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Abstract: This study evaluated the influence of Westernised and traditional African diets on biochemical and haematological profiles in vervet monkeys (*Cercopithecus aethiops*). Twelve adult male vervet monkeys bred at the Medical Research Council, all over 4 years of age and weighing more than 5 kg each, were divided into two groups of six individuals. These monkeys were raised on a standard in-house diet post-weaning, before they were fed for 8 weeks on diets containing milk solids (17.2%) or maize + legume (17.4%), as sources of high crude protein (± 3.5 g/kg). High protein diets had no significant effect on serum biochemical indices such as aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT) concentrations ($P > 0.10$). However, alanine aminotransferase (ALT) concentrations were significantly higher during week 8 ($P < 0.05$) for the maize + legume protein group. Alkaline phosphatase (ALP; $P < 0.07$), total protein ($P < 0.0001$), albumin ($P < 0.02$), and bilirubin ($P < 0.003$) were elevated in the milk solids group, while glucose levels were also significantly higher for the milk solids group ($P < 0.05$) between weeks 2 and 6. Elevated protein intake had no significant effect on haematological parameters such as red blood cells (RBC), platelet and white blood cell (WBC) counts, haemoglobin levels and monocyte and neutrophil concentrations ($P > 0.10$). In contrast, serum lymphocyte levels were significantly raised in the maize + legume protein group ($P = 0.03$), whereas values for the haematocrit ($P < 0.002$), mean cell volume (MCV; $P < 0.03$) and mean corpuscular haemoglobin concentration (MCHC; $P < 0.0001$) were higher in the monkeys that were fed the milk solids.
This investigation showed that the type of dietary protein that is consumed may well affect certain biochemical and haematological indices in vervet monkeys. Compared to the group that were given the traditional African food regime, the animals on the Western-type milk solids diet showed significant elevations in a number of important biological indicators. However, longer-term studies should be completed in this area if we are to make firmer conclusions regarding the link between the nature of dietary proteins that are consumed and its effect on metabolism.

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Introduction

The impact of dietary protein on metabolism and the function of organs is important. Animal protein consumption, in particular, has been associated with among others, the development of certain forms of cancer [3, 41], cardiovascular disease [11, 26, 45], changes in kidney growth and glomerular filtration rates [4, 5, 27], diabetes [40] and disorders of the skeletal systems [9, 16, 17, 37]. Although these diseases are largely absent among indigenous peoples who consume plant protein diets, the potential link between their health profile and the nature of the dietary proteins they eat remains largely unexplored [25].

Africans traditionally consume a combination of grains and legumes as staple foods that contain all the amino acids that are required for supplying complete dietary proteins [25]. Grains are high in methionine and threonine, but low in lysine [52]. Also, when grains are combined with legumes, that

are high in lysine [38] and low in sulphur-containing amino acids, they provide a good source of protein for growth, maintenance and reproduction [22]. The lack of information relating to the link between diet and health is even more important when one considers the increase in diseases suffered by indigenous peoples who, during their cultural transition, alter their feeding patterns by switching from a largely vegetable to animal protein diet [14, 21, 22, 31, 34–36]. Consequently, it is not only informative, but also necessary, to determine whether the type of dietary protein that is consumed has any effect on the health status of the individual. In this regard, non-human primates have been used in various studies to establish baseline blood chemistry values that are important in trying to assess environmentally induced pathology [42, 49].

Besides fulfilling the desirable physiological modelling criteria, vervet monkeys are economical and ideal for laboratory procedures, because they are relatively small. The vervet monkey has been used to study among others, behaviour [18], calcium metabolism [15] and reproduction [12]. However, very little is known about the impact of Westernised animal protein diets or traditional African plant based diets, on their serum biochemistry and haematology.

