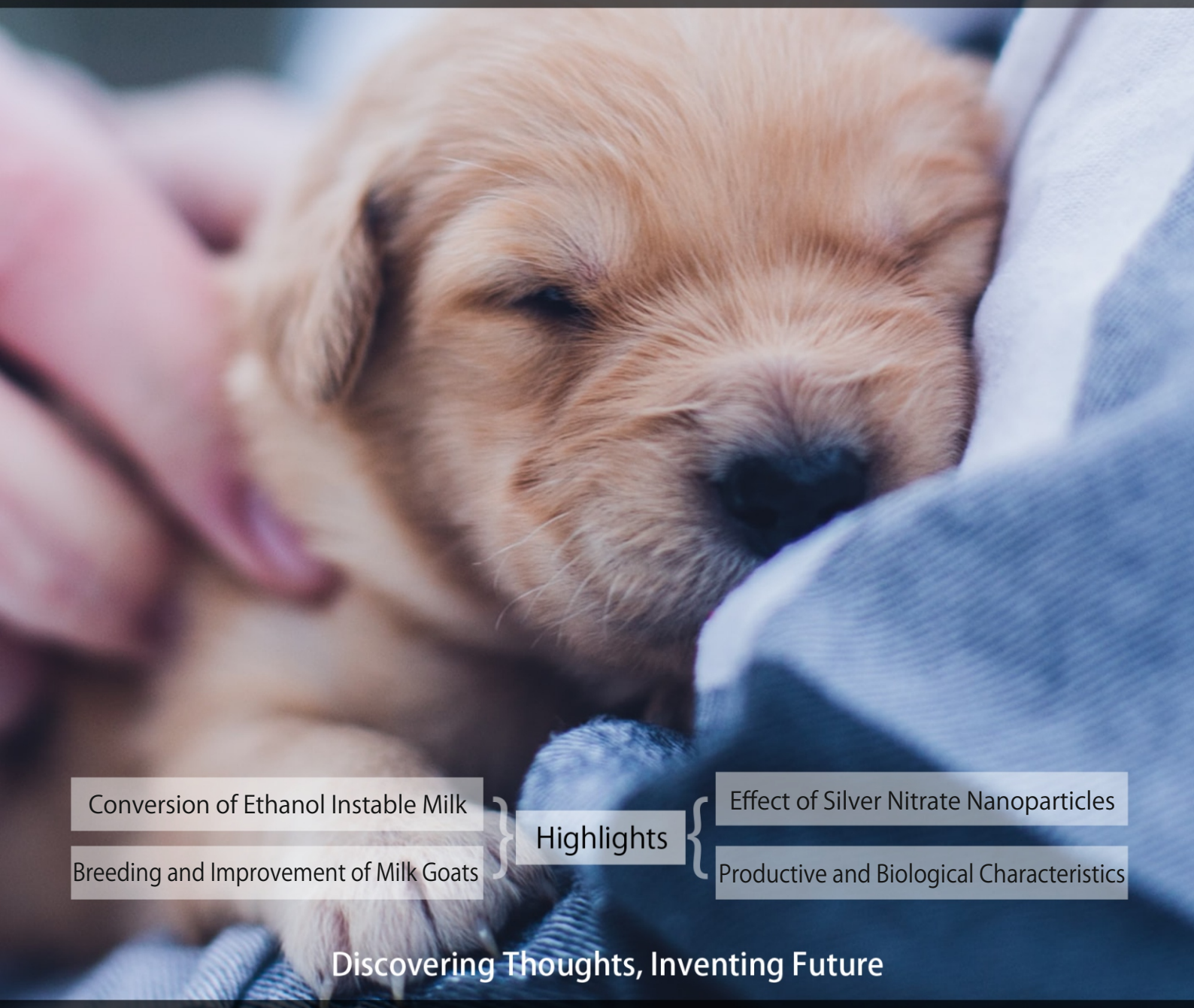

Highlights

- Conversion of Ethanol Instable Milk
- Breeding and Improvement of Milk Goats
- Effect of Silver Nitrate Nanoparticles
- Productive and Biological Characteristics

Discovering Thoughts, Inventing Future

VOLUME 20 ISSUE 4 VERSION 1.0

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Enhancing Effect of Silver Nitrate Nanoparticles as an Adjuvant in Formulation of Polyvalent Foot and Mouth Disease Vaccine

By Hind M. Daoud, Sonia A. Rizk & Nermeen G. Shafik

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Enhancing Effect of Silver Nitrate Nanoparticles as an Adjuvant in Formulation of Polyvalent Foot and Mouth Disease Vaccine

Hind M. Daoud [α], Sonia A. Rizk [σ] & Nermeen G. Shafik [ρ]

Abstract- Vaccination of susceptible animals against foot-and-mouth disease (FMD) is a well-established strategy to combat the disease. The protective immune response induced by vaccines can vary according to the kinds of adjuvants. The advance in nanotechnology has enabled us to utilize particles in the Nano size. So using novel immune adjuvants has an auxiliary role in the amplification of immune responses. Many investigators agree the size of the adjuvant particles is crucial to their adjuvant activities. The main aim of this study is to evaluate the effect of Silver nitrate nanoparticles (AgNPs) 5-10 nm particle size as an adjuvant in the polyvalent foot and mouth disease vaccine (containing FMD viruses O / PanAsia2, A/Iran 05, SAT2/VII/Lib-12 (SAT2/ Lib) and SAT2/VII/Ghb-12(SAT2/Ghb). A comprehensive immunological study was conducted in three calve groups vaccinated subcutaneously with three formulae of polyvalent FMD where group (A) was vaccine formula with AgNPs adjuvant, group (B) the vaccine formula adjuvanted with both MontanidISA 206 oil and AgNPs, while group (C) the vaccine formula with MontanidISA 206 oil adjuvant. A forth calve group kept without vaccination as control. The humeral and cellular immune responses were monitored in all calve groups. The obtained results indicated that incorporating of AgNPs inactivated FMD vaccine induces an increase of the specific protective immune response. The higher level of immune responses found in calves are vaccinated with both oil and AgNPs adjuvanted vaccine up to 40 weeks. In contrast, with AgNPs and with oil vaccine showed protected immunity up to 32 and 36 weeks, respectively. So it could be recommended to use both oil and AgNPs as an adjuvant to polyvalent FMD vaccine to provide adequate long-lasting immunity in vaccinated calves.

I. Introduction

Foot-and-Mouth Disease Virus (FMDV) is the pathological agent of the most important diseases that affect cloven-hoofed livestock. It is a small, non-enveloped single-stranded, positive-sense RNA virus related to the family Picornaviridae. FMD has seven serotypes: O, A, C, Asia 1, and Southern African Territories (SAT) 1, 2 and 3, they cause a highly contagious disease *(Alexandersen et al., 2003).* There are over 60 subtypes within these serotypes. For that, there are no universal vaccines, thus presenting challenges in the selection of vaccine strains *(Brown, 2003 and Arzt et al., 2011).* Infection with FMDV leads to an acute disease that spreads very rapidly. It characterized by fever, lameness, and vesicular lesions on the feet, tongue, snout, and teats, also characterized by high morbidity but low mortality *(Grubman and Baxt, 2004).* Although vaccines extensively used to control FMD, there was no antiviral therapy to treat ongoing infections with FMD virus *(Grubman, 2005).*

The most effective FMD vaccines are consist of chemically inactivated FMDV. They can only offer complete protection after seven days of vaccination because of the time needed to trigger an immune response *(Pacheco et al., 2015 and Zhang et al., 2015).*

As oil as an adjuvants is absorbed more slowly than its gel equivalent, also can cause local reaction in the site of vaccination. To prevent such effect, can use other adjuvant types than the oil, such as nanoparticles *(Batista et al., 2010).* Recently nanoparticles and micro carriers are used in vaccine delivery to enhance the cellular and humeral immunity through an increased presentation of vaccine epitopes to the antigen-presenting cell *(Singh et al., 2010).* The particles in the nanometer size range are of particular interest may be due to their unique cellular uptake and bio-distribution properties. They also play an important role when using as vaccine antigen carriers and adjuvants *(Perni et al., 2014).*

Silver nanoparticles (AgNPs) have attracted significant interest among the emerging Nano products because of their unique properties and increasing use for various applications in nanomedicine *(Gurunathan et al., 2009).* The adjuvanticity effect of AgNPs on rabies vaccine potency shown for the first time, and the results clearly showed the effect of AgNPs on increasing the humoral response to the rabies vaccine *(Vahid et al., 2016).* The immunological adjuvant effect of AgNPs investigated both in vitro and in vivo. The in vivo adjuvant effect of AgNPs evaluated with model antigen ovalbumin (OVA) and bovine serum albumin (BSA) in mice by intraperitoneal and subcutaneous immunization and the results showed the remarkable adjuvant effect of AgNPs. The result is beneficial for the future applications, especially in biomedicine *(Xu et al., 2013).*

This study was carried out to determine the adjuvant effects of Silver nitrate nanoparticles when used as an adjuvant to improve the polyvalent FMD vaccine on the immune response of calves.

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II. Material and Methods

1. Cell culture

Cell line of Baby Hamster Kidney (BHK21) clone 13 was maintained in the Department of Foot and Mouth Disease Vaccine Research (DFMDVR), Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo, according to the technique described by *Macpherson and Stocher (1962),* used for virus propagation and application of serum neutralization test. Using Eagle's medium with 8-10% sterile new-born calf serum obtained from Sigma, USA.

2. Virus propagation and concentration

FMD viruses O / PanAsia2, A/Iran 05, SAT2/VII/Lib-12 (SAT2/ Lib) and SAT2/VII/Ghb-12(SAT2/Ghb) are locally isolated strains of cattle origin. The viruses were typed at VSVRI and confirmed by Pirbright, International Reference Laboratories, United Kingdom and propagated on BHK cells then concentrated using polyethylene glycol 6000 (PEG-6000) according to *Killington et al.(1996) and Hiam and Eman (2010).* The viral suspension was concentrated at 25,000 rpm for 5 hours at 4℃ in a high-speed centrifuge (Avanti J25, Beckman Coulter, and Fullerton, CA, USA). The virus in the bottom was removed and polled. It was further concentrated in an ultracentrifuge at 35,000 rpm /min for 3 hours at 4℃. The viral pelted was polled and preserved at -80℃ to be used in vaccine preparation. Virus concentrations provide virus titers of 10^9; 10^9; $10^{8.5}$, and $10^9 TCID_{50}$/ml for O/PanAsia2, A/Iran 05, SAT2/VII/Lib-12 (SAT2/ Lib) and SAT2/VII/Ghb-12(SAT2/Ghb) respectively.

3. FMD viruses inactivation

Complete inactivation 0f the concentrated virus stock using Binary Ethyleneimine (BEI) according to *Bahnemann (1975) and Ismail et al.(2013).* 1%M BEI in 0.2N NaOH was added to the virus suspension to give a final concentration of 0.001M of BEI. Mixed well the virus and BEI mixture, and the pH then adjusted to 8.0 by sodium bicarbonate. Incubation of the mixture at 37°C for 12 hours.

