



[44]

Amany M Hassan<sup>\*1</sup>, Ebtehag IM Abou Elenin<sup>2</sup>, Etab RI Abd El-Galil<sup>1</sup>, Gouda F Gouda<sup>1</sup>

- 1- Animal Production Dept, Fac of Agric, Ain Shams Univ, P.O. Box 68, Hadayek Shoubra, 11241 Cairo, Egypt
- 2- Animal Nutrition Dept, Animal Production Research Institute, Agricultural Research Center, Dokki, Giza, Egypt

\* Corresponding author: <u>Amanymohamedhassan7@gmail.com</u>

Received 23 April, 2021

Accepted 7 August, 2021

# Abstract

Twelve local Zaraiby goats with average live body weight of 10.71±0.23 kg (5-6 months). Animals were divided into three comparable groups. Goats in first group were fed without supplement (control group) or supplemented with 25% ordinary cobalt and 25% nanocobalt from cobalt requirements (NRC, 1985). The results showed that no significant difference (P>0.05) among experimental groups for DMI and CPI values. However, there was a highest significant difference (P<0.05) in average daily gain, total gain, and feed efficiency (kg gain/ kg DMI) for nanocobalt group. Nutrients digestibility values as DM, CP, CF, NFC, ADF, NDF, cellulose and hemicellulose in addition to percentage of TDN in nanocobalt treatment were higher significantly than those others groups. But the ratio of nitrogen balance in goats' diet with nanocobalt group was less than that using ordinary cobalt and control group. While the DE (Mcal/Kg DMI) ratio was no significant differences observed among groups. Rumen pH and TVFA's values after 3 and 6 hours of feeding, the control and the ordinary cobalt groups increased (P<0.05) more than the nanocobalt group. After 3hrs, adding nanocobalt has lower significant values for NH<sub>3</sub>-N than those others. Where, the control group recorded the lowest value. No significant (P>0.05) differences were observed at 0, 3and 6 hrs. after feeding for all blood parameters. There was a slight increase for most of body measurements with nanocobalt supplement. It could be concluded that adding nanocobalt for goats' rations enhanced growth performance, digestibility coefficients, rumen parameters and slightly body measurements without any diverse effect on animals' health.

**Keywords:** Nano, Cobalt, Zaraiby goats, Digestibility, Weight gain, Body's Muscles Measurements

### **1** Introduction

Cobalt considers an important element for dietary of ruminants, allowing rumen microorganisms to synthesis Vitamin B12 (Tiffany et al 2003). Cobalt is a component of cobalamin. It is as a trace element essential for gastrointestinal microbiota in most of animal species to synthesis cobalamin (EFSA 2012). Sheep need more than twice of cattle Co requirement so, it is extremely susceptible to Co deficiency (Grace et al 2000). Vitamin B12 deficiency in sheep found as weight loss, poor production, (Vellema et al 1997).

It is important to know that the proportion of cobalt (Co) is needed to synthesize B12 is relatively low in small ruminants (sheep) as reported by (Stemme et al 2008, Brito et al 2015), that it has been shown that. In general, it is important to adding Co to the diets' sheep (Shelley et al 2013),( Girard et al 2009), illustrated just 4% of cobalt' diet was used in synthesis of Cobalamin. Also, (Montaña et al 2020) reported that, Cobalt requirements of sheep are so much than others ruminants, it is required by rumen microflora for building vitamin B12, its deficient causes a deterioration of the immune function in lambs. Furthermore, in ruminants, there are many factors affected on cobalamin synthesis by rumen microflora, such as diet composition, ingredients of rations, contribution of cobalt, and ruminal microorganism's species (Montaña et al 2020).

However, (Bishehsari et al 2010) illustrated that adding the lambs' ration with twice NRC recommendations of cobalt sulfate improve concentration of vitamin B12 in plasma, final body weight, average daily gain (ADG) and feed efficiency and that it causes elevated levels of blood vitamin B12. (Kadim et al 2003) illustrated that 0.1 to 0.2mg levels of Cobalt/kg dry matter (DM) can't meet microorganism requirements of Co for B12 synthesis in small ruminants. This may be due to many factors, such that decrease rumen microorganisms (number and type), lowering ration's absorption and B12 building, the essential enzymes for (protein and energy) metabolism which clinically lead to anemia, in appetence, low production, low weight and finally weak immune system (Ulvund, Pestalozzi 1989, Vellema et al 1996). Adding cobalt enhance digested fiber by improve activity of rumen bacteria (Hussein et al 1994). The hypothesis that adding Cobalt with concentration more than those recommended by the (NRC 2007) or adding Cobalt with another form improve B12 in kids, as while, the nanomaterials have strong potential than those conventional sources, so required lower quantity (Sindhura et al 2014).