Table 1. The dietary regimes of the vervet monkey, *Cercopithec cus aethiops* (n = 6/group)

Nutrients (g)	Dietary regimes		
	BL	HP-ML	HP-MS
Energy (kJ)	2412	2415	2415
Total protein	17.3	17.4	17.2
Plant protein	15.5	15.5	5.7
Animal protein	1.8	1.9	11.5
Total carbohydrates	96.6	98.4	96.8
Fibre	11.8	10.8	10.1
Total fat	12.9	12.1	13.3
Calcium (mg/g DM)	1.24	1.33	1.59
Phosphate (mg/g DM)	0.98	1.02	1.07
Ingredients (g)			
Apple with skin	70	70	70
Kidney beans	74	74	–
Mealie meal	83	83	–
Milk powder	–	–	38
Polycose (CHO)	–	–	45
Sunflower oil	4	4	5
Sunflower seeds	7	7	3
White sugar	5	5	10
Whole kernel maize	7	7	–
Wholewheat bread	–	–	31
Whole dried egg	4	4	10

BL (in-house MRC ration for baseline data); HP-ML (high protein maize + legumes); HP-MS (high protein milk solids). The diets were supplemented with a standard vitamin and mineral mix for vervet monkeys. DM = dry matter. The monkeys were fed in the mornings and afternoons with a daily serving of fresh fruit.

Materials and methods

The animal protocol was approved by the ethics committees of the Medical Research Council (MRC) and the University of the Western Cape (UWC). Twelve adult male vervet monkeys (*Cercopithecus aethiops*) bred at the MRC Primate Unit, all more than 4 years of age and over 5 kg each, were randomly divided into two groups of six individuals. They were raised on an in-house MRC diet post-weaning, before being placed on the experimental food rations for 8 weeks and fed *ad libitum*. The in-house ration served as a placebo (baseline), against which the experimental diets were compared. Both groups of animals received similar high protein rations (3.5 g/kg), except for the source of the crude protein, which was largely derived from a Western-type animal protein (milk solids – 17.2%), compared to a combination of indigenous African plant proteins (maize + legumes – 17.4%).

The diets were formulated using the South African Food Fund feed composition computer program, at the MRC. We observed the animals for dietary palatability and found that food compliance was good, and we were also satisfied that food wastage was minimal and insignificant. Water was provided *ad libitum* during the trial. The food constituents of both diets were autoclaved, thereby minimising the possibility of contamination by toxins or bacteria. The diets (Table 1) were balanced to be approximately equivalent in nutrient content, with apples being provided at noon each day. Although the monkeys were provided with varying in-house rations, we specifically chose animals that were kept on standard food regimes consisting of maize and legumes, since these are core constituents of traditional African diets. We established a plant based control diet by slightly adjusting the MRC in-house ration to match the calorie intake of the experimental animal protein diet. The control and experimental groups were now isocaloric, and differed only with respect to the source of protein that they contained. Hence, this study could focus on how the experimental diet containing the Western-type animal protein milk solids, affected the biochemical and haematological profile of the monkeys.

All the monkeys were kept singly and permanently indoors in a $0.6 \times 0.6 \times 0.8$ m and $1.2 \times 0.6 \times 0.8$ m wall mounted stainless steel home cages. Individuals also had access to a $0.6 \times 0.6 \times 2.0$ m exercise cages every 6 days for 24 hours. In

Table 2. Average body weight (kg ± SEM) of the animals (n = 6/group) taken at two week intervals

Time (weeks)						
Diets	BL	2	4	6	8	*P*
HP-ML	5.67 ± 0.32	5.64 ± 0.30	5.71 ± 0.31	5.86 ± 0.33	5.76 ± 0.39	NS
HP-MS	5.14 ± 0.24	5.20 ± 0.17	5.22 ± 0.19	5.33 ± 0.20	5.32 ± 0.21	NS

BL (in-house MRC ration for baseline data); HP-ML (high protein maize + legumes); HP-MS (high protein milk solids). NS = no significant differences for the average body weight of the monkeys within or between the two dietary groups.

addition, each exercise cage housed a female social partner. To enhance psychological well-being, the home cages were fitted with sterilised cattle femurs for manipulations, perches and foraging containers.

The animal room received about 15 air changes per hour, while a 12-hour photo period was maintained. The temperature was kept at 25°C and the humidity at 40%. The monkeys were weighed every second week to two decimal places (0.01 kg) using a Berkel top loading scale (Table 2).