Sodium thiosulphate added to give a final concentration of 2% to neutralize the BEI action. The inactivated viruses used in the preparation of vaccine formulation with AgNPs, ISA 206 oil, and AgNPs with Montanide ISA 206oil adjuvants for animal immunization.

4. Silver nanoparticles (AgNPs) characterization

Sample of Silver nanoparticles (AgNPs) was prepared as 0.001M/ 10mls and subjected to continuous stirring for 6 hours at room temperature, followed by sonication for three times repeated cycles each of 15 minutes according to *Udapudi et al.(2012).*

5. Measuring of Silver nanoparticles (AgNPs) size with Transmission Electron Microscopy (TEM)

For transmission electron microscopy of the samples of Silver nanoparticles, prepared by dispersing in ultrapure H_2O at about 10% concentration and ultra-sonicated at 1000L for 15 minutes. One drop of this liquid immediately transferred by a micropipette to a 3 mm diameter Formvar coated copper TEM grid, slowly evaporated to dryness. The samples on the TEM grid analyzed using a 100cx JEOL TEM at 80 kV at CURP, Giza, Egypt.

6. Silver nanoparticles (AgNPs) cytotoxicity

Baby Hamster Kidney cell line used to investigate the adjuvant inhibitory adverse effect on cell proliferation as an indicator of safety to use Silver nanoparticles (AgNPs) as a biocompatible adjuvant in the vaccine formulation.

7. Montanide ISA 206

The mineral oil-based adjuvant from water-in oil-in-water (double emulsion) mixed with antigen w/w supplied by Seppic, Paris, France.

8. Preparation of polyvalent FMD formulae:

8.1 FMD Silver nanoparticles (AgNPs) adjuvanted vaccine

Silver nanoparticles (AgNPs) were used as 0.1 mg/dose of the polyvalent inactivated FMD virus suspension, according to *Vahid et al.(2016).*

8.2 FMD oil adjuvanted vaccine

Formulation with oil phase carried out according to the method described by *Barnett et al. (2003),* and *Wael et al. (2014)* where the oil phase consisted of Montnide ISA 206 mixed with the inactivated viruses as equal parts of an aqueous and oil phase (w/ w) and mixed thoroughly.

8.3 FMD oil and Silver nanoparticles (AgNPs) adjuvanted vaccine

The inactivated viruses adjuvanted with ISA 206 oil (w/ w), and AgNPs in a concentration of0.1 mg/dose.

9. Animalgroups

Twelve local breed healthy calves and free from antibodies against FMD viruses as proved by using SNT and ELISA were used in this study where they were divided into four groups (3calves/group) as follow:

Group(A): vaccinated with the inactivated polyvalent FMD vaccine adjuvanted with AgNPs vaccine.

Group(B): vaccinated with the inactivated polyvalent FMD vaccine adjuvanted with both oil and AgNPs vaccine.

Group (C): vaccinated with the inactivated polyvalent FMD vaccine adjuvanted with oil adjuvant vaccine.

Group (D): was kept none vaccinated as a control group.

All vaccinated animals received 3ml/animal of the used vaccine formula inoculated subcutaneously.

10. Sampling

Blood samples were collected from all calf's groups on an anticoagulant for evaluation of cell

mediated immunity using Lymphocyte blastogenesis assay on the 3rd day post-vaccination, then every week up to 10 weeks.

Serum samples were collected for the serological tests (SNT and ELISA), weekly for one month then every 2 weeks up to 40 weeks post-vaccination and stored at -20°C until used.

11. In Vitro evaluation of cell-mediated immunity using lymphocyte proliferation (XTT) assay

Cell growth and lymphocyte proliferation determined by the colorimetric tetrazolium-derived XTT (sodium 3′-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis(4-methoxy-6- nitro) benzene sulfonic acid hydrate) assay (Roche Applied Science, Mannheim, Germany) according to *Sulic et al. (2005).*

12. Serum neutralization test (SNT)

The test was performed by the micro titer technique as described by *Ferreira (1976),* and the antibody titer expressed as serum neutralization log10.

13. Indirect Enzyme-linked immunosrobent assay (ELISA)

It was carried out according to the method described by *Voller et al. (1976)* and *OIE (2012).* Serum samples were examined for FMD viral specific IgG antibodies using in-house developed ELISA assay.

III. Results

a) Confirmation of complete virus inactivation

Complete viral inactivation checked by inoculation of BHK cells incubated for two days and compared to the virus-infected cell (virus control) and normal cell (cell control). The inactivated virus showed monolayer of BHK cells and positive control showed viral cytopathic effect at 24-hour post-infection. Complete virus inactivation was obtained by 16hours for O / PanAsia2, A/Iran 05, SAT2/VII/Lib-12 (SAT2/ Lib), and SAT2/VII/Ghb-12(SAT2/Ghb) respectively.

b) Measurement of Silver nanoparticles (AgNPs) size

Transmission Electron Microscopy (TEM) showed the particle size of the Silver nanoparticles (AgNPs) adjuvant of 5-10 nm as shown in the photo (1)

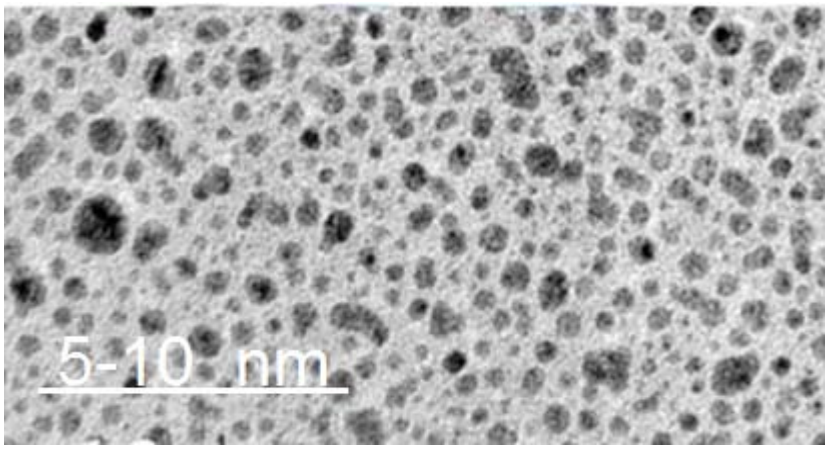

Photo (1): TEM micrograph of silver nanoparticles

c) Testing of adjuvant cytotoxicity

The effect of Silver nanoparticles (AgNPs) adjuvant on the in vitro cell proliferation investigated in BHK cell line monolayers after its exposure to gradient concentrations of Silver nanoparticles (AgNPs) for 48 hours. The percentage of viable cells among all of the preparations was above 50% indicating the safety of Silver nanoparticles (AgNPs) adjuvant.

d) In vitro evaluation of cell-mediated immunity using lymphocyte proliferation (XTT) assay

The obtained results of cell mediated immune response using lymphocyte proliferation test for all animal groups expressed by ΔOD (Delta Optical Density) were as follow:

Group(A) showed ΔOD (0.521) by using FMD viruses at 3rd- day post-vaccination and reached its highest level (1.572) at 3rd- week post-vaccination, then declined after nine weeks post-vaccination.

Group(B) showed ΔOD (0.566) by using FMD viruses at 3rd- day post-vaccination and reached its highest level (1.660) at 3rd-week post-vaccination then declined after ten weeks.

Group (C) showed ΔOD (0.486) by using FMD viruses at 3rd-day post-vaccination and reached its highest level (0.973) at 3rd- week post-vaccination, then declined after seven weeks. These results are demonstrated in a *table (1) and fig (1).*

Table (1): Comparative delta optical density of the cell-mediated immune response of calves vaccinated with the prepared FMD polyvalent vaccine formulae using (XTT) assay

Time Post-vaccination	ΔOD in buffy coat in vaccinated calves			
	Group (A)	Group (B)	Group (C)	Group (D)
Pre-vaccination	0.056	0.042	0.046	0.052
3rd day	0. 521	0. 566	0. 486	0.066
1week	0.863	0.872	0.495	0.054
2 week	1.450	1.633	0.971	0.073
3 week	1.572	1.660	0.973	0.069
4 week	1.265	1.476	0.731	0.055
5 week	0.862	0.932	0.685	0.073
6 week	0.674	0.843	0.642	0.079
7 week	0.621	0.823	0.502	0.054
8 week	0.565	0.753	0.462	0.063
9 week	0.532	0.715	0.374	0.067
10 week	0.404	0.628	0.336	0.056

Group (A)= AgNPs vaccine Group (B)= oil and AgNPs vaccine. Group (C)= oil adjuvant vaccine. Group (D)=control group.

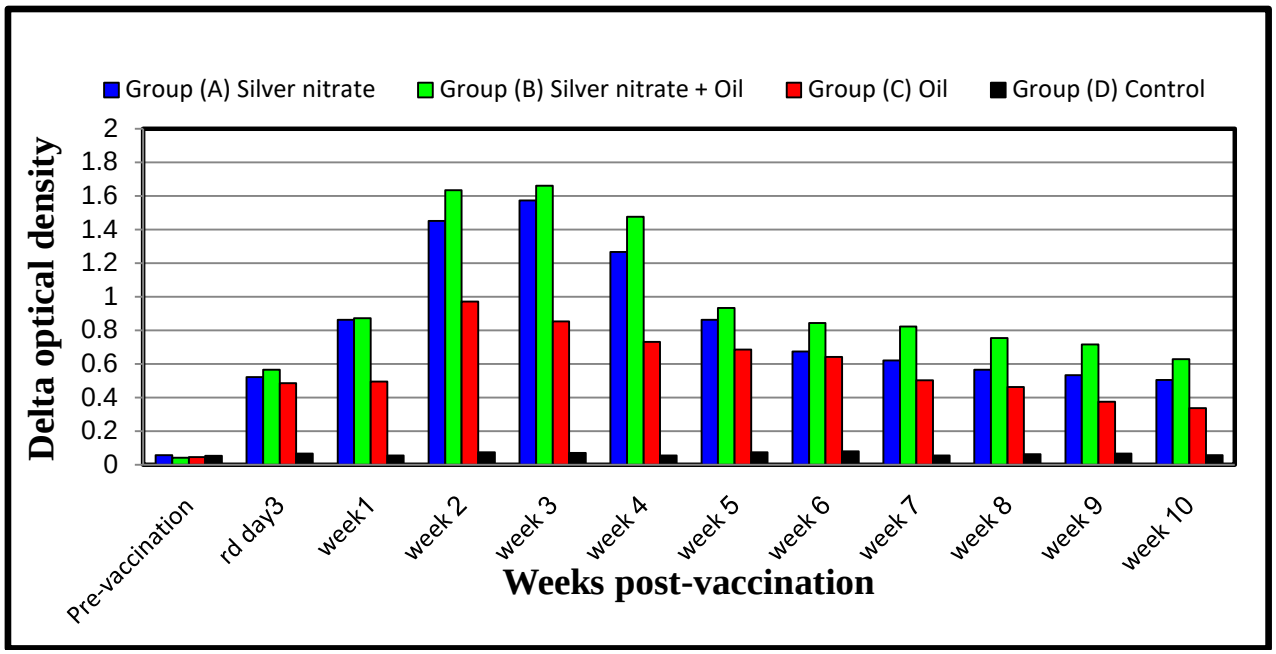

Fig. (1): ΔOD in buffy coat in vaccinated calves

e) *Evaluation of the humeral immune response of calves vaccinated with the prepared FMD vaccine formulae using SNT*