Thus, adding cobalt in nanoparticle may decrease the amount of cobalt supplementation or having the function of high amount of conventional cobalt sources.

The aim of this work was to illustrate ordinary and nanocobalt supplementation effects on growth performance, feed conversion, nutrient digestibility, some blood biochemical changes, and body's muscles measurements of Zaraiby goats.

### 2 Materials and Methods

This research was accomplished at farm belonging to department of animal production, Faculty of Agriculture, Ain Shams University, Egypt. Twelve animals were used from Zaraiby goats. The experiment lasted for 60 days. The average initial animals' weights were  $10.71\pm0.23$  kg (5 to 6 months age). Goats were weighed during the experiment every week before the morning meal to estimate average daily gain (ADG). Feeding requirements were adjusted biweekly according to weight changes.

### 2.1 Animal and feeding

Animals divided into 3 groups. Each group has 4 randomly selected animals. The first group: control group without additives, second group: adding ordinary cobalt with 25%, third group: adding nanocobalt with 25% of sheep and goats' requirements (NRC 1985). Animals fed diet containing 50% roughages (Rice straw) and 50% concentrate feed mixture (CFM). The concentrate feed mixture consisted of 41% yellow corn, 21% wheat bran, 20% un-decorticated cottonseed meal, 5% soybean meal, 5% rice bran, 4% molasses, 2.5% limestone, 1.0% common salt and 0.5 minerals mixture. The feeding was done twice daily, (at 8a.m. and 5p.m.), while, water was free all the day. The chemical composition of feedstuffs is illustrated in (Table 1).

Items	Rice straw	Concentrate feed mixture	Standard ration			
Dry matter(DM)	89.00	90.92	89.96			
0	n dry matter %					
Organic matter(OM)	85.56	82.11	83.80			
Crud protein(CP)	1.92	16.65	9.28			
Ether extract(EE)	2.24	3.48	2.86			
Crud fiber(CF)	45.09	6.56	25.81			
Non-Fiber Carbohydrate (NFC)	36.31	55.42	45.85			
Ash	14.44	17.89	16.20			
Cell wall constituents%:						
NDF	79.09	53.94	66.51			
ADF	48.28	20.66	34.47			
ADL	10.55	1.41	5.98			
Hemicellulose	30.81	33.28	32.04			
Cellulose	37.73	19.25	28.49			

Table 1. Chemical composition of feedstuff ingredients

### 2.2 Nanocobalt characterization

Prepare Cobalt oxide nanoparticles were successfully by thermal decomposition of cobalt hydroxide synthesized from cobalt acetate, ammonium hydroxide and 10% glycerol according to (Manigandan et al 2013). X ray determined (XRD) patterns of nanoparticles calcined at 450°C, points the cobalt oxide has cubic phase structure. The nanoparticles is marked using Scherrer equation relation (Holzwarth and Gibson 2011), and it was found to be average size around 49 nm. It noticed that the particles embrace irregular morphology with different sized particle. In addition, cobalt oxide nanoparticles show rod shape with smooth surface and adsorbed on the surface due to the aggregation with the rod like agglomerates were purely due to the magnetic induction between the particles (Koutzarova et al 2006).

### 2.3 Digestibility trials

The digestibility trial was done during the last 10 days of the feeding experiment. The feces samples were collected before the morning feeding. The feed and feces samples were preserved until they were analyzed. The rumen fluid samples were collected from animals at 0, 3 and 6 hrs. after morning feeding and the samples were saved until laboratory's analysis at a cooling temperature of less than  $25^{\circ}$ C degrees. Rumen pH, ammonia–N (NH<sub>3</sub>-N) and total volatile fatty acids (TVFA's) were estimated at rumen samples .