Baseline samples of blood were taken while the animals were on MRC in-house diets. Before sampling, the animals were starved for ± 20 hours from the afternoon (16:00) of the previous day until material was collected under 10 mg/kg ketamine anaesthetic from both groups the next morning (08:00–12:00). Approximately 10 ml of blood was collected by femoral venipuncture for haematological evaluations. About 3 ml of blood was placed into vacuum tubes containing EDTA as anticoagulant, while smears were made from whole blood with anticoagulant. The remaining samples for plasma biochemistry were placed in 10-ml heparinised tubules, centrifuged in a Beckman model J6B refrigerated bulk centrifuge and the plasma stored at − 40°C until assayed. The samples were analysed on an automated Ciba Corning 550 Express clinical chemistry analyser and Coulter MD 18 haematology analyser within 8 hours following collection. Table 3 contains the methods used for individual analyses and includes the intra- and inter-assay coefficients of variation for the biochemical indices that were evaluated.

Univariate analyses were used to examine the distribution of all variables. The baseline measurements for the two groups differed to such an extent that all measurements over time had to be adjusted for the baseline. This was completed by computing the difference from the baseline for every variable at each time point, and conducting statistical analyses on these values. All further comparisons between the milk solids and maize + legume groups were analysed by repeated measures analysis of variances of the different variables. This statistical tool allowed for the trends of both groups to be investigated over the specified time period.

When no statistical differences were attained at this level, or if group-time interactions occurred,

Table 3. Analytical methods

Test	Method	Units	CV %
Aspartate aminotransferase (AST)	NADH-NAD	IU/l	2.3–4.9
Alanine aminotransferase (ALT)	NADH-NAD	IU/l	2.0–3.0
Gamma glutamyl transferase (GGT)	L-γ-Glutamyl-3-carboxy-p-nitroanilide	IU/l	1.6–2.4
Alkaline phosphatase (ALP)	p-Nitrophenylphosphate	IU/l	3.0–3.5
Glucose	Hexokinase	mmol/l	1.6–2.0
Total protein	Biuret	g/l	1.2–3.1
Total bilirubin	Diazotised sulphanilic acid	µmol/l	2.2–5.5
Albumin	Bromocresol green	g/l	1.8–2.7
RBC	Coulter counter	$\times 10^{12}$/l	–
WBC	Coulter counter	$\times 10^{9}$/l	–
Differentials	Cytochemical staining	%	–
Haemoglobin (Hb)	Coulter counter	g/dl	–
Haematocrit (Hct)	Coulter counter	%	–
Mean cell volume (MCV)	Coulter counter	fL	–
Mean corpuscular Hb [] (MCHC)	Coulter counter	g/dl	–
Platelets	Coulter counter	$\times 10^{9}$/l	–

CV % = intra- to inter-assay coefficient of variation percentages for the biochemical parameters that were measured using six replicates per sample.

data from groups were compared at each individual sampling period where significance was indicated via asterisk when appropriate. Statistical significance was reached at 5%. For marginal significance, a level between 5% and 10% was considered because the sample size was small. This analysis was produced by a General Linear Model procedure using SAS (version 6, 1989, USA).

Results

The animals were food compliant, in that both groups consumed their respective diets normally, and wastage of food was insignificant. We constantly observed their body weights, which remained normal throughout the experimental period (Table 2).

The diets had variable effects on liver enzymes. Whilst both diets had no effect on aspartate aminotransferase (AST; Fig. 1a), the maize + legume group showed an elevation in alanine aminotransferase (ALT; Fig. 1b), at only week 8 of the experimental period ($P < 0.05$). Furthermore, the concentrations of gamma glutamyl transferase (GGT; Fig. 1c) also remained unaffected by the type of dietary proteins that the animals consumed. On the other hand, alkaline phosphatase (ALP; Fig. 1d) decreased in both groups over the experimental period, with marginally lower levels being recorded in the monkeys that were fed the maize + legumes diet ($P < 0.07$).

There was a general trend towards a decrease in total protein levels (Fig. 2a) for both groups (except during week 8), relative to baseline values ($P < 0.001$). On the other hand, the levels of albumin (Fig. 2b; $P < 0.02$) and bilirubin (Fig. 2c; $P < 0.003$) were always higher in the milk solids group than in the maize + legume group. In addition, glucose concentrations were significantly raised ($P < 0.05$) in the milk solids group during weeks 2, 4 and 6 (Fig. 2d).