Table (2) showed that the application of SNT revealed that protective neutralizing serum antibody titer for Silver nitrate AgNPs only started at the 1st-week post-vaccination with average antibody titers of 1.65,1.5,1.5 and 1.5 $\log_{10}$ for type O,A, SAT2/ Liband SAT2/Ghb respectively. Such antibodies reached their peak level at 10th -week post-vaccination with average titers of 3.0, 3.0, 2.85 and 2.7 $\log_{10}$ for the four types respectively and continued as protective levels till the 32 weeks then declined. The protective neutralizing serum antibody titers induced by Montanide ISA 206 oil and AgNPs started at the 2nd-week post-vaccination with average antibody titers of 1.95,1.8,1.8 and 1.7 $\log_{10}$ for type O, A, SAT2/ Liband SAT2/Ghb respectively recording their peak level at the 10th-weeks post-vaccination with average titers of 3.0, 3.0, 2.85 and 2.85 $\log_{10}$ respectively and continued with protective level till the 40 weeks. FMD serum neutralizing antibody titers induced by Montanide ISA 206 oil started at the 2nd- week post-

vaccination with average values of 1.65, 1.5, 1.5 and 1.5 log_{10} for type O, A, SAT2/ Lib and SAT2/Ghb respectively reaching their peak level at 10th-weeks post-vaccination with average titers of 2.8, 2.7, 2.7 and 2.5log_{10} and continued with protective level till 36 weeks then declined as shown in fig (2, 3 & 4).

Table (2): FMD serum neutralizing antibody titers in calves vaccinated with inactivated FMD polyvalent

Time post-vaccination	FMD serum neutralizingantibody titer (log10) in vaccinated calve groups												
	Group (A)				Group (B)				Group (C)				group D
	O	A	SAT2 /lib	SAT2 /Ghb	O	A	SAT2 / lib	SAT2/ Ghb	O	A	SAT2 /lib	SAT2/ Ghb	
0	0.15	0.15	0.3	0.3	0.15	0.15	0.3	0.3	0	0.3	0.3	0.15	0.15
1 week	1.65	1.5	1.5	1.5	1. 4	1.35	1.35	1.2	1.2	1.2	1.05	0.9	0.15
2week	1.8	1.8	1.7	1.7	1.95	1.8	1.8	1.7	1.65	1.5	1.5	1.5	0.15
3 week	2.4	2.25	2.25	2.1	2.4	2.4	2.25	2.25	1.95	1.95	1.8	1.8	0.15
4 week	2.55	2.55	2.4	2.25	2.55	2.4	2.25	2.1	2.1	2.2	2.1	2.1	0.45
6 week	2.8	2.7	2. 5	2.5	2.7	2.7	2.55	2.5	2.4	2.25	2.1	2.1	0.45
8 week	2.85	2.85	2.7	2.55	2.85	2.7	2.7	2.5	2.4	2.4	2.4	2.25	0.45
10 week	3.0	3.0	2.85	2.7	3.0	3.0	2.85	2.85	2.8	2.7	2.7	2.5	0.45
12 week	2.85	2.7	2.5	2.4	2.85	2.7	2.5	2.4	2.4	2.4	2.4	2.25	0.3
14 week	2.7	2.55	2.4	2.25	2.7	2.5	2.4	2.4	2.25	2.1	2.1	2.1	0.3
16 week	2.5	2.55	2.4	2.1	2.5	2.4	2.2	2.25	2.25	2.1	2.1	2.1	0.3
20 week	2.5	2.4	2.25	1.95	2.4	2.4	2.1	2.1	1.95	1.95	1.8	1.8	0.3
24 week	2.25	2.1	1.8	1.65	2.4	2.25	2.1	2.1	1.95	1.8	1.8	1.8	0.3
28 week	1.95	1.8	1.65	1.5	2.25	2.1	1.95	1.95	1.8	1.8	1.7	1.7	0.15
32 week	1.65	1.5	1.5	1.4	1.95	1.8	1.8	1.65	1.65	1.65	1.5	1.5	0.15
36 week	1.4	1.35	1.35	1.2	1.8	1.65	1.65	1.5	1.65	1.5	1.4	1.5	0.15
40 week	1.2	1.2	1.05	0.9	1.5	1.5	1.5	1.4	1.35	1.35	1.2	1.2	0.15

Group (A) =AgNPs vaccine *Group (B)* =oil and AgNPs vaccine. *Group (C)* =oil vaccine.
Group (D) =control group.

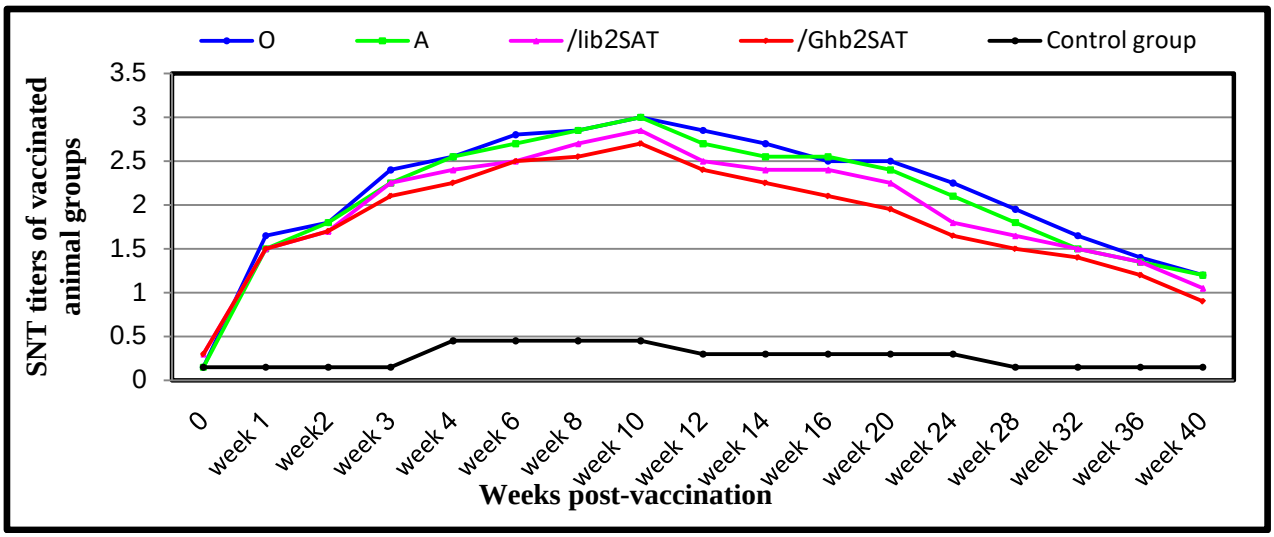

Fig. (2): SNT titer of calve group (A) vaccinated with Silver nitrate FMD vaccine

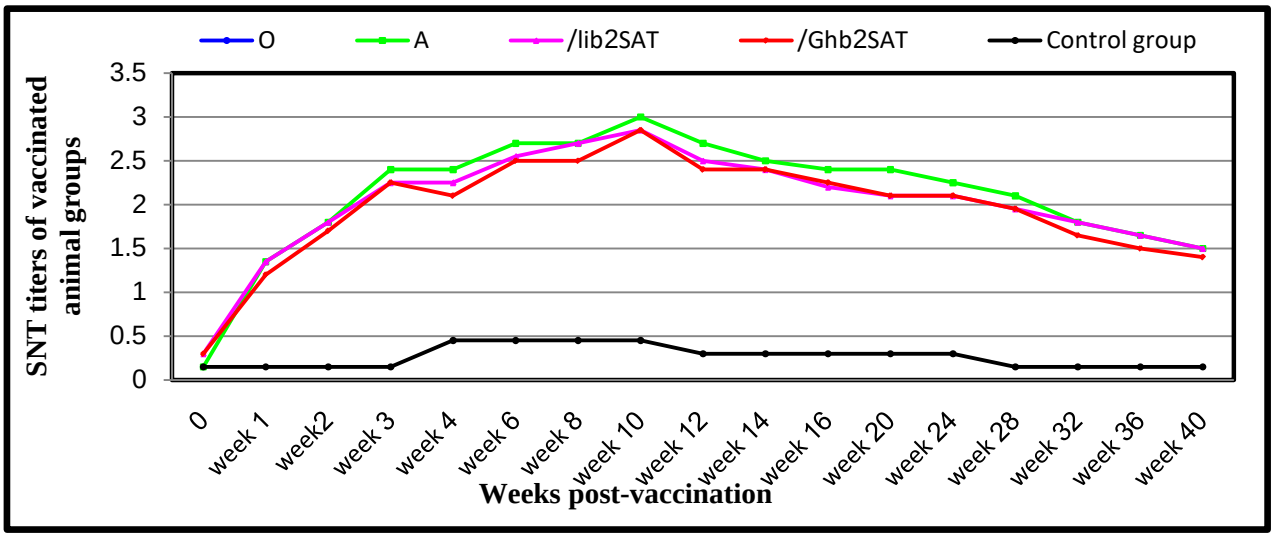

Fig. (3): SNT titer of calve group (B)vaccinated with Silver nitrate and Montaind ISA 206 oil

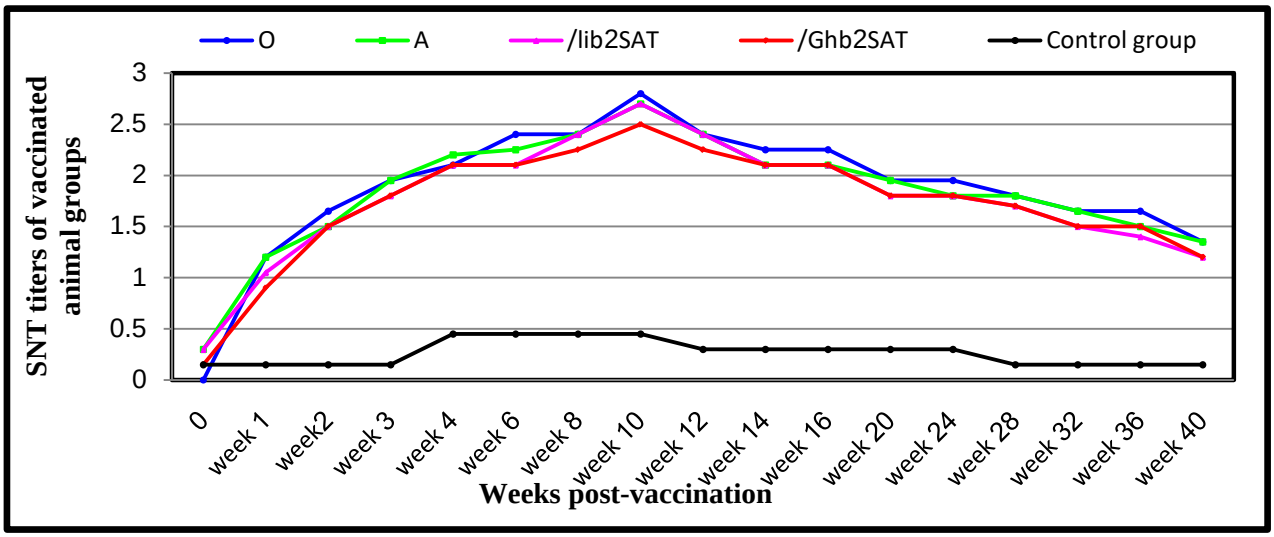

Fig. (4): SNT titer of calve group (C)vaccinated with Montaind ISA 206 oil

f) Evaluation of the humeral immune response of calves vaccinated with the prepared FMD vaccine formulae using ELISA