The samples of feed and feces were analyzed for DM, crude protein (CP), crude fiber (CF), ether extract (EE) contents, the nitrogen free extract (NFE) was obtained by the difference and ash according to (AOAC 1997). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) contents were analyzed sequentially (Van Soest et al 1991) using the Ankom 200 Fibre Analyzer for NDF and ADF and thereafter soaking the residual with 72% sulfuric acid for 3 hours. The NDF content was analyzed with 2 additions of heat-stable  $\alpha$ -amylase and 1:1 g sodium sulfate per gm sample in the neutral detergent solution (Hansen et al 2016). NDF and ADF are expressed inclusive of residual ash and hemicellulose and cellulose calculated by difference between NDF, ADF and ADL values.

Blood samples were taken in the last 3 days of the experiment. Samples were taken through the neck vein of the animals before the morning feeding, the serum was separated and the samples were kept in a cold temperature of less than 25°C degrees. Then the following parameters were measured: TP, glucose, urea, AST and ALT by kits. Total plasma protein concentrations was determined as described by (Henry 1964), albumin concentrations was determined using methods of (Doumas et al 1971), blood plasma urea was determined according to (Batton, Crouch 1977), Alanin amino transferase (ALT) and aspartate amino transfearse (AST) activities were calorimetrically determined according to AST and ALT kits based on reaction of (Young 1990).

### 2.4 Body measurements

In the end of experimental trial, body measurements were recorded. The measured were Rump height (HR), withers height (WH),Sternum height (SH), Body length (BL), Bicostal diameter (BD), Body depth (RD),Heart girth (HG), and Rump width (RW) according to (Cam et al 2010).

### 2.5 Statistical Data analysis

The recorded data statistically analyzed according to statistical analysis system, User's Guide, (SAS 1998). Means Separation was carried out by using Duncan Multiple test (Duncan 1955). The model was used as following:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:  $Y_{ij}$  = the observation of the model,  $\mu$  = General mean common element to all observations,  $T_i$  = the effect of the treatment (i = 1... 3), and  $e_{ij}$  = the effect of experimental error.

### **3** Results and Discussion

### 3.1 Growth performance

The data showed effect of cobalt supplementation on feed intake (FI), total gain, ADG, and feed efficiency (**Table 2**). No significant (P>0.05) difference was observed among experimental groups for DMI or CPI values and feed intake tended to increase with adding nanocobalt. Likewise, (Scholljegerdes et al 2010) reported that total intake was higher (P=0.093) in lambs supplemented cobalt in diets. However, there was a significant difference in favor of the nanocobalt group as it was the highest total gain and ADG, additionally, feed efficiency (kg gain/ kg DMI), then the regular cobalt group, while the lowest value for the control group. These results were in agreement with results of (Schwarz et al 2000) who observed that the diets with no or low cobalt supplementation produced decreasing daily feed intake per animal versus high levels of cobalt. Also, daily gain values were increased significantly with high levels of cobalt. (Stangl et al 2000) reported that cobalt level (adequate supply) for growing cattle is 0.20 mg/kg DM intake. However, using nano cobalt may have more beneficial effect in cobalt efficiency.

# 3.2 Digestibility coefficients and nutritive values

Data of digestibility coefficients and feeding values were illustrated in (Table 3). Nutrient's digestibility values as DM, OM, CP, CF, NFC and NDF in the treatment of nanocobalt were higher significantly than ordinary cobalt then control group except EE. The same trend was observed with those percentages of ADF, hemicellulose and cellulose. It's higher (P<0.05) significantly at nanocobalt than ordinary cobalt after that control group. The percentage of TDN was significantly (P<0.05) highest at nanocobalt treatment than those others. But the ratio of nitrogen balance in goats' diet with nanocobalt group was less than that using ordinary cobalt and control group. While the DE (Mcal/Kg DMI) ratio was no significant differences observed among groups.

(Scholljegerdes et al 2010) reported that lambs fed cobalt, resulted in DM, OM, and NDF digestibility increased significantly (P  $\leq$ 0.098). Furthermore, there were no differences between cobalt levels groups in total nitrogen intake, N digested (g/d), or urine nitrogen. Nitrogen retention did not affect with adding cobalt to diet's lambs.