The diets did not significantly affect haematological indices, such as red blood corpuscles (Fig. 3a), platelet counts (Fig. 3b), hemoglobin (Fig. 3c), white blood corpuscles (Fig. 3d), monocytes (Fig. 3e), as well as neutrophils (Fig. 3f). However, lymphocyte levels (Fig. 4a) were significantly higher ($P = 0.03$) in the group that consumed maize + legumes, while values for the haematocrit (Fig. 4b; $P < 0.002$), mean cell volume (Fig. 4c; $P < 0.03$), and also mean corpuscular haemoglobin concentration (Fig. 4d; $P < 0.0001$) were raised in the monkeys that were given the Western-type milk solids diet.

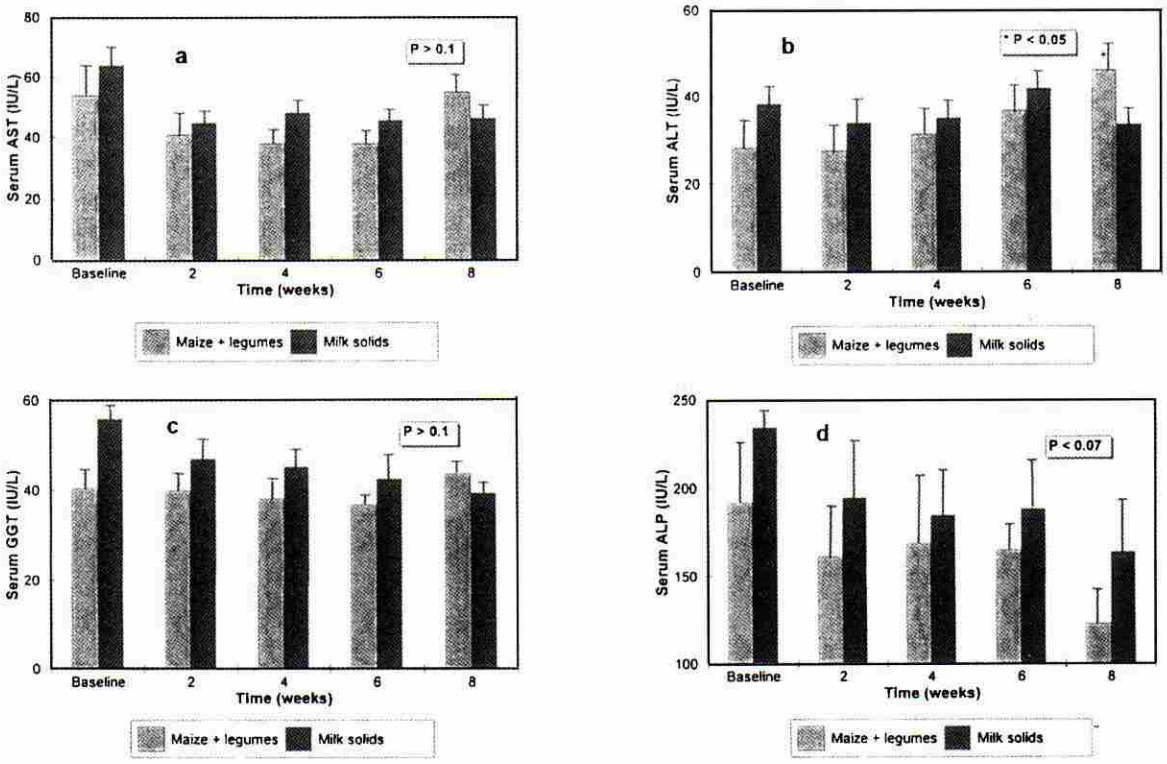

Fig. 1. (**a**) Serum total AST of monkeys on milk solids or maize + legume diets. (**b**) Serum total ALT of monkeys on milk solids or maize + legume diets. (**c**) Serum total GGT of monkeys on milk solids or maize + legume diets. (**d**) Serum total ALP of monkeys on milk solids or maize + legume diets. All biochemical and haematological data are represented as means ± SEM.