From table (3)it is clear that protective FMD-ELISA antibody titers induced by AgNPs only started at the 1st week post-vaccination with average values of 1.95, 1.93, 1.82 and 1.82 $\log_{10}$ for type O, A, SAT2/ Lib and SAT2/Ghb respectively reaching their peak level at the 10th week post-vaccination with average titers of 3.17, 3.17, 3.14 and 3.14 $\log_{10}$ for type O, A, SAT2/ Lib and SAT2/Ghb and remained with protective level till the 32 week then declined

The protective FMD ELISA antibody titers induced by AgNPs and Montaind ISA 206 oil started at the 2nd week post-vaccination with average values of 1.98, 1.97, 1.95 and 1.93 $\log_{10}$for type O, A, SAT2/ Lib and SAT2/Ghb recording their peak level at the 10th week post-vaccination with average values of 3.37, 3.32, 3.25 and 3.25 log10 respectively and continued with protective level till the 40 weeks then declined.

Montaind ISA 206 oil vaccine induced FMD ELISA antibody titers started at the 2nd week post-vaccination with average values of 1.93, 1.97, 1.96 and 1.96 $\log_{10}$ for type O, A, SAT2/ Lib and SAT2/Ghb respectively with peak levels at the10th week post-vaccination with average titers of 3.16, 3.06, 3.14 and 3.12 log10 respectively continued with protective level till the 36th week then declined as shown in fig (5, 6 & 7).

Table (3): FMD ELISA antibody titer in vaccinated calves with the prepared polyvalent FMD vaccine formulae

FMD ELISA antibody titer (log10) of vaccinated calve groups													
Time post-vaccination	Group (A)				Group (B)				Group (C)				Group D
	O	A	SAT2 /lib	SAT2/Ghb	O	A	SAT2/ lib	SAT2/ Ghb	O	A	SAT2 /lib	SAT2/Ghb	
0	0.21	0.18	0.18	0.27	0.26	0.24	0.22	0.25	0.12	0.28	0.19	0.3	0.6
1 week	1.95	1.93	1.82	1.82	1.77	1.73	1.68	1.66	1.55	1.55	1.50	1.50	0.6
2week	2.18	2.18	2.14	2.14	1.98	1.97	1.95	1.93	1.93	1.97	1.96	1.96	0.6
3 week	2.55	2.52	2.54	2.50	2.72	2.70	2.62	2.60	2.19	2.19	2.15	2.13	0.6
4 week	2.75	2.70	2.68	2.64	2.85	2,85	2.78	2.76	2.42	2.43	2.40	2.42	0.6
6 week	2.87	2.80	2.78	2.70	2.90	2.88	2.88	2.78	2.49	2.48	2.46	2.43	0.75
8 week	2.96	2.88	2.80	2.78	2.94	2.98	2.97	2.90	2.86	2.75	2.74	2.73	0.75
10 week	3.17	3.17	3.14	3.14	3.37	3.32	3.25	3.25	3.16	3.06	3.14	3.12	0.75
12 week	3.19	3.17	3.16	3.14	3.25	3.25	3.18	3.18	2.9 8	2.95	2.92	2.93	0.9
14 week	2.86	2.82	2,78	2.76	2.97	2.95	2.84	2.80	2.58	2.57	2.54	2.55	0.9
16 week	2.74	2.74	2.68	2.73	2.88	2.87	2.84	2.80	2.48	2.45	2.41	2.43	0.9
20 week	2.52	2.48	2.42	2.37	2.68	2.62	2.63	2.63	2.38	2.36	2.32	2.28	0.6
24 week	2.35	2.29	2.28	2.27	2.49	2.46	2.44	2.46	2.17	2.14	2.14	2.10	0.6
28 week	2.15	2.12	2.12	2.10	2.28	2.28	2.25	2.25	1.96	1.93	1.93	1.90	0.6
32 week	1.94	1.90	1.85	1.84	2.18	2.16	2.15	2.10	1.92	1.90	1.87	1.88	0.3
36 week	1.73	1.73	1.68	1.62	1.98	1.96	1.96	1.92	1.47	1.42	1.42	1.40	0.3
40 week	1.64	1.63	1.52	1.52	1.92	195	1.93	1.90	1.42	1.38	1.35	1.35	0.3

Group (A) =AgNPs vaccine *Group (B)* =oil and AgNPs vaccine. *Group (C)* =oil vaccine.
Group (D) =control group

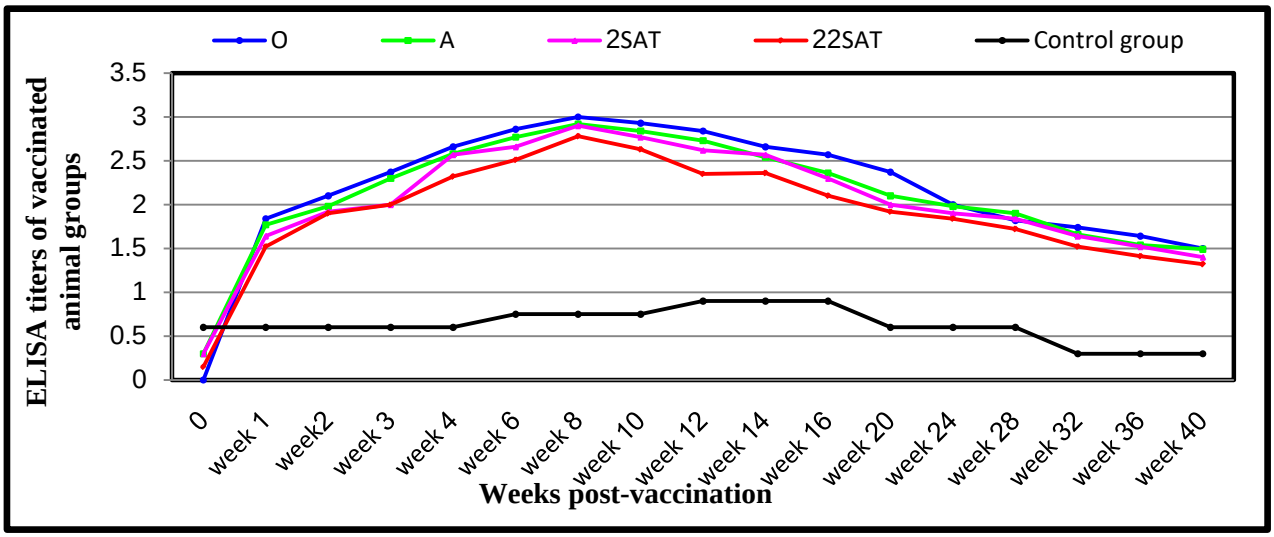

Fig. (5): FMD ELISA antibody titer of calve group (A) vaccinated with Silver nitrate vaccine

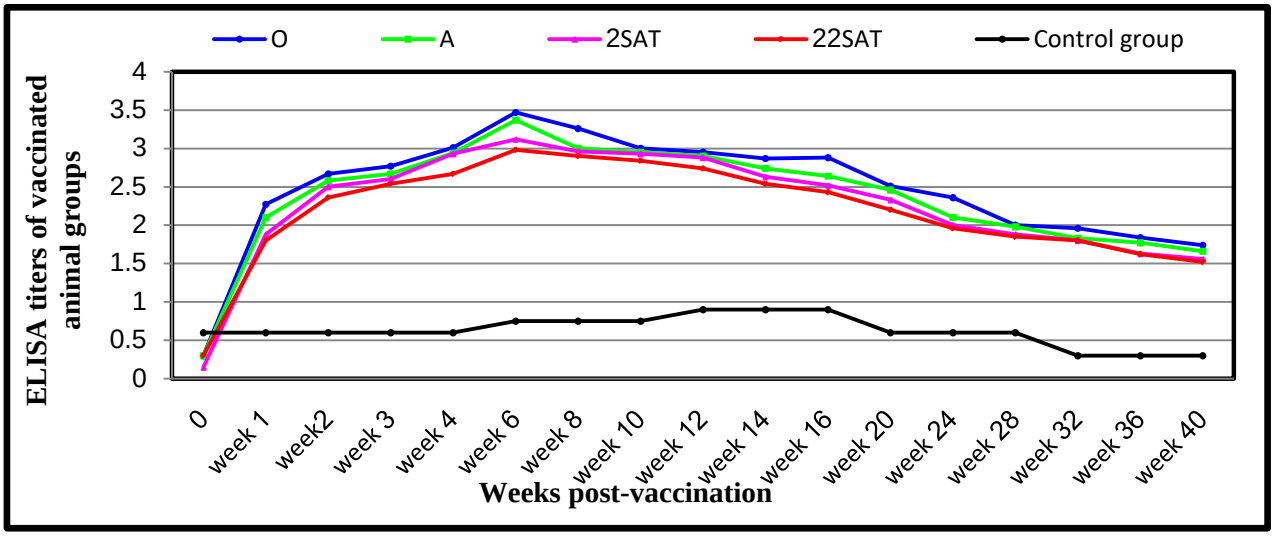

Fig. (6): FMD ELISA antibody titer of calve group (B) vaccinated with Silver nitrate and Montanid ISA 206 oil Vaccine

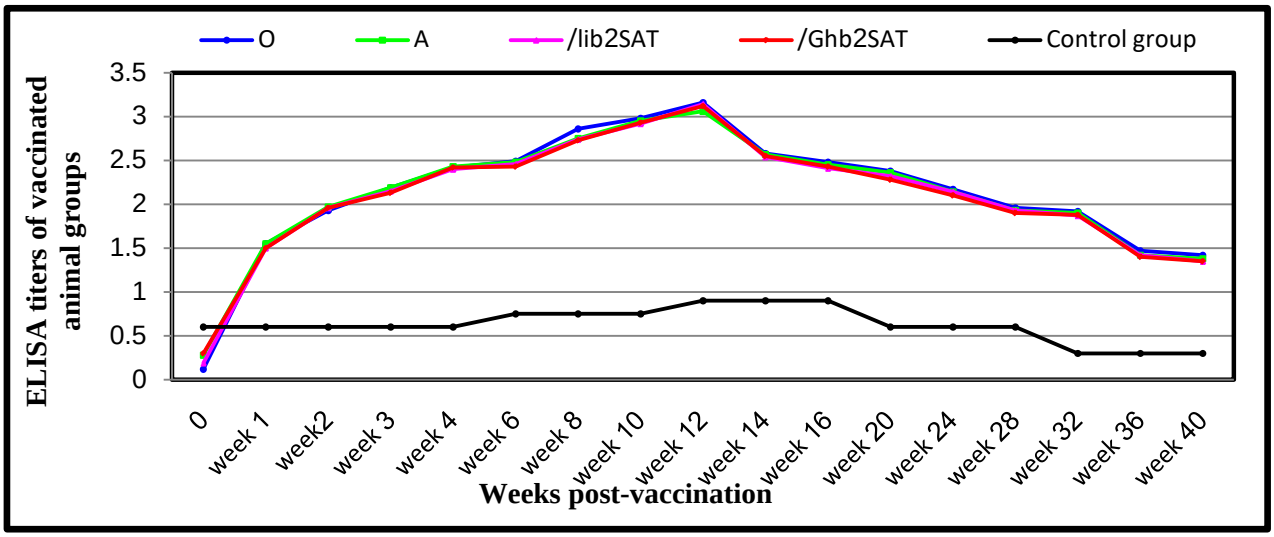

Fig. (7): FMD ELISA antibody titer of calve group (C) vaccinated with Montanid ISA 206 oil Vaccine