Rations	Rations			SE
	Control	Ordinary cobalt	Nano cobalt	±
Exp. Period	60	60	60	-
No. of Anim.	4	4	4	-
Average initial weight(kg)	10.670	10.700	10.750	-
Average final weight(kg)	15.170	15.400	15.850	-
Total dry matter intake(kg/group)	156.00	156.48	159.90	-
DMI (kg/head)	39.00	39.12	39.90	-
DMI (kg/h/d)	0.650	0.652	0.665	-
CPI (g/h/d)	60.32	60.50	61.71	-
Total gain (kg)	4.500 <sup>b</sup>	4.700 <sup>b</sup>	5.100 <sup>a</sup>	0.18
ADG (kg/ h/d)	0.075 <sup>b</sup>	0.078 <sup>b</sup>	0.085 <sup>a</sup>	0.21
Feed efficiency (kg gain/kg DMI)	0.115 <sup>b</sup>	0.120 <sup>a</sup>	0.127 <sup>a</sup>	0.44
Feed conversion (kg DMI/kg gain)	8.66	8.32	7.82	1.30

**Table 2.** Effect of cobalt supplementation (ordinary and nanocobalt) on feed intake, total gain, ADG and feed efficiency for Zaraiby goats

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

**Table 3.** Effect of cobalt supplementation (ordinary and nanocobalt) on nutrients digestibility and feeding values for Zaraiby goats

Item		SE		
	Control	Ordinarycobalt	Nano cobalt	±
DM	65.58 °	70.91 <sup>b</sup>	76.33ª	0.29
ОМ	70.11 °	73.26 <sup>b</sup>	78.05 <sup>a</sup>	0.30
СР	71.27 °	72.45 <sup>b</sup>	77.99 <sup>a</sup>	0.33
CF	66.32 °	69.34 <sup>b</sup>	75.44 <sup>a</sup>	0.31
EE	83.18	84.21	84.65	0.28
NFC	77.98 °	80.33 <sup>b</sup>	83.58 a	0.26
NDF	66.23 °	70.12 <sup>a</sup>	72.46 <sup>a</sup>	0.23
ADF	75.37 °	78.20 <sup>b</sup>	80.98 a	0.22
Hemicellulose	63.55 <sup>b</sup>	66.32 <sup>a</sup>	69.50 <sup>a</sup>	0.26
Cellulose	61.84 <sup>b</sup>	63.91 <sup>b</sup>	68.33 a	0.27
Feeding values(%):				
TDN%	62.87 °	67.22 <sup>b</sup>	72.36 ª	1.60
Nitrogen balance	+2.66	+2.55	+2.04	0.82
DE (Mcal/Kg DMI) A	2.77	2.96	3.19	0.04

a, b and c: Means in the same rows with different superscripts are significantly different at (P<0.05).DE (Mcal / Kg DMI) = 0.04409 x TDN% (NRC, 1988)

### 3.3 Rumen parameters

natural decrease in the pH value occurred after 3 hrs.in all groups due to digestion operation .

Rumen fluid parameters as pH value, ammonia and TVFA's concentration after 3 and 6 hours after feeding were reported at (**Table 4**). The data showed that after 3 and 6 hours the pH value in control group and the ordinary cobalt groups increased (P<0.05) more than the nanocobalt group. It observed that, there were The data at (**Table 4**) showed the effect of adding cobalt on NH<sub>3</sub>-N levels, and the results revealed that after 3 hours, no significant difference between control group and that adding ordinary cobalt, while, adding nanocobalt has lower significant values than those others. However, after 6 hours, there weren't differences (P>0.05) among experimental groups. It

is naturally observed that increasing NH<sub>3</sub>-N levels at 3 hrs. after feeding then decreasing after 6hrs. Generally, data indicated that adding nanocobalt to goats' diets resulted in depress of ammonia-N values comparing with control and ordinary cobalt groups. But the control group remained the highest among them.

**Table 4** contains the data of TVFA's values, at zero time, results illustrated that there was significant difference between the control group and those others (ordinary cobalt and nanocobalt groups). Where, animals in control group recorded the lowest TVFA's values. However, after 3 and 6 hours, value of TVFA's of nanocobalt group was the lowest significant level than other groups, the control group was the highest values, followed by the ordinary cobalt group. Overall, TVFA's values increased after 3hrs according to maximum level of feed digestion process, and then declined again due to many factors' effects on level of TVFA's in the rumen.