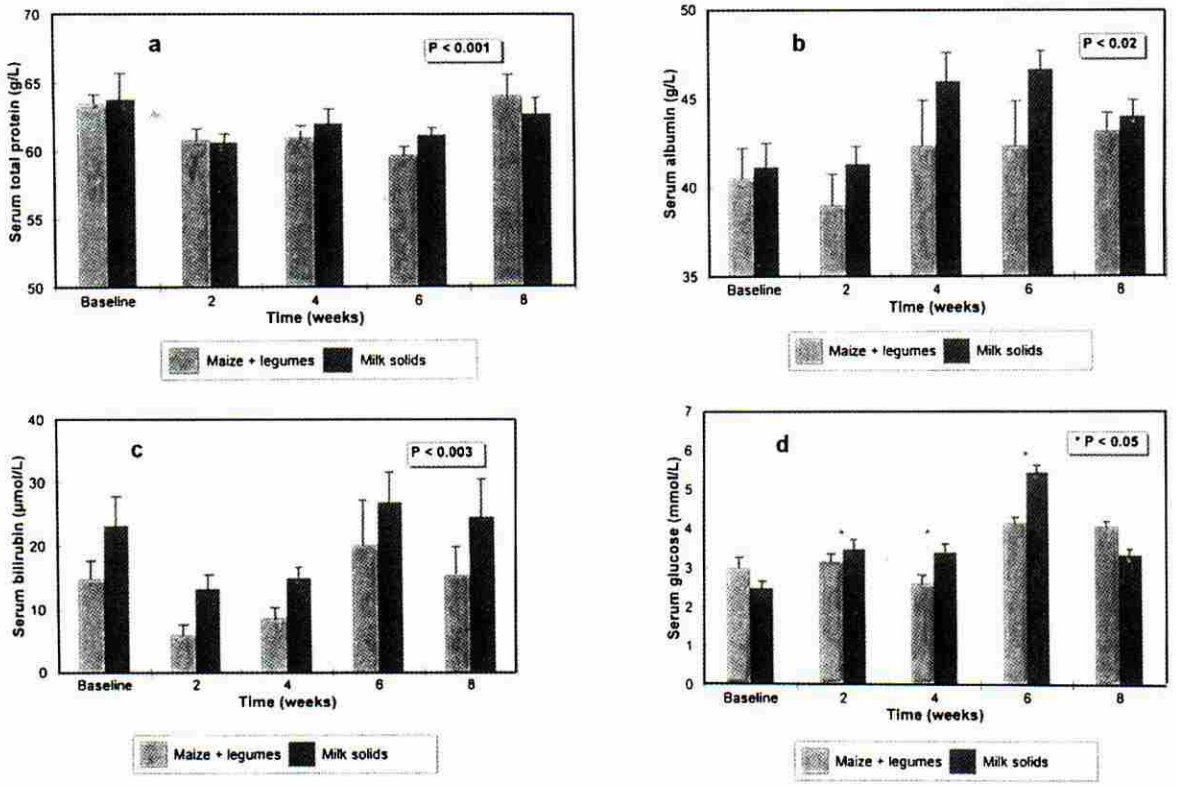

Fig. 2. (**a**) Serum total protein of monkeys on milk solids or maize + legume diets. (**b**) Serum albumin of monkeys on milk solids or maize + legume diets. (**c**) Serum bilirubin of monkeys on milk solids or maize + legume diets. (**d**) Serum glucose levels of monkeys on milk solids or maize + legume diets. All biochemical and haematological data are represented as ± SEM.

Discussion

This investigation demonstrated that certain haematological and biochemical indices were affected by the type of diet that the animals consumed. The data obtained in this investigation are in general agreement with the physiological limits of the same measured parameters reported elsewhere [39, 47], and suggest that environmental cues, such as food, could play an important role in modulating metabolism.

The AST in animals that consumed elevated dietary protein was similar in both groups. Mean ALT, an indicator not related to the age or sex of monkeys [10], was only higher for the high maize + legume monkey group during week 8. Large changes in GGT are related to altered protein metabolism [28]. The types of diets used in this study had no significant effect on this and other enzymes, and may point to the possibility that the amino acid profiles provided by both diets were appropriate for normal liver functioning. Even though there was a general decrease in ALP for both groups of animals, this trend proved only to be marginally significant, with all values remaining within physiologically normal limits.

Serum total proteins were generally higher in the milk solids group throughout the experiment, with values that are in fair agreement with those obtained by other investigators [43, 47, 50]. Albumin is included when reporting the value for total proteins [29], and this protein fraction increased for both groups over the course of the experimental period. Despite the increasing trend in circulating albumin in both groups, this variable remained higher in the animals that were fed the milk solids diet, compared with the monkeys that received the traditional African-type food regime of maize + legumes. Different diets are thought to affect albumin levels in non-human primates [20], with the milk solids in this study improving concentrations of this parameter for a potentially beneficial nutritional effect.