IV. Discssion

Nanoparticle-containing vaccines have attracted tremendous interest in recent years, and a wide variety of nanoparticles have been developed and employed as delivery vehicles or immune potentiates, allowing an improvement of antigen stability but also the enhancement of antigen processing and immunogenicity *(Smith et al., 2015).*

The control of FMD in animals was considered to be important to effectively contain the disease in endemic areas so that vaccination of animals is effective in limiting the spread of FMD. So, this study aimed to improve the inactivated polyvalent FMD vaccine by adding Silver nitrate nanoparticles as an adjuvant.

Table (1) showed that the results of cell-mediated immune response using lymphocyte proliferation test for all animal groups expressed by ΔOD appeared to be supported by *Knudsen et al., (1979) and Sharma et al., (1984)* who reported that cell-mediated immune response was a constitute of the immune response against FMD virus, and agreement-in some points with *Mercedes et al.(1996); El-Watanyet al.,(1999); Mansour (2001); Samir (2002); Hiam et al.(2010) and Wael et al.,(2014)* who found that FMD vaccine stimulated the cellular immune response and lymphocyte stimulation by FMDV were greater than that by mitogens (PHA)with the highest increase in the 1st and 2nd weeks post-vaccination, while the present findings disagreed with *El-Watany et al., (1999); Mansour (2001)* and *Sonia et al.(2010)* who found that cell-mediated immune response reached its highest level on the 14th day. Also our results came in agreement with those of *Shin et al. (2007) and Frial et al. (2018)* who stated that AgNPs act as an activator of the TH1 response. The Th1 type is characterized by the production of antigen-specific IgG2a a Th1 and the secretion of gamma interferon, interleukins which favor cellular immunity. Also these results were in agreement with those of *Lázaro et al. (2017),* who stated that

metallic nanoparticles facilitate the induction of cellular immune response, particularly T-helper 1 and T-helper 17, and their potential functions as adjuvants for subunit vaccines. The obtained results also were supported by *Venier et al. (2007); Castellheim et al., (2009); Yen et al. (2009)* and *Wong et al. (2009),* who mentioned that AgNPs enhanced interleukins which enhance cell mediated immune response and nanoparticles are considered an efficient tool for inducing potent immune responses.

The tabulated results in tables (2 & 3) that SNT and ELISA titers for AgNPs, oil and AgNPs with oil FMD vaccine formulae agreed with *Vahid et al. (2016)* who showed that adjuvant properties of AgNPs as a potent adjuvant induced higher antibody and the protective function is the production of neutralizing antibodies, either IgM or IgG, which are able to prevent the entry of the virus into cells. The results are supported also by *Malyala and Singh (2010) and Rebecca et al. (2010),* who found that AgNPs might help the vaccine work more effectively, increasing antibody production, also agreed with *Gurunathan et al., (2009); Kaba et al. (2009); Zhao et al. (2014) and Daniel et al. (2019),* who found that AgNPs improved B-cells function, mucosal and humoral immunity and protective activity also helped vaccine for induction of strong immunity when used as an adjuvant. The results also go in hand with the results obtained by *Hamblin et al. (1986).* They explained that the SNT measures those antibodies which neutralize the infectivity of FMD virion, while ELISA probably measures all classes of antibodies even those produced against incomplete and non-infectious virus.

Depending on the present obtained results we could conclud that the usage of Silver nitrate nanoparticles (AgNPs) with Montanid ISA 206 oil in inactivated FMD trivalent vaccine induces long-lasting immunity than that induced by oil adjuvant alone and improve both cellular and humoral immunity resulted in earlier and more long-lasting immunity the thing which can aid in companying to control FMD.

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Conversion of Ethanol Unstable Milk to Stable One

By Saadath Pasha & Bala Krishna Rao Dabbir

Abstract- Milk ethanol stability is defined as the minimum concentration of added aqueous ethanol giving rise to milk coagulation. The milk, which shows a positive reaction to the alcohol test, is usually graded as second rate and diverted for butter making but not condensed milk. The ions calcium, potassium, and chlorides are mainly responsible for the alcohol instability of milk. As there is no rational method to convert ethanol sensitive-milk to ethanol-insensitive one, we attempted to test and remove this bad quality with two homeopathic remedies, *KaliMuririaticum* 200 and *Calcarea Phosphorica 12 x*. We gave these medicines for seven days to16 cows whose milk was sensitive to 70% alcohol. The results were dramatic and proved the high efficacy of the homeopathic system of medicine, and gave conclusive evidence that ions calcium and chlorides are responsible for milk instability. *Kali mur 200*(higher potency) removed the detrimental effects of potassium and chlorides and calcarean phos12 x (lower potency) fortified the alcohol stability of milk. Both the remedies acted synergistically.

Keywords: milk ethanol instability, kali muriaticum 200 and calcarea phosphorica 12 x, stability.

GJMR-G Classification: NLMC Code: WA 360

Strictly as per the compliance and regulations of:

Conversion of Ethanol Unstable Milk to Stable One

Saadath Pasha [α] & Bala Krishna Rao Dabbir [σ]

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I. Introduction

Milk ethanol stability is defined as the minimum concentration of added aqueous ethanol that gives rise to milk coagulation(1). The milk, which shows a positive reaction to the alcohol test, usually graded as second class, is used for butter making, not for condensed milk. The instability of milk to alcohol also generates technological problems in the manufacture of creamy liqueurs, such as 'neck plug' and reduced shelf life due to the instability of the emulsion(2). A Japanese study of low alcohol stability of bovine milk showed that the alcohol stability of bovine milk is related to the levels of Na and K in the milk and the blood of the cow (3).

Horne & Parker (4)(5) found that serum phase components, govern the ethanol stability/pH profile. They further confirmed that among serum phase components the ionic calcium concentration played an important role. Salts (calcium, magnesium, phosphorus and citrate) were reported to influence ethanol/pH profile parameters (6)(7).

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Horne, and Parker (8), added sodium chloride to a milk sample and observed that the ethanol stability of its concentrate was enhanced and also reported that if that stability is too low, it is increased to the desired level by reducing the chloride content in a short dialysis step before concentration.

Chavez et al. (9) examined the milk samples of good hygienic quality from dairy farms and classified into two groups according to their alcohol stability. Unstable ones to ethanol (72%, v/v) presented lower values of pH, somatic cells count, casein and non-fat-solids relative to stable ethanol samples (ethanol at 78%, v/v or more); whereas freezing point, chloride, sodium, and potassium concentrations were higher in the unstable group. Joubert & Meeske (10) observed that potassium content in the diet was responsible for ethanol stability in milk.

Factors related to the animals, such as extended lactation period (11) (12), affect milk stability. Feed restriction (13) (14), excess of fiber in the diet (15), or nutrient imbalance (11), and higher permeability of the tight junctions of epithelial mammary cells (16) was probably enrolled as a causal factor of the low milk stability. During the autumn and spring, stability defects were reported in some dairy farms with good milk bacteriological quality (17) with no known reason. Similar behavior was reported by Donnelly & Horne (6), who observed that a decrease in milk ethanol sensitivity occurred frequently during winter in Ireland.

Horne (18) proposed extension measures to minimize the sensitiveness of milk to alcohol. The first one is blending 'unstable' with 'stable' milk, the second one is the mixing milk from cows of early and late lactations. He further opined that if the instability was due to too high a salt balance ratio (SBR), this could be modified directly by the addition of sodium citrate. All his suggestions are not practicable and laborious, especially in the case of small farms.

Some of the options available are to administer calcium parenterally daily, supplementation of sodium chlorides orally or resins to hinder the absorption of potassium, or administration of potassium antagonists, but these are costly and not practicable.

We also initially believed that both Potassium and Chlorides were major ions responsible for milk ethanol instability but there is no agent that can alleviate the instability of milk. We hypothesized that there is an only possible alternative system based on the principle

of the Law of Similars, or cures like (The fundamental law of homeopathy) "A substance that can artificially produce specific disease-like symptoms on a healthy person; only that substance can cure a similar disease when given to the patient in the form of homeopathic medicine".

The homeopathic principle was applied on 16 cows ailing with chronic ethanol instability of milk, located in different farms under the milk shed extension area of Akshaya Kalpa Farms and Foods Private Ltd, Tiptur, Karnataka from July to September2020.

II. Material and Methods

Sixteen milk samples from cows showing sensitivity to alcohol were collected from 6 dairy farms located under the Akshaya Kalpa milk shed area from July-September 2020, and included in the study. Following the method of Huppertz and De Kruif (19), ethanol stability was determined by mixing 2 ml of sample (pH values between 7.0 and 2.0) with an equal volume of aqueous ethanol (0–100%, v/v, at 2.5% intervals) in a petri dish. Ethanol stability was determined by the visual coagulation of the sample at the lowest concentration of aqueous ethanol solution. The milk samples were collected in 100ml sterilized polyethylene bottles and preserved with 0.5 % formalin and were refrigerated. The milk samples were collected from alcohol unstable (before treatment) and stable (after homeopathic treatment) and were analyzed. Sixteen normal milk samples from collected the six selected dairy farms were analyzed. The ions Potassium, Sodium, and Chlorides were analyzed in Easylyte analyzer, manufactured by Medica Corporation, Bedford, USA. The homeopathic medicines Kali muriatic 200 and Calcarea Phos 12 X were procured from SBL, Delhi.

Table 1: Homeopathic treatment of milk ethanol instable cows.