Ruminal fermentation may be improved by increasing intake of cobalt (adding cobalt) specifically the cellulolytic bacteria which related to change in population of rumen microflora, (Scholljegerdes et al 2010). It is estimated that the microbiota of rumen for function efficiently needs 0.11% cobalt in the ration (Goff 2000), there are hypotheses that ruminal fermentation may improve with supplemented cobalt, by enhancing microorganism's growth and it's activity (Nagabhushana et al 2008). Moreover, rumen environment can affect with adding cobalt and cause increasing of fiber digestion (Shelley et al 2013).

### **3.4 Blood parameters**

Data of blood parameters are presented in (**Table 5**). No significant differences were found at 0, 3hrs and 6 hrs. after feeding for all blood parameters, which indicated that there were no harmful effects on the animal's vital organs and animal health. While, regards of comparative treatments, there were slightly (P<0.05) increasing of glucose, urea and total protein (TP) values as overall mean in nanocobalt additive then ordinary cobalt comparing

with control group. These results reflected on enhancing of growth performance, and confirmed by (Bishehsari et al 2010) who found that adding cobalt to sheep's diets caused reduction ruminal succinate, also improving propionate concentrations, which lead to increase blood glucose levels.

In addition to, liver function as AST and ALT levels were increased with adding cobalt whether ordinary or nanocobalt, however, level was highest in nanocobalt. These results are in agreement with results of (Ghoreishi et al 2013) that reported the level of the serum ALT was significantly increased after administration of cobalt nanoparticles (p=0.003). Furthermore, they reported that the effect of adding nanocobalt particle on lamb's liver function were similar to adding conventional cobalt. However, the more beneficial effect may be for using nanocobalt. In addition to, goat kids that received adequate amounts of cobalt prevent damages of liver. Vitamin B12 has a significant role in overall N metabolism and lowering synthesis of vitamin B12 could lead to deficiency of serum N (Johnson et al 2004).

### 3.5 Body measurements

**Table 6** showed the effect of adding cobalt on some body measurements. No significant differences (P<0.05) among experimental groups, but there was a slight increase for the nanocobalt group than the ordinary cobalt group and the control group that confirmed by the other data of growth performance and digestion coefficients where animal received nanocobalt additive have positive results revealed on body measurements.

Enzyme systems need vitamin B12 or cobalamin which plays the important role for basic metabolic functions. It has a positive effect on both of metabolic of energy and cell replication processes, so, it considers as a coenzyme (Rizzo and Laganà 2020). Cobalamin works as a co-factor of carbohydrates, lipids, amino acids metabolism and DNA (Herdt and Hoff 2011, Forrellat et al 1999).

Rations	Rations			SE
	Control	Ordinary cobalt	Nano cobalt	±
pH				
0	6.90	6.87	6.75	0.09
3	6.48 <sup>a</sup>	6.43 <sup>a</sup>	6.03 <sup>b</sup>	0.29
6	6.56 <sup>a</sup>	6.53 <sup>a</sup>	6.16 <sup>b</sup>	0.25
Means	6.64	6.61	6.31	-
NH3-N				
0	19.88	19.85	19.59	0.78
3	31.71 <sup>a</sup>	31.23 ª	29.89 <sup>b</sup>	1.71
6	23.35	23.11	22.59	1.38
Means	24.98	24.73	24.02	-
TVFA's				
0	7.18 <sup>b</sup>	7.63 <sup>a</sup>	7.46 <sup>a</sup>	0.24
3	12.13 <sup>a</sup>	11.43 <sup>b</sup>	10.72 °	0.82
6	9.89 <sup>a</sup>	9.87 <sup>a</sup>	9.25 <sup>b</sup>	0.62
Means	9.73	9.64	9.14	-

Table 4. Effect of cobalt supplementation (ordinary and nanocobalt) on rumen parameters for Zaraiby goats

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

Items	Rations			SE
	Control	Ordinary cobalt	Nano cobalt	
Glucouse (mg/dl)				
0	54.40	56.80	55.99	1.86
3	57.74	58.22	58.63	1.90
6	62.32	61.99	63.21	2.02
Overall means	58.15	59.00	59.28	-
Urea				
0	14.90	16.76	17.87	0.53
3	17.40	18.58	19.99	0.58
6	19.38	21.32	22.96	0.61
Overall means	17.22	18.88	20.72	-
GOT (AST)(unit/L)				
0	18.43	20.19	22.54	0.22
3	21.06	23.52	25.03	0.22
6	25.25	26.97	27.11	0.23
Overall means	21.58	23.56	24.89	-
GPT (ALT) (unit/L)				
0	12.50	13.44	15.24	0.20
3	14.33	16.21	18.02	0.21
6	16.87	18.56	20.95	0.21
Overall means	14.57	16.07	18.07	-
TP (mg/100ml)				
0	10.86	11.43	12.79	0.15
3	11.45	12.67	14.23	0.16
6	13.11	14.34	16.97	0.19
Overall means	11.81	12.81	14.66	-