The nature of the diet affected serum glucose levels. The monkeys on the maize + legume diet had significantly lower levels of glucose during weeks 2, 4 and 6. This may be linked to the fact that plant products, such as grains and beans, contain large quantities of water soluble fibre that prevents glucose and insulin surges, by slowing the rate of glucose absorption [30].

In addition, water soluble fibres have been reported to decrease the rate at which amylase digests foods containing viscous fibres, allowing for an extended period of time over which glucose is absorbed [23], particularly across the intestinal wall [1]. However, complete glucose tolerance tests would have to be performed to evaluate whether glucose metabolism can be detrimentally affected by the type of dietary protein that is consumed.

It has been established that the milk protein casein plays a negative role in blood coagulation [6], which may be related to a decrease in platelet survival, limited platelet production, or elevated pooling of platelets in the spleen [18]. Although

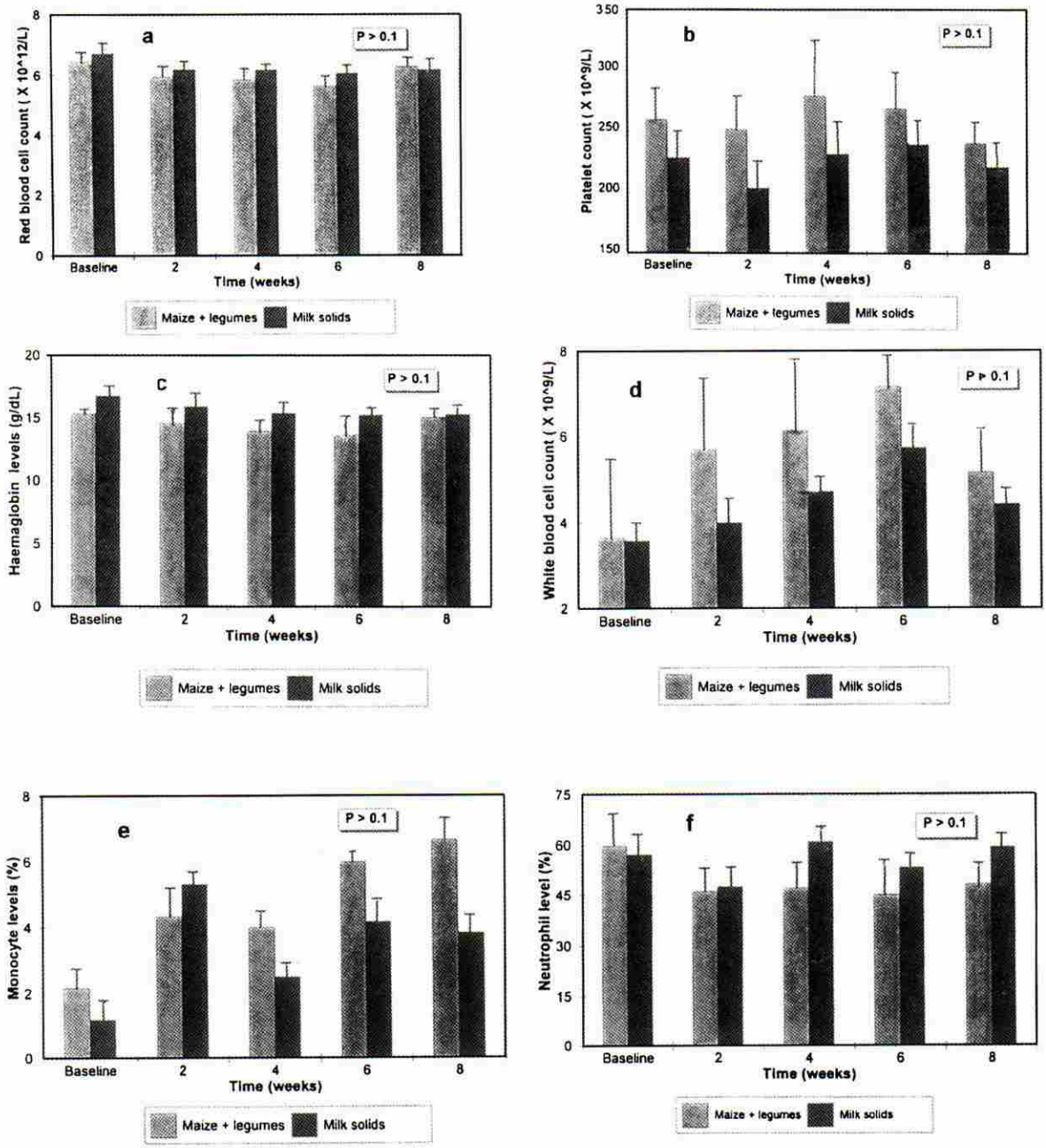

Fig. 3. (**a**) RBC counts of monkeys on milk solids or maize + legume diets. (**b**) Platelet counts of monkeys on milk solids or maize + legume diets. (**c**) The haemoglobin levels of monkeys on milk solids or maize + legume diets. (**d**) WBC counts of monkeys on milk solids or maize + legume diets. (**e**) The monocyte levels of monkeys on milk solids or maize + legume diets. (**f**) The neutrophil levels of monkeys on milk solids or maize + legume diets. All biochemical and haematological data are represented as ± SEM.