Serial number	Treatment	Ionic analysis in ((mmol/L)		
		Sodium	Potassium	Chloride
1	Before treatment Mean	30.36875	35.46688	46.0375
2	After treatment Mean	30.36875	35.375625	39.1375
3	P(T<=t) one-tail	0.045822	0.001325	0.001440167
4	Control milk Mean	25.25	34.48	29.65
5	n	16	16	16

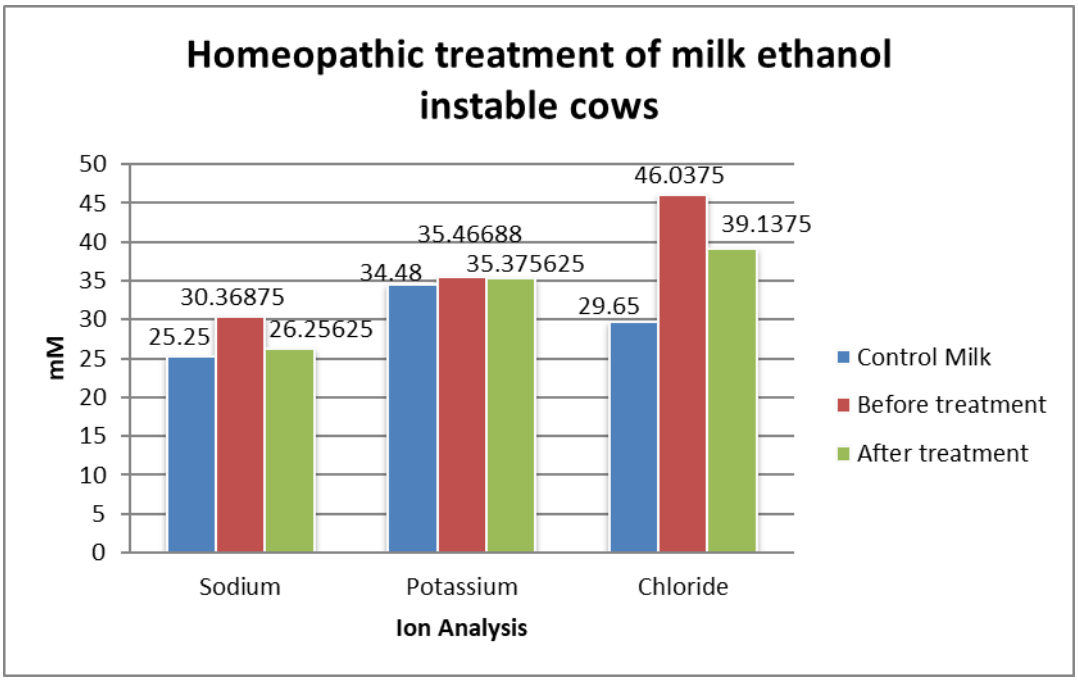

Figure 1: Homeopathic treatment details

Table 2: Ionic analysis of milk by other authors

Table 1	Milk	Ionic analysis (mmol/L)			Reference
		Sodium	Potassium	Chlorides	
1	Stable milk	19.58	38.14	40.89	9
2	Unstable	22.62	39.68	45.41	9
3	stable	23.55 / 5.26	38.50a / 4.26	26.94 / 4.44	22
4	unstable	24.03 / 4.95	43.42b / 6.15	33.62 / 5.35	22
5	Normal	24.2	34.7	30.2	21

Multi star (vitamin liquid), a brand product of Vet Mankind, India, was purchased from the local chemist. Ten ml of Kali muriatic 200 and 90 ml of Multistar were mixed thoroughly. Five ml of mixed liquid were dropped over the tongue in the morning and evening for 5 days and 10 pills of Calcarea Phosphorica12 x, were dropped over the tongue for another two to three days. The data were subjected to ANOVA.

III. Results and Observations

It was observed that all the 16cowswith milk ethanol instability became stable without any side effects. The regaining of stability was gradual after day 2, of the administration of KaliMur 200.It was further observed that there was no recurrence of instability even after 3 months. The ionic values of sodium, potassium, and chlorides of normal milk were lesser than those of affected and treated cows.

It was observed from the Table 1 that, there was no significant reduction of sodium and Potassium, but with regard to chlorides, there was a significant reduction at 1% level, after treatment of the day 7, in stable milk.

IV. Discussion

The milk of 16 cows became stable, dramatically endorsing the homeopathic theory. Administration is simple and the cost of treatment is economical. It was calculated as Rs 100/- Per course. Chlorides in the milk exists as salts of Calcium, Potassium, Sodium, and Magnesium in colloidal and acqueous forms.

In unstable milk there was significantly higher chloride content in unstable milk than in stable milk but there were no significant changes in Sodium and Potassium contents in both instable and stable milk after treatment.

The observations were in agreement with those of Chavez et al (9) Gaucheron(21) and Fagnani et al(22)(Table 2}There was no change in organoleptic character and secretion of milk. The dramatic shift milk from instability to stable within a week after administration of homeopathic-medicine, proved the hypothesis of homeopathy equivocally. The cost of treatment for seven days works out to, in Indian Rupees is 100/-. The results gave conclusive evidence that ions chlorides and calcium were the major ions responsible for alcohol instability than those of sodium and Potassium which are insignificant (higher potency) removed the detrimental effects of Potassium and Chlorides and CalcareaPhos12 x (lower potency) enriched the alcohol stability of milk.

V. Conclusion

We have converted alcohol unstable milk to stable one, after administration of two homeopathic remedies Kali muriatic 200 and Calcarea Phosphorica 12 x, for 7 days, given one after another and proved the efficacy of homeopathic medicines for overcoming the milk ethanol instability that has caused persistent concern since decades. More number of cows with their milk instability may be subjected to homeopathic treatment to get more confidence for the future adoption of the proposed extension strategy.

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Selection on Breeding and Improvement of Milk Goats

By Abdurasulov A.H., Salykov R.S., Madumarov A.K. & Muratova R.T.

Resume- The article includes materials on results of selection on breeding of Kyrgyz milk goats at several farms in different regions, characteristics on phenotypical peculiarities and productivity of breeds of different groups, as well as results of laboratory analyses of biochemical composition of goal milk.

Keywords: selection, cross breeding, milk goats, productivity, biochemical composition and quality of milk.

GJMR-G Classification: NLMC Code: WA 360

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Selection on Breeding and Improvement of Milk Goats

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I. Introduction

Owing to biological peculiarities, goats are well adapted to different natural-climatic, soil and fodder conditions. Animals of this species are extensively distributed throughout the whole global world.

At present dairy production is developed in many countries of the world. Large-scale farms, occupied with breeding of Saanen milk goats, are created in several regions of the RF.

More than 1 million hectares of natural pasture areas are available in Kyrgyzstan, difficult to reach, rocky, shrubby and covered with other low-yield grass, which can be used mainly by goats. Development of goat breeding in Kyrgyzstan is stipulated by a relief of the territory, natural climatic and ecological-geographical peculiarities and traditions of population, which since olden times used products of goats. Around the half of the republic territory is occupied with strong highly dissected mountainous ridges with available large massifs of natural alpine and subalpine pastures of different vertical zonality, which since old times contributed to formation here of the trans-humance grazing of the cattle.

However, in spite of extensive natural pasture areas, available in the republic and fit for specific natural and climatic conditions of management of aboriginal and stud breed of goats, potential capacities of the cheap products-producing industry are far from being realized at full scale.

Maintenance of milk goats does not require large expenses. A basic product – goat milk – is a valuable dietary and medical product, which is in great demand. A source of incomes is sale of young goats for breeding purposes and goats for meat.

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In the result of milk goats breeding production of high quality products of goat husbandry will be increased and incomes of population, private and other farms, occupied with milk goats breeding, will also be increased.

II. Material and Investigation Methods

Kyrgyz milk type of goats was bred by reproductive cross breeding of hybrids of the desirable type, manly of the II and III generation, obtained by cross breeding of local rough-haired goats with Saanen male goats.

Milk-yielding capacity of female goats were defined according to the method of Ya. I. Imigeyev et al. (1976).

All digital materials were processed by a method of variation statistics (Plokhinsky N.A., 1969).

Created goat herds were tested as a new selection achievement in cattle breeding – "Kyrgyz milk goats", approved by the Order of the Ministry of Agriculture, Water Resources and Processing Industry of the Kyrgyz Republic No. 296 as of November 10, 2005 (Patent No. 29 issued by "Kyrgyzpatent").

In subsequent years the work on increase of the livestock and improvement of the milk type of goats was continued by means of pure-breeding at "Jorobay" Breeding Farm of Karasu district, Osh region, and accumulation cross breeding at the goat-breeding farm of "Arstanbek" JSC,. Panfilov district, Chui region.

III. Investigation Results

After approbation and approval in 2005 of the Kyrgyz milk type of goats in the Kyrgyz Republic a dairy system of goat breeding together with basic regions – districts of Chui and Issyk-Kul regions – was developed also in many other regions of the republic. Because of export of the significant livestock of Kyrgyz milk goats, especially from districts of Chui region, the areal of breeding and the livestock of milk goats was changed in regions of the republic.

In connection with a large demand for milk goats and their high cost, a significant part of the livestock, especially from large private farms of Chui region, was sold and transported to districts of Batken, Jalal-Abad, Osh and other regions, as well as exported outside the republic – to Kazakhstan and Tadjikistan.

New farms of milk goats were arranged in several regions based on the pure-breeding of imported animals of Kyrgyz milk type or cross breeding of local coarse- haired and hybrid female goats with male goats of the Kyrgyz milk type and Saanen breeds.

Animals of the desirable type have a strong constitution, a proportional body build, an exterior without defects, well developed udder. The colour is mainly white and grey. Male and female goats are mainly hornless.

Male goats have large values of indices, characterizing the development of chest and skeleton frame. In comparison with male goats, female goats have long legs and a long body. Growth exceeding the quarters or the sacral bone over the shoulder is characteristic both for female and male goats, but there is no significant difference in the overgrowth index between them. Kyrgyz milk goats in comparison with local coarse-haired goats are characterized by a large height of sacral bone and shoulder, they have more extensive chest. The live weight of male goats is 60-70 kg, female goats – 44-48 kg, milk yield during 240-270 days of lactation makes up 450-500 kg with the fat content of 4-5%. The fertility makes up 170-180% and more.

Kyrgyz milk goats are bred at private farms, personal households and other farms in several districts of Chui and Issyk-Kul regions; they are also bread in other regions of the republic and outside.

There are 50 goats of Kyrgyz milk type in the herd of "Jorobay" Breeding Farm, including 28 female goats, 1 male stud goat, 2 rearing goat kids and young goats born in 2013 (Table 1).

Table 1: Number, age and gender composition and productivity of milk goats in the herd of "Jorobay" BF

Age and gender groups of female goats	Number	Including desirable type, %	Live weight, kg	Milk yield per 1 goat, l
Male stud goat	1	100,0	77,0	-
Rearing small goats	2	100,0	35,0	-
Grown up female goats	12	100,0	45-55	2,0-2,5
Female goats born in 2012	8	87,5	35-40	1,2-1,4
Goat kids born in 2013	27	70,0	25-30	-

Pure-breeding is carried out within several years in the herd of the farm, high productive stud male goats of Kyrgyz milk type are used in service. In the result the specific weight in the number of animals of the desirable type was increased up to 80%.

Live weight and milk yield data, indicated in the Table 1, correspond to the established requirements for the Kyrgyz milk type of goats. A number of goat kids per 100 grown up female goats is 160% per 100 female goats, including first lambers – 135%.