Table 5. Effect of cobalt supplementation (ordinary and nanocobalt) on blood parameters for

**Table 6.** Effect of cobalt supplementation (ordinary and nanocobalt) on body measurements for

 Zaraiby goats

Items		SE		
items	Control	Ordinary cobalt	Nano cobalt	±
WH	51.75	53.75	54.00	1.28
HR	58.00	56.50	56.75	2.19
BL	53.25	50.25	51.65	2.26
SH	33.25	33.00	40.25	4.73
RD	58.75	58.00	55.25	4.32
BD	67.75	67.25	68.00	3.08
RW	66.25	64.75	65.75	3.14
HG	62.50	61.00	61.50	2.91
withers height (WH) Rump height(HR) Body length (BL)				

Sternum height (SH)Rump width (RW)Rump width (RW)

RD) Bicostal diameter (BD)

The depression of available cobalt element that reserves for microorganisms in the rumen, consequently, closing the production of B12 that resulting in high level accumulation of blood propionate (Underwood and Suttle 1999). By lowering intake, ruminal microbiota decrease, which convert succinate to propionate by using Vit. B12, consequently, there is decrease of diet fermentation, that causes a depression in supply of energy and clearly low of animal's body weight.

(Girard et al 2009) reported that in sheep only 5% of B12 that produced in rumen was absorbed. Ruminant animals have higher requirements of vitamin B12 than non-ruminant animals, due to use it in metabolism of propionic acid (McDowell 2012). So, depression of (methyl malonyl) CoA mutase activity (as a key enzyme in the gluconeogenesis of propionate) in ruminant animals causes depression of plasma glucose, high pyruvate formed and urine methylmalonate. Appetite control center in the hypothalamus which affected by elevation of methylmalonate and pyruvate in blood, that causing anorexia and lost animal weight (Viglierchio 2000). In addition to, synthesis and supplemental cobalt can effect on energy production (Shelley et al 2013).

### **4** Conclusion

It could be concluded that adding nanocobalt for goats' rations enhanced growth performance, digestibility coefficients, rumen parameters and slightly body measurements without any diverse effect on animals' health.

### References

AOAC (1997) Association of Official Analytical Chemists, Official Methods of Analysis, *Washington* 1, 69–90.

Batton C, Crouch G (1977). Enzymatic colorimetric determination of urea. *Analytical Chemistry* 49, 464-469

Bishehsari S, Tabatabaei MM, Aliarabi H, Alipour D, Zamani P, Ahmadi A (2010) Effect of dietary cobalt supplementation on plasma and rumen metabolites in Mehraban lambs. *Small Ruminant Research* 90, 170–173.

Brito A, Chiquette J, Stabler SP, Allen RH, Girard CL (2015) Supplementing lactating dairy cows with a vitamin B 12 precursor, 5, 6dimethylbenzimidazole, increases the apparent ruminal synthesis of vitamin B12. *Animal*, 9, 67–75 © *The Animal Consortium 2014. Parts* of this are a work of the Government of Canada, represented by the Agriculture and Agri-Food Canada 2014. doi:10.1017/S175173111400220.

Cam MA, Olfaz M, Soydan E (2010) Body measurements reflect body weights carcass yields in karayaka sheep. *Asian Journal of Animal and Veterinary Advances* 52, 120-127.

Doumas BT, Watson WA, Briggs HG (1971) Albumin standards and the measurement of serum albumin with bromocresol green *Clinical Chimistry Acta* 31, 87-97. doi: 10.1016/0009-8981(71)90365-2.

Duncan DB (1955) Multiple range and multiple F tests. *Biometrics*, 11, 1–41. <u>https://doi.org/10.2307/3001478</u>.

Farrell DJ, Perez-Maldonado RA, Mannion PF (1999) Optimum inclusion of field peas, faba beans, chick peas and sweet lupins in poultry diets. II. Broiler experiments. *British Poultry Science* 40, 674-80. *doi:* 10.1080/00071669987070.