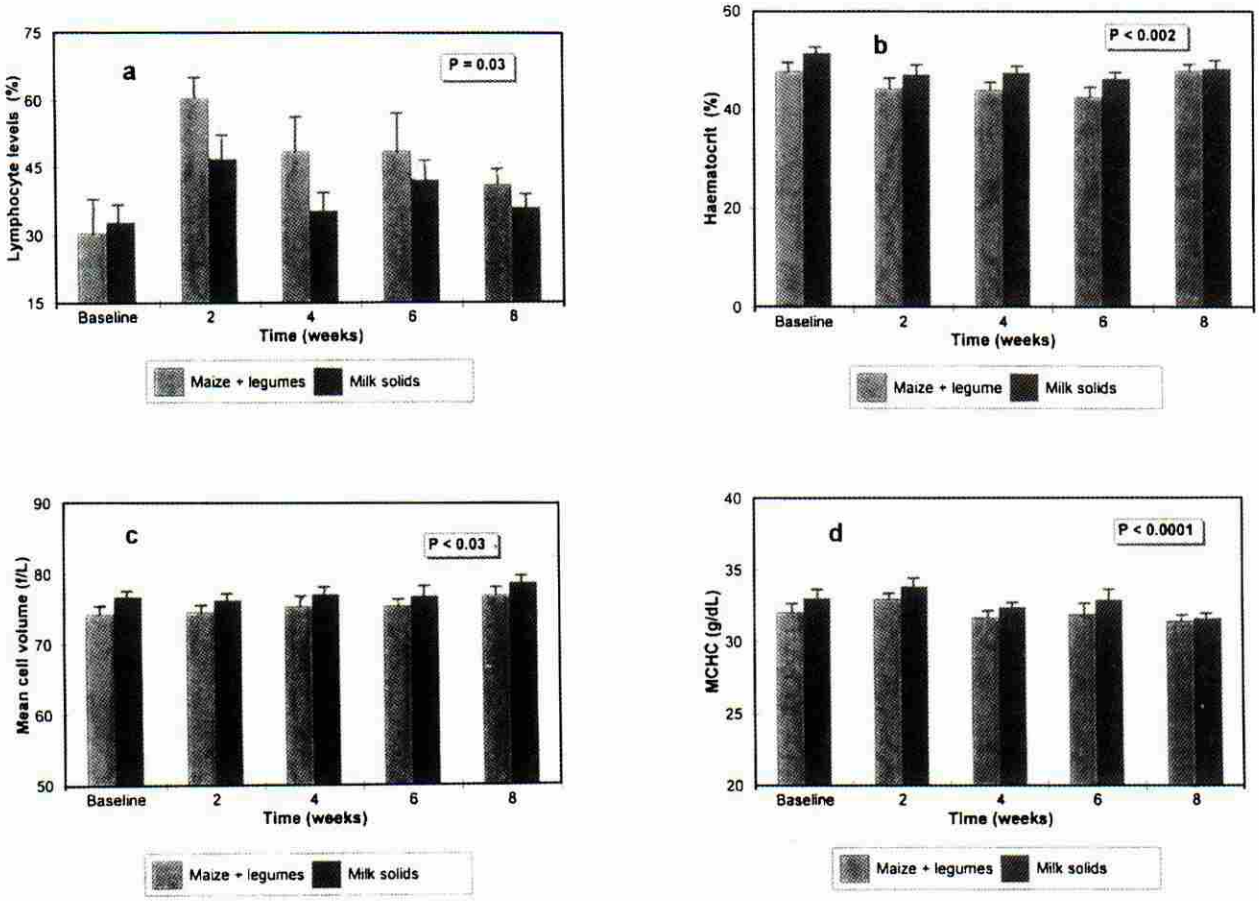

Fig. 4. (**a**) The lymphocyte levels of monkeys on milk solids or maize + legume diets. (**b**) The haematocrit of monkeys on milk solids or maize + legume diets. (**c**) The mean cell volume of monkeys on milk solids or maize + legume diets. (**d**) The MCHC of monkeys on milk solids or maize + legume diets. All biochemical and haematological data are represented as ± SEM.

platelet counts in animals on milk solids appeared to be diminished when compared to those on maize and legumes, these differences did not reach statistical significance. In addition, the concentrations of RBCs in both groups were statistically similar and unrelated to the type of diet that the animals consumed.

The animals on the milk solids diet also had higher levels of indices, such as mean cell haemoglobin concentration, mean cell volume and the haematocrit, when compared with those on the maize + legume food regime. Since the values for these parameters in both groups were within the range of data collected from other monkeys, there was no danger of any of the animals becoming anaemic [29]. Because RBCs contain hemaglobin, and the haematocrit is an indicator of RBC numbers [20], one would expect changes in these three parameters to be similar. In this study, though, significant changes in haematocrit measurements follow more closely similar trends observed in mean cell haemoglobin concentration and mean cell volume parameters. It has been reported that diets do influence RBC membrane fatty acids [33, 44], possibly affecting proper functioning, while species and sex differences affect haemoglobin, haematocrit and RBC indices [20]. However, we cannot, as yet, pinpoint the specific reasons for the relational differences we report for these indices.

Evidence from animal models suggests that dietary fat that is associated with increased animal protein consumption may affect the immune system by interfering with its immunosurveilance capabilities [2, 8]. In this regard, it has been suggested that high polyunsaturated fatty acids (PUFA) depress natural killer cell activity [19, 48]. In the current study, fat levels were controlled and were roughly similar in both groups. Nonetheless, the diets did differentially affect immune cell counts.

Total WBC counts were not significantly affected by the diets that the animals were given, but the levels were nevertheless higher in the maize + legume group when compared with the milk solids group. Differential WBC counts for monocyte levels in the maize + legume group showed the same