In the herd of the goat-breeding farm JSC "Arstanbek" accumulation cross breeding of local coarse-haired and hybrid female goats is carried out since 2008 with male goats of the Kyrgyz milk type. Within the elapsed years a number of hybrid goats was increased in the desirable milk type and productive properties of hybrid and desirable type of animals were studied.

As of November 1, 2013 there were 70 reproductive goats in the herd, including 45 grown up female goats and first lambers. Data on herd characteristics by gender and age composition and other indicators are given in Table 2.

Table 2: Characteristics of reproductive part of herd of the Kyrgyz milk type of goats at JSC "Arstanbek" by gender and age composition and phenotypical features

Groups of goats	Age	Goats, available in herd			Chest girt, cm	Live weight, kg
		Total, nos.	Specific weight, %			
			White colour	Hornless		
Stud goat	3 years	1	100,0	100,0	92,0	63,0
Rearing male goat	Up to 1 year	1	100,0	100,0	67,0	30,0
Female goats	Grown up	30	90,0	50,0	79,0-100,0	42,0-80,0
Female goats (first lambers)	1.5 years	15	60,0	40,0	72,0-86,0	34,5-52,5
Doe kids	Up to 1 year	23	100,0	48,0	48,0-62,0	12,0-23,0

Data, indicated in Table 2, show that a major part (around 87%) of grown up goats and youngsters are of while colour. A specific weight of hornless female goats makes up 50 %, first lambers – 40 % and doe kids born in 2013 – 48 %. In general, breeds of the desirable milk type make up 80%.

A number of youngsters per 100 female goats makes up 176 %, the average daily milk yield – 2.5 kg per 1 grown-up female goat, and 1.6 – 2.0 kg in two-tooth lambing. The chemical composition of milk is studied, the analysis of laboratory investigation data of biochemical composition of selected milk samples is indicated in Table 3.

Table 3: Indicators of milk biochemical composition in Kyrgyz milk type of goats (n = 6)

Indicators	Meas. unit	In average	Fluctuations	Local coarse-haired	Saanen
Content:					
moisture	%	87,24	87,08-87,85	83,9	87,61
dry substance	%	12,76	10,84-15,77	16,1	12,39
incl. – fat	%	4,08	3,00-4,60	3,6	4,30
protein	%	3,55	3,30-3,80	4,46	2,70
ash	%	0,83	0,77-0,89	0,79	0,70
lactose	%	4,30	3,43-6,88	5,31	4,62
nonfat milk solids	%	8,68	7,84-11,37	10,56	8,15
calcium	g/kg	0,65	0,57-0,71	-	-
phosphorus	g/kg	0,69	0,60-0,72	-	-
Density	units	27,8	25,0-30,0	-	-
Acidity	units	22,0	20,0-24,0	-	-

It is evident from the data of Table 3, that in average the content makes up: moisture – 87,24, dry substance – 12.76 %, including fat – 4.08 %, protein – 3.55 %, lactose – 4.3 %, ash – 0.83. Separate breeds have certain differences by value of the major part of indicators.

It should be pointed out that the major part of indicators of milk biochemical composition of the Kyrgyz milk goats (MG) differs from the local coarse-haired (LCH) and is approaching to indicators of Saanen goats.

IV. Discussion

Based on results of the laboratory analysis of the milk biochemical composition of Kyrgyz milk goats (KMG) it differs from that of local coarse-haired (LCH) and is approaching to indicators of Saanen goats.

"Maksat" Association was organized in Aksy district, Jalal-Abad region on the territory of Karajigachaiylokmotu (village council), which unites amateur goat-breeders, occupied with breeding of the Kyrgyz milk type of goats.

At present 13 members of the Association maintain from 2 to 5 milk goats, 45 goats in total, including 3 stud male goats, 25 female goats, 17 kids in the age up to 1 year. The productivity of female goats is as follows: live weight 30-45 kg, daily milk yield 2.6-3 l.

Almeyev I.A. (1973) points out that a valuable peculiarity of goats is their high ability to fattening. During the maintenance on high mountainous summer grazing (July-September) they not only restore losses in live weight, but also show significant body weight gains.

Rako A. (1987) reports that in France the average milk yield of 1 goat during the lactation period makes up 600-800 l, in separate animals - 1000 l and even 2000 l, a record is 3175 l. Goat's milk is used mainly for production of cheese.

According to data, obtained by Kumar R. et al. (1986), milk yielding capacity of goats of Bengal Djamnopory, Barbare, Saanen and its half-bred hybrids with Bengal breeds differs at double milking per day: the highest milk yield of Djamnopory goats was already within the first month of lactation (23.3 l/month), the remained goats had the highest milk yield within the second month (15.9 l/month), hybrids and Bengal goats - 8-9 l/month, the highest milk yield was in Saanen female goats (392 liters within 272 lactation days), very low milk yield was in Bengal goats (26 – 28 liters within 106 – 110 days).

Chawla D.S., Bhatnagar D.S. (1986) point out that the milk yielding capacity within the first 150 days of lactation in hybrid goats of Alpine and Saanen breeds with a local beetle breed indicate that these two breeds have an advantage in the first and in average in the first four lactations, milk yield in AB goats was increased by 32 and 25%, accordingly, and in ZB goats – by 45 and 30%, accordingly. At cross breeding of AB with Saanen goats milk productivity was increased by 63 kg during the first lactation and by 45 kg in average during all other lactations.

In comparison with cow milk, goat milk has more calories, it contains the increased quantity of dry substance, fat, protein and mineral salts. According to the opinion of Z.F. Nazarov, the amino acid composition of the goat milk is close to the human milk.

According to the data of E.V. Eidrigevich (1939) goats are characterized by strong constitution, high viability and fitness to the all year round pasture management, live weight of female goats is 42.6 kg. Basic products were meat and milk.

Iolchiev B.S., Marzanov N.S., Chalykh E.A. (2000) point out that milk yield of Saanen goats, bred in Moscow region, is characterized by the average level or depending on the lactation varied from 765 kg (4th lactation) up to 435 kg (1st lactation). The impact of age on protein content in milk was also authentic and made up 79.2 % (P 0.95).

According to reports of Dauletov B.S. and Musazhanov E. (1993), cross breeding of coarse-haired female goats with male goats of the soviet wool-bearing breed contributed to the increase of the productivity level and improvement of the goat wool quality, and cross breeding with male goats of Saanen milk breeds –

increase of live weight and thus to the increase of incomes, obtained from coarse-haired goat breeding.

Bello A., Babiher S. (1989) report that observations over the growth of 20 pure-bred (desert breed – DB), 20 hybrid goat kids (Saanen male goats x DB female goats) and recording of slaughter products indicators allowed to define superiority of hybrid animals by weekly increment of live weight (0.8 in comparison with 0.6 kg in DB), by live weight before slaughter and after fasting (26.9 in comparison with 25.7 kg) and by feed consumption (7.7 kg SV/kg of mass increment in comparison with 8.4 kg, accordingly). At practically equal weight at slaughter (28.2 and 28.6 kg) hybrid goat kids were distinguished by higher mass of cold semi-carcass (7.56 in comparison with 7.07 kg in DB) and less fat mass in the carcass (1.31 kg of carcass in comparison with 1.71 kg) and better ratio of meat-bones (3.0 in comparison with 2.28).

Jaitap D.Z. (1989) reports that hybrid animals at birth were heavier than original animals. Less variability by live weight at birth was observed in animals 3/4A. The breed of stud male goats made influence on the live weight of goat kids. Thus, goat kids from stud male goats, imported from the USA, were heavier than pure-bred goat kids.

Gursoy D., Ozean L., Pekel E. (1988) point out that wool clip in Kil breed is equal to 0.5 kg per year, live mass of female goats: 33-38 kg, of male goats: 55-60 kg. Milk yield of Kil goats in average makes up 225-308 kg, reproductivity is 1.5 goat kids per year. At cross breeding of Saanen x Kil goats, milk yield of the first generation was increased up to 310.1 kg, of the second generation – up to 417.1 kg, third generation – up to 710.2 kg.

Masazhanov E. (1993) reports that at cross breeding of local coarse-haired female goats with Saanen milk male goats and soviet wool-bearing breed, soviet-wool-bearing hybrids by fattening properties were inferior to local kids, and Saanen hybrids had relatively high indicators of body weight gains. Slaughter yield, carcass mass in Saanen hybrids were higher, than in local coarse-haired and hybrid peers.

It should be pointed out that a number of inhabitants, wishing to raise milk goats, is increasing in many mountainous regions, because it is difficult to manage milk-cows due to shortage of fodder, especially within a winter period.

In this connection breeding farms on breeding of milk goats (JSC "Arstanbek", "Jorobay") must be occupied with raising and sale of young breeders, moreover, it brings significant incomes.

V. Conclusion

Kyrgyz milk goats by their size are attributed among other milk breeds to middle and large ones. Live weight of stud male goats is 60-80 kg, of female goats – 46-50 kg, which in comparison with Kyrgyz wool breed female goats by 10-12 kg and in comparison with local coarse-haired breed by 6-8 kg is higher.

Indicators of reproductive capacities of Kyrgyz milk goats is by 40-50% higher than in wool and local coarse-haired goats. Semen answers requirements of artificial insemination. Kid crop per 100 female goats makes up in average 160-170%. Slaughter yield in milk goats is higher. Content of fat in meat of milk goats is by 5.86% lower and content of protein, on the contrary, is by 5.19% higher than in local coarse-haired goat kids.

Milk yield in milk goats makes up in average 450-500 liters, in separate top producers – up to 1000 liters. In comparison with Kyrgyz wool and local coarse-haired goats these indicators are 4-5 times more. A lactation period in milk goats is 8-10 months, in Kyrgyz wool breed and local coarse-haired breed is two times less.

According to the chemical composition of milk in goats of different breeds there are differences. More dry substance is contained in milk of milk goats and makes up 18.02 %, in Kyrgyz wool breeds- 15.2%, in local coarse-haired goats – 16.1%, in Saanen goats – 12.39%. Fat content in milk of milk goats is also higher than in goats of other breeds.

Goats of Kyrgyz milk type are extensively distributed and are bred in many regions of the republic and outside. Breeding of Kyrgyz milk type of goats is economically effective. Earnings from sale of received products of milk goats make up 8,355 soms. A difference with other breeds makes up 4,760 soms and 4,505 soms, respectively, or earnings obtained from milk goats is almost 2 times higher, than in Kyrgyz wool and local coarse-haired goats.

The project is funded by the Islamic Development Bank and the Republic of Indonesia conducted research work in the field of cattle and goat breeding. More than 80 professionals are trained on the reproduction of cattle in the center of Singosari Malang. To improve the productivity of the Kyrgyz dairy goats imported from Indonesia 300 doses of frozen semen from Saanen goats and to increase meat productivity of local coarse wool of goats 500 doses of goat meat breed. At this time, formed herds goats for artificial insemination.

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Productive and Biological Characteristics of Growing Sheep with Different Genotypes

By Abdurasulov A.H., Salykov R.S., Abdymazhitov N. K., Madumarov A.K. & Muratova R.T.