Forrellat BM, Gómis HI, Gautier DGH (1999) Vitamina B12: Metabolismo y aspectosclínicos de sudeficiencia. *Revista Cubana de Hematología, Inmunología y Hemoterapia* 15, 159–174.

EFSA (2012). EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Scientific Opinion on safety and efficacy of cobalt carbonate as feed additive for ruminants, horses and rabbits *EFSA Journal 10, 2727. doi: 10.2903/j.efsa.2012.2727.* Available online: www.efsa.europa.eu/efsajournal

Ghoreishi SM, Najafzadeh H, Mohammadian B, Rahimi E, Afzalzadeh MR, Varnamkhasti MK, Darani HG (2013). Effect of cobalt nanoparticles on serum biochemical and histopathological changes in liver and kidney of lambs. *Iranian Journal Veterinary Science & Technolog* 5, 1-8.

Girard CL, Santschi DE, Stabler SP, Allen RH (2009) Apparent ruminal synthesis and intestinal disappearance of vitamin B12 and its analogs in dairy cows. *Journal of Dairy Science* 92, 4524–4529.

Goff JP (2000) Determining the mineral requirement of dairy cattle. In Proceedings of the 11th Annual Florida Ruminant Nutrition Symposium, University of Florida, Gainesville, FL, USA, pp. 106–132.

Grace ND, West DM (2000) Effect of an injectable micro encapsulated Vitamin B on serum and liver vitamin B concentrations in calves. *New Zealand Veterinary Journal* 48, 70-73.

Hansen J, Sato M, Hearty P, Ruedy R, Kelley M, Masson-Delmotte V, Russell G, Tselioudis G, Cao J, Rignot E, Velicogna I, Tormey B, Donovan B, Kandiano E, von Schuckmann K, Kharecha P, LeGrande AN, Bauer M, Lo KW (2016) Ice melt, sea level rise and super storms: Evidence from paleoclimate data, climate modeling, and modern observations that 2°C global warming could be dangerous. *Atmospheric Chemistry and Physics* 15, 20059-20179

Henry RJ (1964) Clinical chemistry, principles and techniques, 2nd Edition, Harper and Row, pp. 525.

Herdt TH, Hoff B (2011) The use of blood analysis to evaluate trace mineral status in ruminant livestock *Veterinary Clinics of North America: Food Animal Practice* 27, 255–283.

Holzwarth U, Gibson N (2011) The scherrer equation versus the Debye-scherrer equation. *Nature nanotech.* 

534.10.1038/nnano.2011.145

Hussein HJ, Capp SP, George WK (1994) Velocity measurements in a high Reynolds number, momentum conserving, axisymmetric, turbulent jet. *Journal of Fluid* Mechanic 258, 31-75.

*DOI:* <u>https://doi.org/10.1017/S002211209400</u> 323X.

Johnson EH, Al-Habsi K, Kaplan E, Srikandakumar A, Kadim IT, Annamalai K, Al-Busaidy R, Mahgoub O (2004) Caprine hepatic lipidosis induced through the intake of low levels of dietary cobalt. *The Veterinary Journal* 168, 174–179. Kadim, IT, Johnson, EH, Anandarajah, OM, Al-Ajmi SD, Ritchie A, Annamalai K, Al-Halhali AS (2003) Effect of low levels of dietary cobalt on apparent nutrient digestibility in Omani goats. Department of Animal & Veterinary Sciences, College of Agricultural and Marine Sciences, Sultan Qaboos University, P.O. Box 34, Al-Khoud 123, Muscat, Oman Animal Feed Science and Technology 109, 209–216.

Koutzarova T, Kolev S, Ghelev Ch, Paneva D, Nedkov I (2006) Microstructural study and size control of iron oxide nanoparti-cles produced by microemulsion technique. *physica status solidi* 3, 1302–1307.

Manigandana R, Giribabua K, Suresha R, Vijayalakshmib L, Stephenc A, Narayanana V (2013) Cobalt oxide nanoparticles: characterization and its electrocatalytic activity towards nitrobenzene. *Chemical Science Transactions* 2, 47-50.

McDowell LR (2012) Vitamin B12. in Vitamins in Animal and Human Nutrition, Iowa State University Press: Ames, IA, USA, pp. 523–563.