trend, albeit statistically insignificant. It is doubtful that these responses may relate to food contaminants, since we autoclaved our diets, but could well be linked to the nature of the fats, inherent in the milk solids diet. With a lower level of pathogen-fighting cells, the immune system would be more vulnerable to infection in monkeys on milk diets that have also been linked to ailments such as asthma, hay fever, sinusitis, atopic dermatitis and insulin-dependent diabetes [7, 13, 32, 46, 51].

The high consumption of an animal product, such as milk solids, may raise fatty acid intake. Increased amounts of fatty acids may influence membrane fatty acid composition, and hence, its functional properties, thereby altering how cells respond to stimulation. This is essential because cells of the immune system are dependent on cell membrane function for secretion of antibodies, antigen receptors and other bioactive compounds [24]. Despite our finding of depressed lymphocyte levels in monkeys on milk solids, it remains an isolated immune function. It is not indicative of the functioning of the whole immune system, nor can these WBC indices be used to assess the risk profile for tumourigenesis or viral infections.

Furthermore, variables such as eosinophils, basophils and immunoglobulins should be assessed in further studies, since they would provide a more comprehensive indication of whether the immune system is challenged. In addition, only a single analysis was performed on each animal at baseline, and at the individual sampling stages post-dietary intervention. Further studies should include replicates of these indices at each time interval to better control for variation and to provide more comprehensive evidence of a dietary induced effect.

This investigation showed that in many instances, except for certain immune cell counts that were depressed, most of the biochemical and haematological variables in monkeys that consumed the Western-type milk solids diet were raised, when compared to values in animals on the traditional African diet. However, it would be prudent to complete longer-term studies in this area, if we are to make firmer conclusions regarding the link between the nature of dietary proteins that are consumed and its effect on metabolism.

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