Osh State University

Annotation- In order to assess the productive and biological characteristics of growing sheep of different origin we have carried out commercial cross breeding of Kyrgyz hair fat-tailed sheep with dairy Awassi sheep imported from Israel. As a result of basic measurements of body type it was established that crossbred descendants differed with better proportion of majority of body statures with well demonstrated meat and milk shapes. The duration of lactation period with crossbred females of first generation is longer for 50 days, but milk yielding capacity - 88,7 l more than of females of Kyrgyz hair sheep population.

Generally having analyzed the effectiveness of crossbreeding, including the received gains in weight, milking capacity, we have become convinced of its advantage. The advantage of crossbred growing sheep has to do with a degree of expression of heterosis effect. The quantity of products derived from crossbred lambs was significantly greater at fewer costs for its production.

Keywords: sheep, breeds, raising, breeding, selection, dairy productivity, chemical composition of milk, hematology, correlation.

GJMR-G Classification: NLMC Code: QW 70

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I. Introduction

The breeds cultivated in the republic were mainly of wool-mutton, mutton-wool and mutton-fat-wool productive direction. Along with that no less important was sheep's dairy productivity upon which the preservation, vitality, growth and development of lambs depend, and generally the state of herd reproduction.

The sheep's milk is a full value food product notable for its highest dietary properties and well digested. The valuable types of hard and soft cheese and different fermented milk products were made of it.

The world experience shows that in many countries a sheep breeding of dairy line has a priority meaning. More than 15 names of food products produced from sheep's milk are highly appreciated in Bulgaria, France, England, Italy, Germany, Israel, Syria, Jordan, and Turkey. For a long time an independent cheese industry was functioning in these countries.

Many scientists and professionals in the CIS countries have studied the sheep's dairy productivity at different times [1;4;6;].

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Author ¥: Ph.D., Associate Professor, Osh State University.

In Kyrgyzstan a number of scientists have studied the issue of sheep's dairy productivity or raised it as a problem at different periods of creation and improvement of breeds [2;3;5;6].

II. The Material and Research Methods

We have organized two groups of ewes according to analog method. The breeding stock in both groups was represented by local hair sheep. The first experienced group of ewes was artificially bred by sperm of sheep of Israeli dairy breed Awassi. The second control group of ewes was bred by local hair sheep.

We have studied a growth and development of growing sheep, change in body weight, by individual weighing at different age period; characteristics of exterior were determined by calculation of body type indexes.

The ewes' dairy productivity was studied according to methods of Y.I.Imigeev and others (2007).

The milk yield was taken into account by the following method. In the evening prior to record milking, lambs were separated from ewes and maintained individually at night and in the morning, till the end of milking. When lambs separated, all ewes are stripped rapidly but the milk obtained was not taken into account.

In the morning of record day, i.e. precisely in 12 hours after evening stripping, they conducted record milking of ewes, made individual measurement of milk quantity derived per sheep for the half of day. The milk yield was done per day by redoubling the quantity of drawn milk for the half of day. And for the control period we made calculations by multiplying obtained amount to number of days of such period. By summing up the sheep's milk yielding capacity for control periods, we have identified them as lactation and calculated the average daily milk output.

We have determined the amount of days of the first control period by summing up the days from lambing to record day + record day + 7 days thereafter.

The second record was done at the 14th day after the first and it was equal to 14 days. The third record was done at the 21st day after the second. The control period was equal to 28 days. The following sequential control records were conducted after each 28 days.

The chemical composition of sheep's milk has been studied according to methods of G.S.Inikhova, K.P. Brio. (1971).

We have studied clinical and hematological indications in animals per group based on the commonly accepted methods. We have examined a pulse, respiratory rhythm, body temperature, number of formed elements and percent of content of hemoglobin in blood by methods of Kudryavtsev A.A. and Kudryavtseva L.A. (1974), Vasilieva A.A. (1948), Nikitina V.N. (1949) and others.

All digital materials were processed by methods of variation statistics (Plokhinskiy N.A. 1969).

III. The Results and their Discussion

The first generation crossbreeds had the body weight by birth: growing sheep 4,4 kg, ewes - 4,2 kg., the body weight of crossbred sheep at weaning was 34,7±0,41 kg on average or by 2,2 kg (6,8%) more than in controlled group. The body weight of ewes in the experienced group was equal to 31,8±0,38 kg or 1,6 kg more (5,3%) more than in controlled group.

The body weight of crossbred sheep aged 8 month was 43,3±0,53 kg on average with fluctuation between 35,6 - 48,1 kg, of ewes -40,8±0,32 kg on average ranging from 35,1 to 44,5 kg, in percent to control group, the advantage among crossbreeds was attributed to growing sheep -7,4 %, ewes - 6,0 %.

The body weight of growing sheep at one year of age was on average: crossbreeds - 50,3 kg, local hair sheep - 45,8 kg or 4,8 kg more in crossbreeds. The body weight of crossbred ewes was equal to 44,4 kg against 41,2 kg of local hair sheep or was greater to 3,2 kg.

At the age of 1,5 the crossbred animals surpassed their herd mates in body weight, growing sheep by 12,1%, ewes by 8,5 %. The variation factor on crossbred growing sheep was 6,8%, on local hair sheep - 5,7% or by 1,1% higher in crossbreeds. The correlation factor on crossbred ewes was also by 0,3% higher than in control group.

The absolute growth of growing sheep from weaning to eight-month was 8,6 kg, ewes - 9,0 kg, daily average growth -9,5 and was 10 g more in crossbreds than in control group. During from 8 to 12 month the crossbred growing sheep had 7,0 kg, local hair sheep - 5,5 kg, i.e. was 1,5 kg more in crossbreeds. The absolute growth of body weight in crossbred ewes for the given period was 3,6 kg, in control - 2,7 kg or was 0,9 kg more in crossbreeds.

In general, for the whole period of breeding, the absolute growth of body weight since birth till the age of 1,5 year in crossbred growing sheep was 68,89 кг, in local hair sheep - 61,4 kg or was 7,85 kg more in crossbreeds. The indications of ewes were accordingly 53,53 kg and 49,13 kg, or 4,4 kg more in crossbred animals.

In order to have more complete and objective assessment of growth and development of crossbred and well-bred growing sheep, we have done the exterior measurements and calculated the basic body type indexes of animals with various genotypes.

When studying the age specific changes in measurements within the experienced and control groups of lambs, the substantial difference was found in growth intensity and development of individual parts of body of growing sheep with different genotypes. All body statures of crossbreeds have surpassed local herd mates in growth and development.

With a view to study the impact of cross breeding of local hair sheep with sheep of dairy breed Awassi for milk producing ability, we have determined ewes' milking capacity. The milk producing ability has been determined by methods of Imigeev Y.I. and others. (2007).

Daily milk yield in crossbreeds was on average 0,774 l, in local hair sheep -0,461 l, or was 0,313 l more in crossbreeds. Milk yield for lactation was equal to 162,5 l. and 73,8 l accordingly, or was 88,7 l. lower in control group.

Table 1: Chemical composition of sheep's milk of different breed

Indicators	Unit of measure	Breed	
		AwxLh	LhxLh
Moisture	%	81,70	80,1
Dry basis	%	18,30	19,92
Protein	%	4,96	5,28
Fat	%	6,92	6,42
Lactose	%	5,55	7,32
Nonfat milk solids	%	11,4	13,5
Ash	%	0,89	0,90

Selection and quality assessment. Milk yield of ewes was 135,5 l. for 1 lactation and 204-173 l for 4-5 lactations (190-177 days), with content of fat up to 6,8 %, and protein -6,2%.

The content of dry basis in milk of crossbreeds Aw x Lh was 18,30 %, of local hair sheep - 19,92 % or higher by 1,62 %. The content of fat in crossbreeds' milk was higher by 0,50% than in control. For content of remained components such as protein, lactose, nonfat

milk solids and ash in sheep of control group, indicators are rather higher than in crossbreeds' milk.

We have studied the biochemical indicators of blood of experimental animals during researches of results of commercial cross breeding (table 2.).

Table 2: Biochemical composition of sheep's blood with different breed

Indicators	Unit of measurement	Breed	
		Awx Lh	Lh x Lh
Red cells,	ml.	10,84	12,6
White cells,	thou.	9,46	9,77
Hemoglobin,	g/%	14,7	13,0
Protein,	mg/%	7,59	7,73
Alkaline reserve	mg/%	4,60	4,85
Calcium,	mg/%	7,73	7,67
Phosphorus	mg/%	5,25	6,16

The red blood count of crossbred animals was 10,84 mln. or by 1,76 mln less than in blood of local hair sheep. The content of hemoglobin in crossbreeds Aw x Lh -14,7%, in hair sheep -13,0%, or by 1,7% higher than in crossbreeds. For other indicators there were no substantial differences. Repetition of characteristics. In order to have animals to have high and sustainable productivity during their life, first of all, they must have good adaptability to paratypic living environment. Or in other words the breed to be created or internal breed type shall conform to specific environmental conditions.

In view of the above stated the analysis of results of our researches on age specific repetition of body weight of half-blooded crossbreeds derived from breeding of local hair sheep with Awassi dairy breed is of great interest (table 6).

Table 3: Age specific repetition of body weight of half-blooded ewes (AwxLh)

Age	f
By birth – weaning	0,01
At weaning – 8 months	0,10
At weaning – 12 months	0,21
At weaning – 18 months	0,01

The higher value of repetition of body weight in ewes is obtained at the age of 4 and 12 – months, since this indicator is significantly affected by genetic structure of herd associated with level of stock breeding. Generally in this case the low indicators of repetition in ewes are conditioned by insufficient provision of herd's genetic uniformity and by application of outbreeding of two breeds.

Correlation of characteristics. Without touching a great variety of economic and useful characteristics of sheep, we have studied the nature of relation between the body weight and basic exterior measurement, like shoulder height, height at hips, chest depth, chest breadth, chest girt and cross body length of sheep. Mainly, positive correlation dependencies – from 0,03 to 0,65 were obtained between body weight and basic exterior measurements. Positive relation – 0,10 - 0,12 in crossbreeds between body weight and shoulder height, chest depth, medium positive correlation – 0,24 between body weight and cross body length. The highest degree of dependency 0,65 is between body weight and chest breadth, statistically correct.

IV. Conclusion

As a result of application of crossbreeding by use of genetic resources of local population of hair fat-tailed sheep and Awassi breed, a new genotype of animals was derived which serves as a valuable breeding material in creating new direction of sheep's productivity in Kyrgyzstan – dairy sheep breeding.

The duration of lactation period of the first generation crossbred ewes is longer for 50 days, but milk yielding capacity – 88,7 l more than those of Kyrgyz hair sheep population.

Some low hematologic and clinic-physiological indicators have been identified in crossbred animals in comparison to those of local hair sheep that has to do with undergoing adaptation processes in new genotypes sheep body.

Within the conditions of market relations in order to raise profitability of farmers, farm households, individual owners of animals, it is recommended to diversify the production of new innovative solutions – introduction of elements of dairy sheep breeding.

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Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

Abbreviations

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Eletronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

Tips for writing a good quality Medical Research Paper

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of medical research then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.

6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.

Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

The Administration Rules

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.

Segment draft and final research paper: You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

Written material: You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.

Criterion for Grading a Research Paper (Compilation) by Global Journals

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.

Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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