Montaña JRG, Valente FE, Alonso AJ, Lomillos JM, Robles R, Alonso ME (2020) Relationship between Vitamin B12 and Cobalt Metabolism in Domestic Ruminant: *An Update. Animals* 10, 1855, doi:10.3390/ani10101855http://www.mdpi.com/journal/animals

Nagabhushana V, Sharma K, Pattanaik AK, Dutta N (2008) Effect of cobalt supplementation on performance of growing calves. *Veterinary World* 1, 299–302. http://www.veterinaryworld.org/.

NRC (2007) National Research Council, Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids .Washington, DC: The National Academies Press .<u>https://doi.org/10.17226/11654</u>

Rizzo G, Laganà AS (2020) A review of vitamin B12. In Molecular *Nutrition Elsevier: Amsterdam, The Netherlands,* pp. 105–129. SAS (1998) Static Analysis, 5th International Symposium, SAS '98, Pisa, Italy, September 14-16, 1998, Proceedings. Lecture Notes in Computer Science 1503, Springer 1998, ISBN 3-540-65014-8.

Scholljegerdes EJ, Hill WJ, Purvis HT, Voigt LA, Schauer CS (2010) Effects of supplemental cobalt on nutrient digestion and nitrogen balance in lambs fed forage-based diets. *Sheep& Goat Research Journal* 25, 74-77.

Schwarz FJ, Kirchgessner M, Stangl GI (2000) Cobalt requirement of beef cattle — feed intake and growth at different levels of cobalt supply. *Journal of Animal Physiologycal and Animal Nutrition* 83, 121–131.

Shelley CL, Marchetti K, Salazar AL, Tracey, LN, Schmitz L, Scholljegerdes EJ, Lodge-Ivey SL, Larson CK (2013) Effect of Cobalt Supplementation on Rumen Fermentation and Blood Metabolites. In Proceedings of the Sheep Symposium. Integrating Advanced Concepts into Traditional Practices, *Western Section American Society of Animal Science: Bozeman, MT, USA* 64, 107–111.

Sindhura SK, Selvam PP, Prasad TNV, Hussain OM (2014). Synthesis, characterization and evaluation of effect of phytogenic zinc nanoparticles on soil exo-enzymes. *Applied Nanoscience Volume 4, Issue 7, pp.819-827 DOI: 10.1007/s13204-013-0263-4.* 

Stemme K, Lebzien P, Flachowski G, Scholz H (2008) The influence of an increased cobalt supply on ruminal parameters and microbial vitamin B12 synthesis in the rumen of dairy cows. *Archives of Animal Nutrition 62, 207-218 doi: 10.1080/17450390802027460.* 

Tiffany ME, Spears JW, Xi L, Horton J (2003) Influence of dietary cobalt source and concentration on performance, vitamin B-12 status, and ruminal and plasma metabolites in growing and finishing steers. *Journal of Animal Science* 81, 3151-3159. doi: 10.2527/2003.81123151x.

Ulvund MJ, Pestalozzi M (1989) Ovine white liver disease (OWLD) in Norway: clinical symptoms and preventive measures. *Acta Veterinaria Scandinavica* 31, 53-62. doi: 10.1186/BF03547577.

Underwood EJ, Suttle NF (1999) The Mineral Nutrition of Livestock. In: Ibid. (Eds), 3rd ed. CABI, New York, Cobalt, pp. 251-274.

Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* 74, 3583–3597. Vellema P, Moll L, Barkema HW, Schukken YH (1997) Effect of cobalt supplementation on serum vitamin B12 levels, weight gain and survival rate in lambs grazing cobalt-deficient pastures. *Veterinary Quarterly 1, 1-5.* 

Vellema P, Rutten VP, Hoek A, Moll L, Wentink GH (1996) The effect of cobalt supplementation on the immune response in vitamin B12 deficient Texel lambs. *Veterinary Immunology and Immunopathology* 55, 151-161.

Viglierchio MC (2000) Aportes de la Bioquímica a la InterpretacióndelMetabolismo del Cobalto. Anuario.Facultad de CienciasVeterinarias. Universidad Nacional de la Pampa: La Pampa, Argentina, 2000, pp. 22–28.

Young DS (1990). Effects of drugs on clinical laboratory tests. Third edition 3, 6-12.